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Review Article

Metformin, Microbiota and Health

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Abstract

The intestinal microbiota is considered a large organ in the human body performing functions in the host that range from supporting digestion and absorption of nutrients from the diet to regulating the various processes in the host. Maintaining a diverse and stable microbiota is critical to maintaining host homeostasis and health. Studies have suggested the relationship between the microbial changes and the development of several pathologies. In this context, metformin, has shown to be a promising drug for the regulation of the microbiota, thus favoring the prevention and treatment of type 2 diabetes mellitus (T2DM), obesity, cancer, the inflammatory state of human immunodeficiency virus (HIV), heart disease, Alzheimer's disease and aging, and pathologies associated with dysbiosis. In this review, the main aspects on the importance of metformin's action on dysbiosis, and the factors that regulate the metformin uptake and activity as genetic polymorphisms and GLP-1 receptor activation were discussed.

Microbiota and its role in the human body

Microbiota, whose composition and activity are associated with health and the development of diseases,[1] are composed of different microorganisms, including bacteria, bacteriophages, eukaryotic viruses, and fungi that inhabit body surfaces and cavities usually in a beneficial relationship for both parties.[2] Most microbiotás microorganisms are found in the gastrointestinal tract (mainly colon), and their composition are influenced by the type of birth, lifestyle, diet in the first years of life, medications, and host genetics.[2]

Human microbiota composition comprises approximately 1,000 species, where bacteria are considered the main constituents corresponding to the five main phyla: Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia.[3] Studies have pointed out that the number of these microbial cells is around 1×10^{14} against 1×10^{13} of eukaryotic cells, thus indicating that the number of microbial cells is 10 times greater than the number of human cells.[4] Hence, the microbiota can be considered as a metabolic organ supporting important functions in the host, such as digestion and absorption of food nutrients, the regulation of the immune system, regulation of the endocrine and neurological functions, alteration of the mechanism of the action of drugs, removing toxins, and the production of various compounds, including vitamins and short chain fatty acids (SCFAs).[2,5] The maintenance of diverse microbiota with a functionally stable microbiome contributes to the homeostasis and health of the host.[6]

Modulations in the composition of the microbiota, compromising the symbiotic relationship between the host and microorganisms in a process called dysbiosis, are often associated with the development of autoimmune diseases, obesity, diabetes, cancers, neurological diseases among others.[7,8] Dysbiosis can be classified into three types: (1) loss of beneficial microorganisms, (2) an increase in opportunistic pathogens, and (3) loss of microbiota diversity; despite this categorization, the three scenarios usually occur simultaneously.[9] Thus, factors capable of promoting changes in the composition of the microbiota are considered risk factors for diseases associated with a microbial imbalance.[9] Therefore, drugs able to restore the microbiota balance have become promising candidates for the treatment of these pathologies. In this context, metformin is an important candidate, so are several factors that regulate its uptake and activity.

Metformin: Brief history and the main mechanisms of action

Metformin (1,1-dimethylbiguanide) is a drug of the biguanide class derived from guanidine, the active substance in Galega officinalis. The processes for its synthesis began in 1920, from the discovery of the antidiabetic properties in 1918 of guanidine.[10] Due to its high antihyperglycemic efficiency, without causing

Keywords: Gut microbiota; Dysbiosis; Metformin; GLP-1; Polymorphism.

Abbreviations: AD, Alzheimer's disease; AMPK, AMP-activated protein kinase; BCAAs, branched-chain amino acids; CRC, colorectal cancer; CVD, cardiovascular diseases; FMT, fecal microbiota transplantation; GLP1, glucagon-like peptide; HIV, human immunodeficiency virus; LPS, lipopolysaccharide; SCFAs, short chain fatty acids; T2DM, type 2 diabetes mellitus.

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hypoglycemia and with a low risk of developing lactic acidosis, metformin has been used for about a century, and for some years, it has been considered the therapy of choice for type 2 diabetes mellitus (T2DM).[10,11]

In recent years, the action of metformin has been observed on other diseases like various types of cancer, cardiovascular and neurological diseases, obesity, among others.[12] Metformin's mechanism of action on adenosine monophosphate (AMP)-activated protein kinase (AMPK) is central to most diseases in which metformin acts[13–15] and involves the inhibition of complex I (CI) of the mitochondrial electron transport chain (ETC), consequently leading to mitochondrial dysfunction and energy impairment. Metformin's entrance into the mitochondrial matrix has led to a reduction in the adenosine triphosphate (ATP) levels and an increase in the adenosine diphosphate (ADP) and AMP levels, thus promoting the activation of AMPK.[16] AMPK works as an intracellular energy sensor that restores the energy homeostasis disrupted by metformin, which increases the catabolic processes and reduces the anabolic processes. Thus, processes such as protein, lipid, and nucleic acid synthesis end up being reduced compromising the progression of diseases dependent on these processes.[17]

Metformin, microbiota modulation, and T2DM

T2DM is considered a metabolic syndrome characterized by impaired glucose metabolism resulting from a combination of genetic, environmental, and behavioral factors.[18,19] Epidemiological data for 2017 showed 462 million people worldwide diagnosed with T2DM, or corresponding to 6.28% of the world population.[20] This pathology is characterized by hyperglycemia associated with the scenario of insulin resistance leading to hyperinsulinemia, and in some cases, it could also be associated with reduced insulin production by pancreatic beta cells (β-cells) given the functional impairment.[21]

Some studies have pointed to differences in the microbiota between patients with T2DM and healthy patients,[22,23] which has been characterized by a reduction in the butyrate-producing strains (Faecalibacterium prausnetzii and Roseburia intestinalis) and an increase in the opportunistic pathogens (E.coli and Clostridium ramosun).[18,24] From this observation and several others, it was suggested that the involvement of the microbiota in the development and progression of T2DM passed through its metabolites. The involvement of the microbiota with T2DM was due to (1) compromising the integrity of the intestinal barrier, (2) reduced production of SCFAs, (3) the metabolization of bile acids, and (4) increased production of branched-chain amino acids (BCAAs)[18,25,26] (Fig. 1).

