Chapter

Metformin and Its Benefits in Improving Gut Microbiota Disturbances in Diabetes Patients

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Abstract

The human gastrointestinal tract presents a vastly population of microorganisms, called the microbiota. The presence of these microorganisms offers many benefits to the host, through a range of physiological functions. However, there is a potential for these mechanisms to be disrupted condition, known as dysbiosis. Recent results are showing important associations between diabetes and the gut microbiota and how the intestinal flora can influence the prognosis of this illness. Microbial intestinal imbalance has been linked to alterations in insulin sensitivity and in glucose metabolism and may play an important role in the development of diabetes. Metformin is one of the most important and widely used first-line medications for the management of type 2 diabetes (T2D). It is a complex drug with multiple sites of action and multiple molecular mechanisms. In recent years, attention has been directed to other modes of action, other than the classic ones, with increasing evidence of a major key role of the intestine. By analysing the effects of metformin on the homeostasis of the microbiota of diabetes patients, our present topic becomes one of the major importance in understanding how metformin therapy can improve gut microbiota dysbiosis and thus provide a better outcome for this illness.

Keywords: metformin, diabetes mellitus, gut dysbiosis, improvement, microbiota

1. Introduction

The human gastrointestinal tract hosts a complex population of microorganisms. The function and composition of the gut microbiota vary from an individual to another, factors contributing to its differences being various. The mode of birth, the type of diet, exercise, body mass index, different diseases and therapies are factors that influence the gut microbiota composition and function. Type 2 diabetes (T2D), a highly prevalent metabolic disease, is lately characterized as a disease with significant alteration of the composition and function of the gut microbiota. New therapeutic targets are revealed, and researchers are thoroughly exploring these possible pathways and hypotheses to understand the pathogeny of

the disease better and also to better manage the treatment options. Metformin, one of the most widely used first-line medication for the management of type 2 diabetes, looks to present other modes of action than the classic ones involving liver metabolism. Studies proved that metformin could modulate the gut microbiota disturbances encountered in type 2 diabetes, in this way improving the outcome of the disease.

1.1 The gut microbiota: definition, development and structure

Among other things, the cohabitation of the man with the environment is at the root of the human evolution, an extraordinary example in this sense being the relationship between humans and microorganisms.

The digestive tract hosts a complex, vast and dynamic community of microorganisms, called the microbiota. Together they form a mutualist relationship, with profound implications for the host both during homeostasis and disease [1].

It is worth mentioning that the gut is not the only place where there is a population of microorganisms with which the human organism is in such a connection (e.g., the skin also harbouring a plethora of bacteria) [2].

The composition of the microbiota varies from individual to another but also from segment to segment of the digestive tract and includes species from all three domains of life: bacteria, Archaea and Eukarya. All of the species are classified into 12 different phyla, of which more than 90% belong to *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* [3].

The process of colonizing the digestive tube with microorganisms is classically believed to begin at birth by "seeding" the newborn with microorganisms originating from the mother's genital area (vaginal passage, mother's areola), the skin, and the microbiota of the contacts in the surrounding environment, and from then the development continues throughout life. In recent years, however, this theory is challenged by a series of studies that have shown the presence of microorganisms in uterine tissues (e.g., the placenta, suggesting that colonization could be initiated before birth, by haematogenous sowing) [4]. At the age of 3–4 years, the core of the microbiota is relatively defined, and its structure is similar to that of the adult but is continuously subject to change depending on various external and internal factors.

Even if the core of the microbiota is established from an early age, several factors contribute to carve its form, explaining its variations from an individual to another [5]:

- Type of birth
- Gestational age
- Diet (starting with breast milk that plays an essential role in the development of the flora)
- Ageing
- Geographic region and cultural habits
- Physical activity
- Diseases
- Drugs (especially antibiotic therapy)

As stated before, the composition also depends on the digestive tract region (biogeography), this being explained by the physiological properties of the digestive segment. For instance, in the small intestine, the pH is lower and the transit time is shorter, which is why only rapidly growing bacteria, with the ability to adhere to the surface, are thought to survive. On the other hand, the colon shows a favourable environment for the development of microorganisms. It is worth mentioning that there are differences in the composition between faecal/luminal and mucosal bacteria [6].

In its final form, this whole microsystem consists of over 2000 species, which make up altogether more than 100 trillion cells, about 10 times more than the cells of the human body, hence the name of "superorganism".