Metformin is orally administered and its concentration in the jejunum is estimated to be 30 to 300 times greater than in plasma indicating that the intestine is one of the main targets of metformin, in addition to constituting an important reservoir of this drug.[27]

This high uptake of metformin by the gastrointestinal tract influences the microbial profiles of T2DM patients treated with metformin compared to those who are untreated,[28] consequently revealing the impact of metformin in dysbiosis. At present, several studies have shown the ability of metformin to restore the present microbial profile before the development of the disease.[18,29,30] Microbiota regulation by metformin may also be induced by increasing or decreasing a particular strain.[31] Although changes in the microbiota are specific for each pathology in which metformin acts, in general, it is associated with an increase in various strains: Verrucomicrobiaceae, Porphyromonadaceae, Rikenellaceae, Akkermansia muciniphila, Prevotellaceae, Escherichia, and Shigella sp., and reduction of the strains: Lachnospiraceae, Rhodobacteraceae, Peptostreptococcaceae, and Clostridiaceae.[32]

SCFAs produced by certain bacterial strains are involved in increasing insulin sensitivity and intestinal barrier integrity, in addition to mediating the activation of intestinal gluconeogenesis (mainly butyrate and propionate), thereby ensuring energy homeostasis and better regulation of glucose metabolism.[33–35] Reduction of strains that produce SCFAs is associated with T2DM, while its increase was observed in metformin-treated T2DM patients.

Microbial profile alterations induced by metformin in T2DM treated patients showed (1) an increase in the Firmicutes phylum, (2) an increase in the Roseburia, Butyrivibrio, and Bifidobacterium genera, (3) an increase of the strain Akkermansia muciniphila, and (4) a reduction of the Bacteroides fragilis strain.[7]

By modulating the microbiota, metformin was able to (1) preserve the gastrointestinal barrier by increasing the Akkermansia muciniphila strain and reducing Lipopolysaccharide (LPS)-producing strains, such as Bacteroides fragilis, therefore enabling the production of mucus in the gastrointestinal barrier that guaranteed its integrity and reduced intestinal permeability. It also prevented the release of LPS and the subsequent activation of toll-like receptor 4 (TLR4) on the surface of the macrophages leading to an inflammatory cascade that involved the activation of JNK and IKKβ and phosphorylation of IRS-1/2, which are considered as markers of insulin resistance and damage the glucose metabolism, respectively.[5,30] (2) This increased the synthesis of the SCFAs mainly through the increase of the Akkermansia and Butyricoccus strains by increasing the intestinal integrity through the activation of AMPK which in turn regulated tight junctions between the enterocytes, thus reducing inflammation and improving the metabolism of glucose and lipids.[18] (3) Metformin regulated the bile acids, associated with the reduction of the Bacteroides fragilis strain that allowed an increase in the level of glycoursodeoxycholic bile acid (GUDCA), which plays an anti-inflammatory role as a farnesoid X receptor (FXR) antagonist leading to an increase in insulin sensitivity and maintenance of glucose homeostasis.[36] (4) It reduced the levels of the BCAAs (leucin, isoleucine, and valine), which were considered as risk factors for T2DM due to their action on the mammalian target of rapamycin (mTOR) pathway to induce insulin resistance (Fig. 1).[7,37–39]

Even though the use of metformin became widespread, in recent years, the development of glucagon-like peptide (GLP)-1 receptor agonists came to aid in controlling T2DM. GLP-1 is a 30-amino-acid peptide hormone produced in intestinal epithelial endocrine L-cells through the processing of proglucagon. Along with insulin secretion and a sensitivity increase, GLP-1 reduces glucagon secretion and improves hepatic glucose metabolism,[40] therefore leading to a reduction in body weight, inflammation, and altered bile acid composition resulting in an improvement of the patient's health.

Moreover, it was suggested that treatment with GLP-1 receptor agonists could influence the composition of microbiome,[41] and target both T2DM and obesity simultaneously.[41,42] GLP-1 peptide was augmented for its microbial diversity in hyperglycemic and obese mice, but it was unclear if this effect was due to the treatment with the peptide or a secondary effect of weight loss.

Associated with the modulation of gut microbiota by metformin, a study by Koh et al. showed that the microbial metabolite imidazole propionate, generally found in high levels among T2DM patients and related to glucose intolerance,[43] impaired the effect of metformin on the glycemic reduction in diabetic mice inhib-

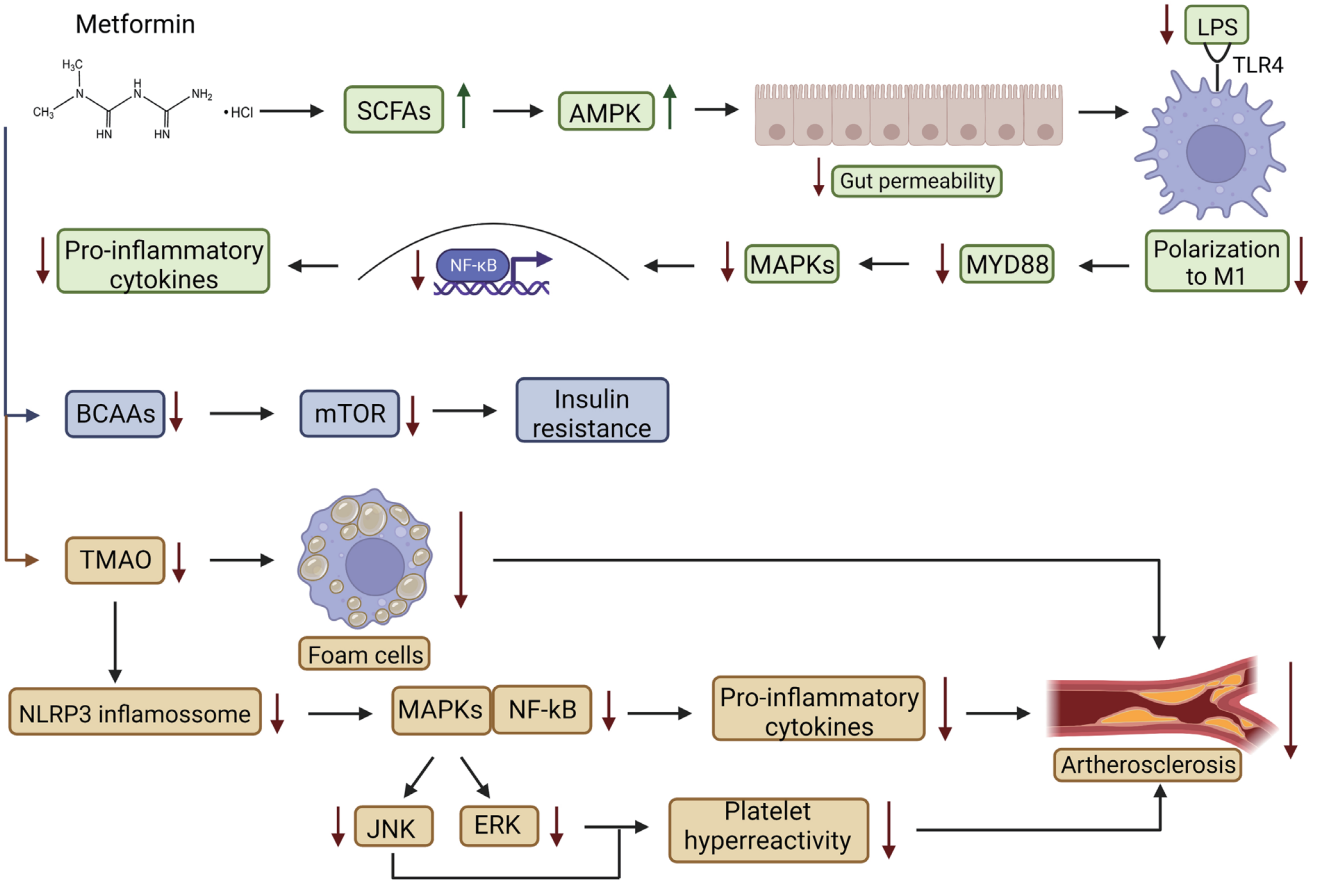

Fig. 1. Metformin action against T2DM, obesity, CRC, HIV inflammation, and CVDs through microbiota and molecular alterations. Through microbiota modulation, metformin enhanced the SCFAs strains leading to AMPK activation and the reduction of gut permeability reducing the LPS release in circulation and its recognition by TLR4 in macrophage, as well as avoiding its polarization to the M1 phenotype, the recruitment of protein adapters (such as MYD88), and the activation of MAPKs and NF-κB reducing the secretion of pro-inflammatory cytokines. Beyond its action against inflammation in T2DM and CVDs, metformin reduced the BCAAs levels in these patients by avoiding mTOR activation and insulin resistance, a risk factor for T2DM and CVDs. Due to the importance of the TMAO levels for the development of CVDs, metformin reduced TMAO through microbiota modulation by avoiding macrophage transformation into foam cells (a hallmark of AS) and the activation of NLRP3 inflammasome, consequently reducing MAPKs (JNK and ERK), NF-κB activation, platelet hyperreactivity, the secretion of pro-inflammatory cytokines, and the risk of AS development. Green squares: metformin action in T2DM, obesity, CRC, HIV inflammation and CVDs; blue squares: metformin action in T2DM and CVDs; orange squares: metformin action in the CVDs. AMPK, AMP-activated protein kinase; AS, atherosclerosis; BCAAs, branched chain amino acids; CRC, colorectal cancer; CVDs, cardiovascular diseases; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MYD88, myeloid differentiation protein 88; NF-κB, nuclear factor kappa B; NLRP3, NOD-LRR- and pyrin domain-containing protein 3; SCFAs, short chain fatty acid; T2DM, type 2 diabetes mellitus; TLR4, toll-like receptor 4; TMAO, trimethylamine N-oxide. Created with www.biorender.com.

iting the phosphorylation of the AMPK active site T172 through the activation of p38 gamma, but the effects of metformin were recovered after the inhibition of p38 gamma. This indicated that a microbial metabolite could be associated with metformin response variations among T2DM patients.

Understanding the importance of the regulation of microbiota exerted by metformin, so that it could exert its mechanisms of action, a study by Pryor et al. evaluated the relationship between nutrients, microbiota, and metformin action. In this study, it was demonstrated that the nutrients from the amino sugars, peptides, amino acids, and nucleotides classes would be important to the regulation of dysbiosis found in diseases, such as T2DM, by metformin. These nutrients would favor the growth of strains like Escherichia coli, where metformin would promote the enhancement of galactose and the phosphotransferase system (PTS) of the transcription factors Crp and ArgR, thereby indicating the importance of the bacterial PTS-Crp to the metformin action. The PTS is a system associated with the uptake of carbohydrates and downstream regulation of Crp; as such, possibly the Crp with its cofactor AMP could control the transcription of different genes that could alter the metabolism and be associated with an extended lifespan, including the enhancement of the agmatine production by the bacteria that would exert an important role on metformin action in T2DM.[44]

The glycoregulation action of metformin could also be explained by its effect on the enhancement of the expression of the sodium glucose cotransporter-1 (SGLT1) in the upper small intestine that was demonstrated in rodents[45] and humans.[46] This transporter acts on the uptake of glucose and the secretion of GLP-1.[47] As a way to elucidate how metformin promotes the expression of SGLT1, Bauer et al. showed that metformin induced changes in the microbiota of the upper small intestine by enhancing strains of Lactobacillus genus

that acted on the enhancement of SGLT1, an important transporter for the anti-hyperglycemic effect of metformin.[48]

Metformin, microbiota, and gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a common metabolic complication among pregnant women, which is characterized by hyperglycemia that brings risks to the mother and baby.[49] The treatment involves changes in the lifestyle to the use of insulin and metformin, in which the latter has gained more preference due to less side effects.[50,51] Even though it was already demonstrated that metformin altered the microbiota, there have been a number of studies on the microbiota alteration in women with GDM under metformin use. In this context, the study of Molina-Vega et al. showed that women treated with metformin had lower blood glucose and weight gain, and these effects would be associated with the enhancement of the Proteobacteria, Enterobacteriaceae, and Coprococcus strains involved with a lower body mass index (BMI) and blood glucose, and with the decrease of the Firmicutes and Peptostreptococcaceae, strains associated with a higher BMI and blood glucose. Therefore, metformin promoted changes in the microbiota of women with GDM as a way to corroborate its effects in GDM.[52]