1.2 Functions

The microbiota exerts a significant influence on the host during homeostasis and disease, with profound implications for the proper body's physiological functions, considering the microbiota as a "forgotten organ".

The leading roles of the gut flora are the following:

- Mechanic barrier—strengthening the gut integrity, shaping and regenerating the intestinal epithelium, protecting against pathogens [7]
- Biologic active barrier—consuming the feeding substrates for pathogens [7]
- Key regulators of digestion—involvement in the metabolism of biliary salts, short-chain fatty acids (SCFAs), lipids and glucides [8]
- Harvesting energy [9]
- Regulating host immunity [10]
- Synthesis of vitamins—principal reservoir for B complex vitamins [11]
- Synthesis of dopamine, serotonin and other neurotransmitters [12]

1.3 Dysbiosis: definition, causes and consequences

Any perturbation of the healthy gut microbiota that disrupts the mutualist relationship between the organism and the associated microbes is called dysbiosis. The antagonist term of dysbiosis is eubiosis.

The underlying cause of a gut dysbiosis may be the following [13]:

- Unbalanced diet
- Drug therapy: antibiotics, chemotherapy, antiviral drugs and hormone therapy
- Diseases: cancers, hepatopancreatic diseases and diabetes
- Chronic and acute infections
- Local inflammation
- Presence of intestinal parasites
- Frequent enemas

The effects of dysbiosis are reflected in the processes of the internal environment, contributing to the emergence of numerous pathological conditions such as [14]:

- Autoimmune diseases [15]
- Allergies [16]
- Atherosclerosis [17]
- Obesity
- Diabetes
- Cancers [18]
- Neurological disorders [19]
- Prematurely ageing

2. Dysbiosis: microbiota in type 2 diabetes

The relationship between gut microbiota and diabetes is not fully understood, but changes in its composition and function can contribute to the onset and maintenance of insulin resistance, thus influencing the prognosis of this illness. Both T2D patients and those that are at high risk of developing this disease seem to have an imbalance in the composition and function of the microbiota, just like a "metabolic dysbiosis".

Analysing the literature, the main changes observed in the microbiota composition of diabetic patients are [20–23]:

- Reduced Gram-positive bacteria such as bacteria from phyla *Firmicutes*
- Reduced butyrate-producing bacteria, such as *Roseburia* and *Butyrivibrio*
- Decrease in bacteria that regulate intestinal permeability, such as *Akkermansia muciniphila*
- Increased Gram-negative bacteria, such as *Bacteroides*, *E. coli* and *Proteobacteria*
- Increase in various opportunistic pathogens such as *Clostridium symbiosum* and *Eggerthella lenta*

The Gram-negative bacteria (*E. coli*, *Bacteroidetes* and *Proteobacteria*) present lipopolysaccharides (LPS) at the surface of the membrane. Lipopolysaccharides are also known as endotoxins. They are large molecules consisting of a lipid and a polysaccharide composed of O-antigen with an outer core and an inner core joined by a covalent bond [24]. LPS are found to be elevated in the plasma of diabetic and obese patients by crossing an altered intestinal barrier (leaky gut). Accumulating, they trigger an inflammatory reaction called endotoxinemia. This systemic inflammatory response is associated with dyslipidaemia, increased blood pressure, but

also, with insulin resistance and earlier onset of diabetes through a variety of mechanisms such as [25]:

- Activation of pro-inflammatory kinases: mitogen-activated protein kinases and I kappa B kinase complex
- Increased expression of inflammatory proteins: tumour necrosis factor-α (TNFα), monocyte chemotactic protein and interleukin 6
- Impaired insulin signalling at the level of insulin receptor substrate 1
- Inhibition of glucose transport

The passage of LPS through the intestinal mucosa is due to increased intestinal permeability (so-called leaky gut) that can be explained by the diminishing of butyrate and mucin-degrading bacteria such as *Roseburia*, *Butyrivibrio* and *Akkermansia muciniphila*. Furthermore, this epithelial dysfunction can determine an important translocation of intestinal bacteria into the adipose tissue, which maintains a low-grade inflammation and insulin resistance, process called "metabolic infection" [26, 27].