The impact of probiotics, prebiotics, and metformin in a microbiome

The use of probiotics (live microorganisms administered to the host at a certain concentration) and prebiotics (non-digestible nutritional ingredients that stimulate the growth of strains) beneficial to the host in combination with metformin could promote valuable changes in the composition of the intestinal microbiota[53] by corroborating an antihyperglycemic effect and improving the response to metformin in intolerant T2DM patients.[54,55] The combination of metformin and probiotics (Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus gasseri, Bifidobacterium breve, Bifidobacterium animalis sbsp. lactis; Bifidobacterium bifidum, Streptococcus thermophilus and Saccharomyces boulardii)[55] increased the SCFA-producing strain Anaerotruncus colihominis and the metabolic pathways associated with butyrate production (SCFA) in patients treated with this combination, which was not observed with treatments using only one or the other. In addition, associated with the increase in the SCFAs in patients treated with the combination, an improvement in blood glucose and attenuation of insulin resistance was observed. Hence, this indicated that the use of probiotics could increase the antihyperglycemic efficiency of metformin in T2DM patients through microbiota modulation.[55]

Prebiotics could also aid the effects of metformin. In Shin et al.'s clinical study with T2DM patients, the subjects were subjected to a placebo or Scutellaria baicalensis (SB) root (antidiabetic action) with metformin for a period of eight weeks, and its effects on the pro-inflammatory markers (TNF-α and IL-6) and on the antihyperglycemic action of metformin were evaluated. A reduction in glucose intolerance associated with changes in the intestinal microbiota was observed in patients treated with SB+metformin with an increase in the Lactobacillus and Akkermansia strains,[53] which was considered important for glycemic and insulin homeostasis.[56,57] Therefore, SB acted as a prebiotic and improved the antidiabetic efficiency of metformin by modulating the intestinal microbiota.[53]

It is also important to emphasize that because T2DM is a risk factor for obesity, cardiovascular diseases (CVDs) and cancer, in addition to compromising healthy aging, the use of probiotics and prebiotics as adjuvants to metformin in T2DM could reduce the risk of developing these diseases.

Metformin and obesity

Obesity is a multifactorial disease whose incidence has tripled since 1975, and responsible for affecting more than 650 million adults, and 340 million children and adolescents according to data from the World Health Organization (WHO) in 2016.[58] Furthermore, the pre-obesity stage affected more than 1.9 billion adults according to the WHO data in 2016.[58] In Brazil, between 2016 and 2017, obesity increased by 60% among people over 18 years of age.[59] Overweight and obesity are defined as the high accumulation of fat that causes important impacts on health and is related to the development of several diseases, such as arterial hypertension, dyslipidemia, CVDs, T2DM, and cancer.[60]

Even though some genetic and epigenetic factors can contribute to the development of obesity, the interaction between genetic and environmental factors, such as diet and lifestyle leads to the imbalance between energy obtained and spent.[61]

In addition, many studies suggest the participation of the intestinal microbiota in the development of obesity.[62,63] The first study that demonstrated the association between dysbiosis and obesity was carried out by Ley et al, where a 50% reduction in the genus Bacteroidetes and 50% increase in the genus Firmicutes in obese animals was observed through metagenomic analysis; moreover, these animals showed an increase in the methanogenic Archaea strains, such as Methanobrevibacter smithii, which in turn were associated with a higher efficiency of fermentation.[64] The study was extended to humans showing a similar profile.[65] Other studies also observed an increase in the Firmicutes/Bacteroidetes ratio in obese people.[66–69] However, some others found a contrary profile or did not detect changes between these strains.[70–73]

Other strains were also altered in obesity. Some studies showed a lower abundance of the genus Bifidobacterium in obese individuals.[68,71,74] However, only a few species belonging to this genus had their anti-obesity action confirmed in animals,[75] which was associated with their anti-inflammatory effect. Additionally, opportunistic pathogens were often associated with obesity like Fusobacterium, Escherichia/Shigella, Staphylococcus aureus that were observed in pregnant women and overweight children, and Enterobacteriaceae was observed in pregnant and overweight women.[71,68–77] Unlike opportunistic pathogens, beneficial strains were generally present in low concentration in obese individuals, such as Akkermansia muciniphila.[78] Hence, the development of obesity is mainly associated with its involvement with the reduction of SCFAs, inflammatory processes, and energy regulation.[79–81]

The intestinal microbiota are also able to ferment polysaccharides present in the diet that are not digested by humans, thus generating SCFAs.[80] Once absorbed, SCFAs could be transferred to hepatocytes, where they would mediate lipogenesis and gluconeogenesis as substrates.[82,83] Additionally, they would act as signaling molecules binding to free fatty acid receptors 2 (GPR43 or FFAR2) and 3 (FFAR3 or GPR41), and would be expressed mainly by intestinal epithelial cells.[84] Through these receptors, SCFAs would promote greater energy uptake from food and fat accumulation leading to an increase in body mass.[79] SCFAs also promote the inhibition of the fasting-induced adipocyte factor (FIAF), which inhibits lipoprotein lipase (LPL) activity resulting in the accumulation of triglycerides in adipocytes.[80]

Furthermore, the inflammatory process is an important contributor to obesity, and some studies have already demonstrated the

presence of pro-inflammatory cytokines during the progression of obesity.[85,86] The relationship between microbiota, obesity, and inflammation was found to be associated with LPS, which is related to the deposition of adipose tissue, the inflammatory process, and insulin resistance.[87,88]

The microbiota of obese individuals is also considered a little diverse and contributes in different ways to the development and progression of obesity.[89] Thus, drugs capable of regulating the microbiota and circumventing obesity-associated dysbiosis are promising candidates for the treatment of obesity.[18]

Likewise, several studies have reported weight loss promoted by metformin both in diabetic patients[90,91] and in non-diabetic patients.[92,93] Metformin plays its anti-obesity effects through 1) increased expression of the fibroblast growth factor 21 (FGF21), which is associated with increased lipolysis,[94] 2) increased activity of brown adipose tissue (BAT), a tissue rich in mitochondria capable of increasing thermogenesis,[95] and 3) reduced appetite associated with lactate production[96] and increased levels of GLP-1, a molecule that acts in the regulation of glucose metabolism.[97]

The activation of AMPK by metformin plays an important role in its actions, as it is associated with the reduction of blood glucose, insulinemia, cholesterol synthesis, and lipogenesis, thereby allowing metformin to perform its anti-obesity and antidiabetic effects.[98–102] AMPK is expressed in different tissues; however, little is known about the role of intestinal AMPK in the regulation of energetic and glucose homeostasis. In this context, a study by Zhang et al. created a knockout mice model for intestinal AMPK and observed its importance for metformin's action. The knockout group treated with metformin showed a reduction in the effects of this drug on (1) weight loss, (2) the improvement of glucose tolerance, (3) the accumulation of lipids in the liver, and (4) the reduction of the triglycerides, cholesterol, and insulin levels. These observations indicated the importance of intestinal AMPK for the metformin action in obesity, diabetes, and associated diseases. Throughout the study, the inhibition of intestinal AMPK was observed for its association with the impairment of microbiota alterations that would be important for metformin action.[103]