2.1 Dysbiosis: microbiota in type 1 diabetes (T1D)

At the moment, the amount of information regarding an alleged link between gut microbiota and T1D is modest. Several studies showed similarities between the disturbances of the microbiota found in T2D and T1D patients: reduced population of *Firmicutes* and increased the population of *Bacteroidetes* and increased in intestinal permeability. Increased gut permeability might contribute to pancreatic β-cell damage due to the increased absorption of exogenous antigens such as Streptomyces toxin—streptozotocin—that has tropism for pancreatic tissue and can cause lesions at its level [28].

2.1.1 Dysbiosis: protective anti-inflammatory- and anti-insulin-resistant mechanisms

There are also mechanisms mediated by the gut microbiota such as the production of short-chain fatty acids and secondary bile acids (SBA) that counteract those pro-inflammatory- and insulin-resistant effects. These mechanisms can be affected in the case of dysbiosis.

SCFAs are produced from dietary fibres that are fermented by the intestinal bacteria. Acetate, butyrate and propionate are the three most common SCFAs. They exert an essential role in the metabolism of carbohydrates, lipids, in maintaining the integrity of the intestinal barrier and in modulating inflammatory reactions through a variety of functions [29]:

- Maintaining the integrity of the colon epithelium: Butyric acid is the primary energy source of the colon's epithelial cells. It stimulates the proliferation but also the differentiation and apoptosis of the colonocyte, thus participating in the coordination of its life cycle. It also participates in the regulation of tight junction proteins (claudin 1 and zonula occludens).
- Improves carbohydrate metabolism: Propionate lowers the accumulation of lipids in the adipose tissue and reduces hepatic lipogenesis thus decreases the

insulin resistance. Propionate and acetate also stimulate the production of glucagon-like peptide-1.

- Anti-inflammatory role: Butyric acid plays an essential role in maintaining the integrity of the intestinal mucosa, preventing endotoxemia and metabolic infection. Butyric acid also inhibits the nuclear factor kappa-beta from the macrophages that cause a suppression of TNF-alpha, IL-6 and myeloperoxidase activity.

At the intestinal level, bacteria metabolize primary bile acids (cholic and chenodeoxycholic acids) to secondary bile acids (deoxycholic and lithocholic acids). Bile acids are involved in multiple metabolic pathways, research over the last decades, demonstrating an essential role against inflammation and insulin resistance. Secondary bile acids contribute to a decrease in insulin resistance through:

- Stimulating the production of glucagon-like peptide-1 by binding to G-protein-coupled receptor 1 [30]

- Modulating glucose absorption through interaction with farnesoid X receptor (FXR)

- Modulating energy expenditure: increase energy expenditure in brown adipose tissue by activating enzyme type 2 iodothyronine deiodinase and oxygen consumption, thus contributing to the prevention of obesity [31]

- Increasing triglyceride clearance

- Bile acids are the major pathway for catabolism of cholesterol, thus regulating the metabolism of lipids

In terms of their anti-inflammatory role, lithocholic acid inhibits the release of pro-inflammatory cytokines TNF-alpha, IL1 and IL6 from colon epithelium [32].

3. Metformin and the gut

Metformin presents as a sophisticated drug having multiple sites of action and various molecular mechanisms. Lately, attention has been directed to other modes of action, different than the classic ones. Its action at the intestinal level was suggested by the results of several studies that showed the following:

- A delayed-release formula is retained almost entirely in the gut, with minimal systemic absorption. It is effective at lowering blood glucose as the standard immediate-release formulation in individuals with type 2 diabetes [33].

- In diabetic rats, intravenous administration of metformin is less effective than intra-duodenal administration for lowering blood glucose levels [34].

- Human genetic studies proved that variants in SLC22A1 gene (the gene encoding OCT1), which reduce hepatic uptake of metformin, do not impact upon the efficacy of metformin to lower HbA1c in individuals with type 2 diabetes [35].

Regarding the effects of metformin on the gut microbiota, studies have shown that administration of metformin produced several changes in the composition of the intestinal flora such as the following [36]:

- Increase microbes from *Verrucomicrobiaceae*, *Porphyromonadaceae*, *Rikenellaceae*, *Akkermansia muciniphila*, and *Prevotellaceae* spp. moreover, species from *Escherichia-Shigella* sp.