Along with its molecular action, metformin could exert its anti-obesity effects through the modulation of the microbiota. Studies have also shown that metformin promoted the increase of the Akkermansia muciniphila strain usually found in low concentrations in overweight people, thus performing an anti-obesity action.[56,104] In addition, metformin was able to reduce the level of Escherichia coli usually associated with being overweight.[105] Probiotic strains are reported to have beneficial effects for weight loss; in this context, metformin has been shown to increase the beneficial bacteria Prevotella and Lactobacillus strains associated with weight loss.[104,106] In obese people, there is a large production of SCFAs; however, this high production is contrary to the diversity of SCFA-producing strains in the microbiota of these individuals.[89] In this context, metformin was able to increase the diversity of SCFA-producing bacteria, such as Allobacum, Bacteroides, Blautia, Butyricoccus, and Phascolarctobacterium.[106] By increasing the diversity of the producers, the probability of SCFA absorption and performance of beneficial effects would be increased, such as increased intestinal integrity, preventing the release of inflammatory molecules, and triggering anti-obesity effects.[89]

Associated to microbiota modulation to exert its anti-obesity effect, some studies demonstrated that metformin reduced the LPS-associated inflammation through the downregulation of TLR4, a key regulator of this process. Other studies indicated that through AMPK activation, metformin promoted the macrophage polarization to M2 phenotype triggering anti-inflammatory effects (Fig. 1).[107]

Therefore, metformin may have anti-obesity effects either through its molecular mechanisms of action or through the modulation of the microbiota.

Metformin and colorectal cancer (CRC)

Data from GLOBOCAN in 2020 pointed to colorectal cancer (CRC) as being responsible for 1.9 million cases and 935,000 deaths, thereby ranking third among the 10 most common types of cancer and second among those that most lead to death worldwide.[108] Advanced age and male individuals were highly associated with the risk of developing CRC.[109] Both genetic and environmental factors were also among the risk factors for CRC among which environmental factors could be highlighted: smoking,[110] alcoholism,[111] obesity,[112] the consumption of red and processed meats,[113] T2DM,[109] and microbiota with emphasis on the Fusobacterium nucleatum[114] and Bacteroides fragilis[115] strains.

The development of CRC begins with the formation of an aberrant crypt that evolves into a neoplastic lesion (polyp) providing progression to CRC, whose process takes around 10–15 years.[109] Surgical removal of polyps is usually indicated to reduce the risk of progression to CRC;[116] however, the chance of new polyps and of CRC development is high.[117] In this context, some chemotherapy drugs have been used as the prophylaxis for the development of CRC, such as non-steroidal anti-inflammatory drugs; however, the risk of developing cardiovascular diseases as a side effect is high.[118] This scenario has led to the search for safer drugs to exercise this chemoprophylaxis and even treatment for CRC with metformin as a strong candidate.[119]

The use of metformin in cancer is mainly associated with the inhibition of CI of the ETC resulting in energy depletion (increased AMP/ATP and ADP/ATP ratio) and activation of the energy sensor AMPK limiting tumor proliferation.[120] Several studies have already reported the antitumor effect of metformin on CRC of being able to reduce or inhibit the formation of aberrant crypt foci (ACFs) and colorectal polyps in in vivo studies,[121] clinical,[122] and in vitro.[123] In addition to its molecular antitumor effects, metformin plays an anti-CRC action through its ability to regulate the microbiota.[29]

CRC patients have microbiota with low diversity compared to healthy individuals.[124] However, it is not possible to standardize the characteristic microbiota of CRC.[125] Although the dysbiosis found in patients with CRC is mainly associated with bacterial strains, some fungi are also related to this neoplasm. Even though fungal diversity was not affected in patients with CRC, it was found to alter the Basidiomycota/Ascomycota ratio and the Malasseziomycetes class, in addition to the reduction of Saccharomycetes and Pneumocystidomycetes.[126]

One of the main bacterial strains associated with CRC is Fusobacterium nucleatum,[127,128] This strain is responsible for creating a tumor microenvironment for CRC through the TLR4/MYD88/NF-κB pathway by generating a pro-inflammatory environment and enabling epithelial-mesenchymal transition (EMT) through the activation of the nuclear factor kappa B (NF- κB), which is considered an important factor for CRC invasion and metastasis.[129] Thus, F. nucleatum is considered a biomarker for CRC, and its detection at high concentrations is related to a poor prognosis and low survival expectancy.[130,131] Additionally, Enterococcus faecalis is generally found at high levels in patients with CRC and is associated with oxidative stress through the generation of hydroxyl radicals

that act as mutagens, promote genetic instability, and increase the risk of developing CRC.[132,133]

Another important feature related to CRC is the high levels of E. coli.[134] Even though it is considered a commensal strain, E. coli produces the colibactin genotoxin inducing DNA damage and increasing the risk for CRC.[85,135] On the other hand, probiotic bacteria (SCFA producers), such as Clostridium butyicum (producing butyrate), are generally found in low concentrations or are nonexistent in CRC patients.[125] Moreover, strains producing SCFAs, mainly butyrate, have anti-inflammatory, pro-apoptotic action and protect DNA against damage, which exerts beneficial effects in the fight against CRC.[136,137] Metformin interacts with F. nucleatum by reducing its levels and antagonizing the progression of CRC.[138] Beyond reducing the levels of this strain, metformin is associated with the inhibition of the TLR4/MYD88/NF-KB pathway,[139] thus reducing the inflammation and metastasis in CRC (Fig. 1). F. nucleatum is associated with chemotherapy resistance and post-chemotherapy recurrence in CRC.[138] Therefore, metformin, by reducing the F. nucleatum levels, could increase the chemotherapeutic efficiency and reduce the risk of recurrence.

Patients with CRC usually have low levels of Akkermansia muciniphila and Bifidobacterium,[140,141] a scenario that is reversed by metformin. A. muciniphila is a butyrate-producing strain associated with the antitumor effects of metformin, and mediates the anti-inflammatory, pro-apoptotic, and antitumor effects of this drug,[142,143] while Bifidobacterium is a probiotic strain performing antitumor effects through its pro-apoptotic action.[144]

Bile acid levels are generally elevated among CRC patients and are considered important for the carcinogenesis of this type of cancer.[145,146] Studies have shown the ability of metformin to reduce the abundance of bile acids producing strains, such as Bacteroides fragilis, which is found in high concentrations in CRC patients[147] antagonizing CRC carcinogenesis.[36]

Another strain associated with CRC is Helicobacter pylori, a bacterium that infects the mucosal layer above the gastric epithelium and causes gastritis that can progress to chronic stomach inflammation and in some cases lead to CRC.[148] Metformin is able to inhibit H. pylori by preventing this bacterial strain from triggering the development of CRC.[149]

Therefore, metformin antagonizes the development of CRC both by its molecular effects and by modulating the intestinal microbiota.

Metformin and its action on HIV-associated inflammation

The human immunodeficiency virus (HIV) targets the host's immune system. In 2020, it was estimated that about 1.5 million people had been infected with HIV, thereby reaching a total of 37.7 million people infected worldwide.[150]

HIV targets CD4+ T cells of the intestinal mucosa by promoting the reduction of this cell type and compromising the intestinal epithelium, which allows the translocation of microbial products to the circulatory system and triggers a systemic inflammatory response.[151] By compromising the immunity of the intestinal epithelium, HIV triggers dysbiosis.[152,153] Antiretroviral therapy (ART) has also revolutionized HIV treatment and increased the life expectancy and quality of life of HIV-positive individuals. However, even regulating a viral load, CD4+ T cell concentration and reducing inflammation, ART cannot restore the immune response to the level of HIV-negative individuals.[154] Therefore, intestinal impairment, dysbiosis, and chronic inflammation remain in HIV-positive patients leading to the development of dyslipidemia, cardiovascular diseases, depression, and cancer.[155–157]

Furthermore, the gastrointestinal tract is essential for the HIV pathogenesis, as it is the main site of replication and viral reservoir[158] given the high expression of the CCR5 receptor on CD4+ T cells that functions as a co-receptor for HIV.[159] By compromising intestinal immunity, HIV triggers dysbiosis and systemic inflammation.[151] In HIV positive individuals, dysbiosis is characterized by an increase in Proteobacteria, Enterobacteria, and Fusobacteria, in addition to a reduction in Ruminococcaceae, Bacteroidia, Anaerovibrio, Bifidobacterium, and Clostridium.[157,160,161] HIV-associated dysbiosis is characterized by the reduction of strains related to the maintenance of intestinal integrity among which are A. muciniphila and the butyrate-producing strains, Roseburia, Coprococcus, Faecalibacterium, and Eubacterium.[162–165]

Likewise, metformin is able to benefit HIV-positive patients by reducing systemic inflammation through the (1) inhibition of NF-κB, (2) reduction of cytokine production, such as TNF-α and IL-1, and (3) through changes in the microbiota.[33,36,104,166–168] In this context, previous studies have demonstrated the increase of the A. muciniphila and butyrate-producing strains in HIV-positive patients by metformin, which has enabled an increase in intestinal integrity, consequently avoiding LPS release and associated inflammation.[169,170]

Thus, by regulating the microbiota in HIV-positive patients, metformin promotes an increase in intestinal integrity and a reduction in systemic inflammation, where it supports the ART treatment and reduces the risks of development of pathologies associated with inflammation.

Metformin and cardiovascular disease (CVD)

CVDs consist of a group of pathologies associated with the heart and blood vessels, including coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, pulmonary embolism, and deep vein thrombosis.[171] The WHO's data indicate that CVDs were responsible for 17.9 million deaths in 2019, which corresponded to 32% of global deaths.[171] Studies have further shown that the main risk factors for CVDs involve T2DM, obesity, hypertension, dyslipidemia, platelet hyperactivity, and atherosclerosis (AS).[172–176] While genetic factors are responsible for 20% of CVD cases,[177] a growing number of studies have pointed to the importance of the intestinal microbiota for CVDs.[178,179]

Additionally, Bacteroidetes and Firmicutes strains comprise more than 90% of the intestinal microbiota, and in this context, an increase in the Firmicutes/Bacteroidetes ratio was observed among patients with CVD,[180,181] which was associated with inflammation and diabetes mellitus, both considered risk factors for CVDs.[5] The same was observed in individuals with CVDs regarding Enterobacteriaceae and Streptococcus spp., in addition to a reduction in SCFA-producing strains, such as Roseburia intestinalis, Faecalibacterium prausnitzii, and Eubacterium rectale.[182,183] which could lead to the development of risk factors for CVDs, such as obesity, dyslipidemia, insulin resistance, and oxidative stress.[184,185] An increase in trimethylamine oxide (TMAO) producing strains, an important metabolite for the development of CVDs, was also observed.[186–188] TMAO is produced from the metabolism of phosphatidylcholine and choline by microbial enzymes that generates trimethylamine (TMA), which in turn is converted in the liver by flavin-containing monooxygenases, mainly by FMO3, resulting in TMAO.[189,190] Moreover, the increase in TMAO production is associated with increased inflammation and CVDs through the activa-

tion of SR-A and CD36 receptors on the macrophages that promote the recognition and uptake of oxidized low-density lipoprotein (ox-LDL), a risk factor for CVD associated with AS. The uptake of ox-LDL stimulates the transformation of the macrophages into foam cells, which is an early hallmark of AS. The high levels of TMAO also induce the activation of the NLRP3 inflammasome, a lipoprotein complex formed through the activation of pattern recognition receptors (PRRs) of great importance for AS that promote the activation of caspase 1 and the consequent secretion of pro-inflammatory cytokines IL-1β and IL-18 contributing to AS. TMAO is also associated with the activation of MAPKs, JNK, and ERK, which can lead to platelet hyperreactivity and to AS, in addition to promoting the activation of NF-κB that acts on the secretion of pro-inflammatory cytokines, such as IL-1 and TNF-α contributing to the development of AS (Fig. 1).[191]

Another microbial metabolite associated with CVDs are bile acids, especially secondary ones.[192] Primary bile acids are metabolized by bile salt hydrolase enzymes (BSHs) present in bacterial strains, such as in Methanobrevibacter smithii, Clostridium, and Enterococcus,[193,194] into secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA) which in turn are agonists of the TGR5 and FXR receptors.[195,196] By binding to their receptors, secondary bile acids promote macrophage activation, inflammation, and AS.[197,198]