- Decrease of the *Lachnospiraceae*, *Rhodobacteraceae* spp., *Peptostreptococcaceae* and *Clostridiaceae*

Furthermore by comparing the modified microbiome profile by metformin treatment, with the microbiome profiles under various disease situations, these changes have been negatively correlated with multiple diseases that have an inflammatory pathogenic substrate such as colitis, chronic diarrhoea and irritable bowel syndrome, suggesting that its anti-inflammatory proprieties can be determined through regulation of the microbiota homeostasis.

The main side effects of metformin are gastrointestinal: nausea, vomiting, diarrhoea and abdominal pain. These side effects occur most frequently at the beginning of treatment, and in most cases, they disappear spontaneously. The cause of these side effects is not fully understood and may be due to the growth of opportunistic pathogenic bacteria from *Escherichia* to *Shigella* spp. which are shown to increase at the beginning of treatment. If we relate to the increase of these opportunistic pathogens, the further reduction of side effects can be caused by a reduction of the substrate to which these microorganisms are dependent (substrates provided by polysaccharide-degrading anaerobes) through diet and an increase of anaerobic mucus-associated bacteria such as *Akkermansia muciniphila* [37].

4. Metformin and the microbiota of type 2 diabetes

As stated before, the gut microbiota profile is profoundly modified in T2D patients in terms of its structure and composition. Administration of metformin results in improved glucose metabolism, but the way this is achieved is not fully understood, and its implications upon the intestinal flora are incompletely discovered. Analysing data from the literature, administration of metformin causes the composition to change and, therefore, the physiology of the microbiota as well.

5. Metformin and *Bacteroides fragilis*

Administration of metformin is associated with an essential decrease in *Bacteroides fragilis* [38].

Bacteroides fragilis is an obligately anaerobic, Gram-negative, rod-shaped bacteria, whose essential feature in metabolic pathology is the presence of capsular lipopolysaccharides. LPS are found to be elevated in the plasma of diabetic and obese patients and are associated with dyslipidaemia and increased blood pressure but also with insulin resistance and earlier onset of diabetes through a plenty of mechanisms that have been described previously. Colonizing mice with *Bacteroides fragilis* by transferring stool samples enriched with these bacteria determines an increase in body weight, impaired glucose tolerance and a decrease in insulin sensitivity.

Mechanisms by which metformin has determined the decrease of this species have not been elucidated but have been assumed since *Bacteroides fragilis* were reduced in mice that received stool samples from patients who had been given metformin.

Besides reducing *Bacteroides fragilis*, the bile acid glycoursodeoxycholic (GUDCA) is increased through decreasing the bacteria's bile salt hydrolase activity. GUDCA is a glycine-conjugated form of the secondary bile acid deoxycholic acid, which has been known to have anti-inflammatory proprieties by reducing the levels of pro-inflammatory cytokines. Another biological function of GUDCA is to antagonize the farnesoid X receptor.

The FXR is predominantly found at the intestinal and hepatic tissue. Bile acids are the major ligands (activators) of this receptor. It is mainly involved in the metabolism of bile acids but also of carbohydrates and lipids.

The primary functions of FXR activation is the suppression of cholesterol 7 alpha-hydroxylase (CYP7A1), which reduces the synthesis of bile acids (via the feedback mechanism, FXR is activated by bile acids and further determines the suppression of this enzyme, thus reducing the synthesis of bile acids). FXR inhibition produces an increase in bile acids improving metabolic endpoints due to their anti-inflammatory and insulin sensitivity effects [39].

6. Metformin and *Akkermansia muciniphila*

As stated before, the epithelial barrier of T2D patients is affected by an increase in its permeability (so-called leaky gut) followed by a migration of different toxins such as LPS in the systemic circulation causing inflammatory responses, insulin resistance and impaired glucose tolerance. In addition to these changes, a decrease in the *Akkermansia muciniphila* population was observed.

Akkermansia muciniphila is a mucin-degrading bacterium of the phylum *Verrucomicrobia* that resides predominantly in the mucus layer of the colon, where it is involved in maintaining intestinal integrity by promoting mucus secretion and making the barrier mechanism more stable and therefore decreasing its epithelial permeability. Oral supplementation with this bacterial population was shown to reduce intestinal permeability and improve glucose metabolism [40, 41].