Beyond SCFAs, TMAO, and bile acids, another microbial metabolite important to CVDs is the BCAAs that are associated to many risk factors for the development of CVDs, such as obesity, insulin resistance, arterial hypertension, dyslipidemia, and coronary disease indicators.[37,199–202] In this context, Tobias et al. demonstrated the relationship between the high levels of BCAAs in plasma and CVDs, which was probably due to the impairment of the BCAAs catabolism leading to its accumulation and enhancing the risk of AS associated with insulin resistance.[203]

Metformin acts on cardioprotection due to its antidiabetic, anti-obesity and anti-dyslipidemia mechanisms of action.[96,204,205] Furthermore, the cardioprotective effects of metformin may be mediated by its action on the regulation of the intestinal microbiota.[206] Akkermansia muciniphila is a strain associated with health and good cardiac parameters,[78] which increased concentration has been linked to metformin, hence preventing pro-inflammatory conditions and reducing the risk of developing CVDs.[56,207–209] In addition, the role of metformin in the production of bile acids exerts its cardioprotective effects since metformin is associated with a reduction in the BSH activity of the Bacteroides fragilis strain and the lower content of Firmicutes, such as Clostridium perfringens, reducing inflammation and the progression of AS.[210–213]

Metformin is able to increase the concentration of the SCFAs producing strains of Butyrivibrio, Bifidobacterium bifidum, and Megasphaera contributing to an anti-inflammatory effect and cardioprotection.[167,56] Studies have indicated that metformin could reduce the levels of TMAO by regulating the producing microbiota as a way to exert its cardioprotective effects.[214,215] In the study by Su et al., metformin was able to reduce the TMAO levels through increasing the Akkermansia and Bifidobacterium strains (negatively related to TMAO) and reducing the Lachnoclostridium and Ruminiclostridium strains (positively related to TMAO) in vivo (Fig. 1).[215] Furthermore, metformin was able to reduce the levels of TMA and TMAO through its action on TMAO metabolism[209] leading to a reduction of the inflammatory cascade associated to TMAO.

Associated with the metformin action in the BCAAs, thus inducing its reduction in T2DM patients through the modulation of the microbiota and its action as an mTOR antagonist, metformin reduces insulin resistance and the contribution of this risk factor for the development of CVDs.[7,38–40]

Metformin and cognitive function: Therapeutic contribution to Alzheimer's disease

Dementia is a pathology associated with impaired cognitive function and aging. It is estimated that 55 million people suffer from dementia worldwide with an annual incidence of about 10 million new cases per year. It is considered the seventh most fatal disease worldwide; additionally,[216] AD is responsible for 60–80% of diagnosed cases of dementia.[217]

AD is clinically characterized by (1) cognitive and memory impairment, (2) the onset of psychiatric symptoms and behavioral problems, and (3) impairment of daily tasks.[218] The initial symptomatology of AD involves impairment of recent memories, changes in unconscious behavior, as well as changes in language and speech.[219] At the molecular, biochemical and cellular level, AD is characterized by (1) cell death, (2) impairment of energy metabolism, (3) hyperactivation of signaling pathways, (4) deposition of amyloid beta proteins (Aβ), (5) mitochondrial impairment, (6) oxidative stress, and (7) DNA damage.[220–223]

Despite the various cellular, biochemical, and molecular factors related to AD, the deposition of Aβ is considered its main feature.[219] The Aβ deposition promotes the hyperphosphorylation of the tau protein (p-tau), neuroinflammation, oxidative stress, and neuronal degeneration.[224] Studies have indicated that the accumulation of Aβ proteins is considered the initiating factor of AD and is important for the formation of the tau aggregates, in addition to promoting the activation of microglia cells and astrocytic recruitment culminating in local inflammation and providing neuronal impairment and death.[219–221,225] This neurodegenerative disease can be caused by both hereditary and non-hereditary factors, such as aging, lifestyle, and environmental factors.[220] Among the environmental factors, the intestinal microbiota stands out.[226]

Studies have already demonstrated that individuals with changes in the microbiota are more likely to develop neurological diseases, such as AD.[227,228] Based on this observation, other studies found the ability of the microbiota to regulate the behavior and brain function contributing to the development of neurological diseases due to the microbiota-gut-brain axis.[229–234] In this context, the study by Morris et al. showed that 85% of individuals with dementia had a different microbiota from healthy individuals,[235] hence indicating the importance of dysbiosis for the development of neurological diseases, such as AD. Table 1 highlights the main studies related to dysbiosis with AD.[228,235–237]

A clinical study performed on AD patients showed that the Bacillus subtilis, E. coli, Klebsiella pneumoniae, Mycobacterium spp., Salmonella spp., Staphylococcus aureus, and Streptococcus strains were related to the production of the amyloid fibers.[230] The bacterial amyloids could pass through the gut barrier and access the circulation resulting in increasing the secretion of pro-inflammatory cytokines, such as IL17A and IL-22. These proteins and cytokines could pass through the blood-brain-barrier (BBB) to reach the brain and promote the activation of NF-κB, which provided neuroinflammation and the upregulation of miRNA-34a. miRNA-34a promotes the downregulation of the triggering receptor expressed in the microglial/myeloid cells-2 (TREM2) expression leading to the impairment of phagocytosis and the accumulation of amyloid proteins in the brain, which is an important risk factor to AD (Fig. 2).[236,238–240] Another microbial metabolite

Table 1. Dysbiosis in patients with Alzheimer's Disease

Authors	Cohort	Type of samples	Results
Cattaneo et al.[228]	Patients amyloid +/– and healthy	Fecal samples	↓Eubacterium rectale (anti-inflammatory); ↑Escherichia/Shigella (pro-inflammatory strains related to↑IL-1β, NLRP3, and CXCL2
Vogt et al.[235]	Patients with or without AD	Fecal samples	↓Abundance and diversity; ↓Phylum Firmicutes and Actinobacteria; ↑Phylum Bacteroidetes; ↓Genus Bifidobacterium, Dialister, Clostridium and Turicibacter; ↑Genus Blautia, Phascolartobacterium, Gemella, Bacteroides, Alistipes, and Bilophila
Ling et al.[236]	Patients with or without AD	Fecal samples	↓Genus Faecalibacterium (anti-inflammatory strain related with good results of the cognitive tests MMSE and WAIS); ↑Genus Bifidobacterium (strain related with bad results of the cognitive tests MMSE and WAIS)
Ling et al.[237]	Patients with or without AD	Fecal samples	Maintanance of fungal diversity; Altered fungal composition; ↑Candida tropicalis and Schizophyllum commune (related to pro-inflammatory factors such IP-10 and TNF-α; ↓Rhodotorula mucilaginosa (anti-inflammatory strain)

AD, Alzheimer's disease; CXCL2, C-X-C motif chemokine ligand 2; IL-1β, interleukin 1 beta; IP-10, interferon gamma-inducible protein 10; MMSE, Mini-Mental State Examination; NLRP3, nucleotide-binding domain leucine-rich repeat containing protein 3; TNF-α, tumor necrosis factor-alpha; WAIS, Wechsler Adult Intelligence Scale.