A significant change in the composition of the microbiota under metformin treatment regarding intestinal permeability is represented by an increase in the population of *Akkermansia muciniphila*. The mechanism by which this process is accomplished is not fully understood, but it seems that these bacteria metabolise unabsorbable carbohydrates and mucin in short-chain fatty acids, which in turn will be used as fuel for goblet cells. Stimulated goblet cells will further produce mucin, in this way leading to the thickening of the mucus layer and thus to a decrease in the epithelial permeability. Besides increasing the population of *A. muciniphila*, administration of metformin is associated with an increase in the density of mucin-producing goblet cells probably through the indirect mechanism stated above [42, 43] **Figure 1**.

6.1 Metformin and SCAF-producing bacteria

One of the main features of the dysbiosis found in T2D patients is the decrease in butyrate-producing bacteria such as *Roseburia* and *Butyrivibrio*.

Butyrivibrio is a Gram-negative, anaerobic bacteria belonging to the *Clostridia* class, which was first described in the mid-twentieth century [44].

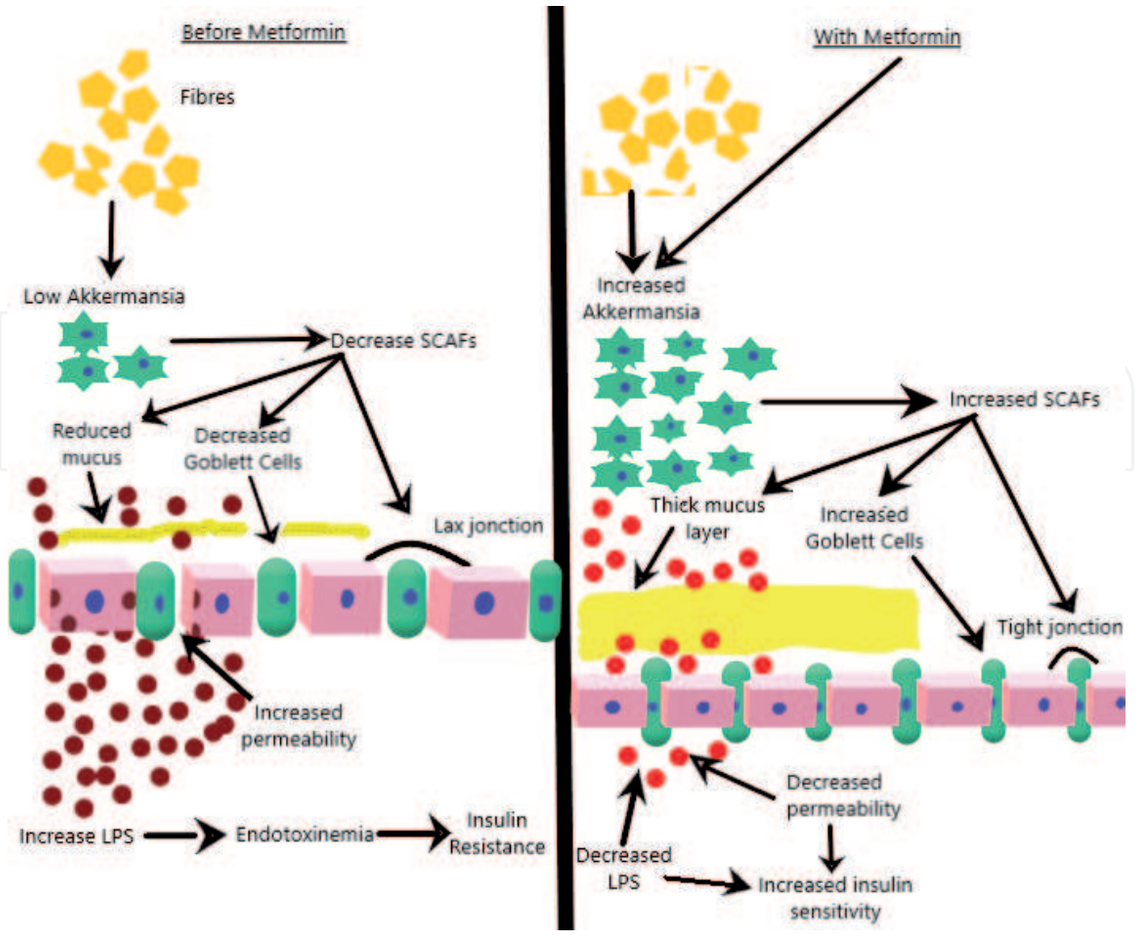

Figure 1.
Akkermansia muciniphila *mode of action [42, 43].*

Roseburia is a Gram-positive anaerobic bacteria member of the *Firmicutes* phyla named in honour of distinguished microbiologist Theodor Rosebury [45].

As stated above, short-chain fatty acids such as butyrate, propionate, and acetate are the product of gut microbiota activity, resulting from the fermentation of the carbohydrates that escapes the absorption process, playing an essential role in the process of enhancing intestinal integrity, reducing inflammation and improving the metabolism of glucose and lipids.

Significant increase of butyrate-producing bacteria, especially *Butyrivibrio* and *Roseburia*, is observed in T2D patients treated with metformin [43].

6.2 Metformin and probiotics

The genus *Bifidobacterium* is a Gram-positive microorganism, member of the *Bifidobacteriaceae* family, belonging to the great *Actinobacteria* phylum, one of the most abundant species of the gut microbiota.

Lactobacillus is a Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria that produces lactic acid from converting carbohydrates.

Oral supplementation of *L. casei* and *B. bifidum*, which are frequently used as a probiotic treatment option, alone and in combination, has been shown to improve insulin resistance (decreased fasting blood glucose, decrease HbA1C) and lower the serum lipid levels by enhancing short-chain fatty acids production, and thus improving the outcome of T2D patients [46].

Administration of metformin has been shown to increase the population of *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, and also *Lactobacillus* [47].

6.3 Metformin and *Adlercreutzia*

The soybean, a legume species native from East Asia, is widely grown for its edible bean, which has numerous uses. It has been assumed that soy foods contribute to reducing the risk of T2D and the progression of this disease in diabetic patients although opinions are divided by the results of studies which inform this theory or rather confirm it [48].

At the gut level, the main species that metabolizes soybean isoflavonoids to equol are the ones from *Adlercreutzia*. It is worth mentioning that not in all people isoflavonoids are metabolized to equol (so-called equol producers). It was speculated that the health benefits of soy-based diets might be higher in equol producers than in equol nonproducers [49].

It seems that metformin treatment increases the population of *Adlercreutzia* in diabetic patients and therefore stimulating the production of equol, thus enhancing soy-based diet health benefits [50].

6.4 Summary of changes found after and before metformin treatment

These tables help summarise the changes found in the gut microbiota both before and after metformin treatment in T2D patients **Tables 1** and **2**.

Structure before metformin treatment	Structure after metformin treatment
Reduced Gram-positive bacteria, such as bacteria from phyla *Firmicutes*	Increased *Firmicutes*
Reduced butyrate-producing bacteria, such as *Roseburia* and *Butyrivibrio*	Increased *Roseburia* and *Butyrivibrio*
Decrease in bacteria that regulate intestinal permeability, such as *Akkermansia muciniphila*	Significant increase of *Akkermansia muciniphila*
Increased Gram-negative bacteria, such as *Bacteroides*, *E. coli* and *Proteobacteria*	Significant decrease of *Bacteroides fragilis*
Increase in various opportunistic pathogens, such as *Clostridium symbiosum* and *Eggerthella lenta*	Increased probiotic bacteria, such as *Bifidobacterium* and
	Increase *Adlercreutzia*

Table 1.
Summary of changes in microbiota composition before and after metformin treatment of T2D.

Mechanisms before metformin	Mechanisms after metformin
Decrease production of SCFAs	Increased production of SCFAs
Decrease production of bile acids	Increased bile acid production, especially GUDCA Inhibition of farnesoid X receptor
Epithelial dysfunction and increased intestinal permeability	Enhancing the intestinal barrier, decreasing its permeability
Increased systemic LPS	Decreased in LPS migration, reduced systemic LPS
Endotoxemia and metabolic infection	Reduced endotoxemia
Inflammation	Decreased inflammation
Insulin resistance	Increased insulin sensitivity
	Increased production of equol

Table 2.
Summary of changes in the functions of microbiota before and after metformin treatment.

7. Conclusions

Alterations of the intestinal microbiota are a key element in understanding the pathophysiology of diabetes and maybe to explain the variability in terms of its therapeutic response and complications occurrence in different patients.

Metformin exerts a significant influence on the bacterial constellation found in the gut, bringing a significant contribution to restoring its balance.

With changes in both composition and function, modulation of the intestinal flora of patients with type 2 diabetes mellitus, obtained by various methods, can bring a better outcome of diabetes patients and can improve the morbidity and mortality rates of this widely present metabolic disease.

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