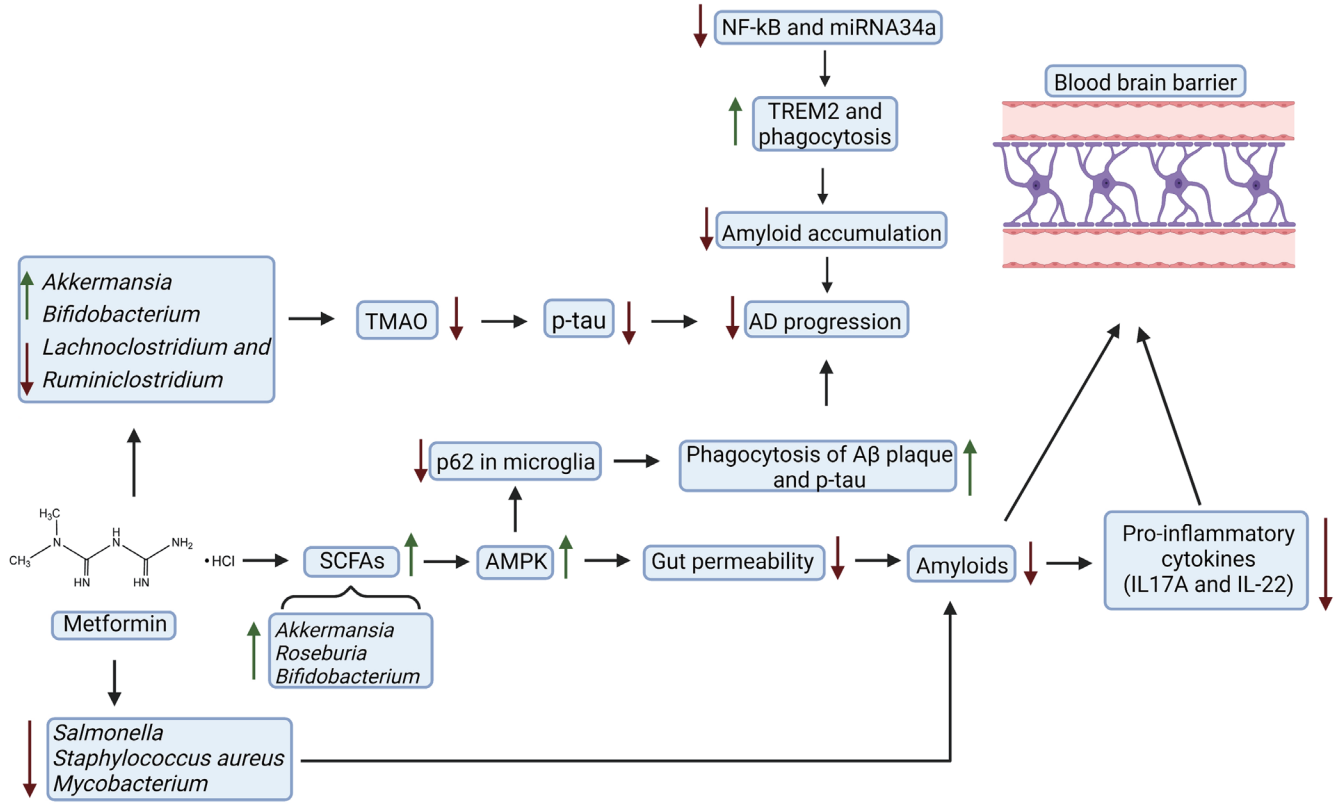

Fig. 2. Metformin acts as an antagonist for AD progression. Metformin enhanced the SCFAs producers, such as Roseburia, Akkermansia, and Bifidobacterium, and was generally reduced in AD patients. SCFAs induced the activation of AMPK, which by regulation of tight junctions in enterocytes reduced (1) the gut permeability, (2) the release of amyloid proteins in the circulation, and (3) the secretion of pro-inflammatory cytokines, such as IL17A and IL-22. Due to the reduction of the amyloid proteins and cytokines, their transport to the brain through BBB were reduced, thereby promoting (1) the reduction of NF-κB activation, (2) the downregulation of miRNA34a, (3) the upregulation of TREM2, and (4) the increase in the phagocytosis process, thus reducing amyloid accumulation, an important risk factor for AD. Through AMPK activation, metformin reduced the p62 accumulation in microglia (a marker of autophagic impairment) enhancing the phagocytosis of the Aβ plaques and p-tau proteins, and reducing the deposit of these risk factors for AD. Due to the importance of TMAO for AD, metformin reduced the strains related to TMAO metabolism (Lachnoclostridium and Ruminoclostridium) and enhanced the negative strains correlating to the TMAO levels (Akkermansia and Bifidobacterium), as a result promoting the reduction of TMAO and p-tau protein, an important AD biomarker. In this context, metformin reduced the levels of the amyloid proteins by reducing the amyloid strain producers (Salmonella, Staphylococcus aureus, and Mycobacterium). AD, Alzheimer disease; AMPK, AMP-activated protein kinase; Aβ, amyloid beta plaques; BBB, blood brain barrier; IL, interleukin; miRNA34a, micro-RNA 34a; NF-κB, nuclear factor kappa B; SCFAs, short chain fatty acid; TMAO, trimethylamine N-oxide; TREM2, triggering receptor expressed on myeloid cells 2. Created with www.biorender.com.

that could contribute to neurological diseases is TMAO, which has been found elevated in the cerebrospinal fluid (CSF) of AD patients and associated with an important Alzheimer biomarker, p-tau protein,[26] so that the reduction of TMAO would work as a therapeutic tool to improve cognitive impairment.[86]

The ability of metformin to elevate anti-inflammatory SCFA-producing strains, such as Roseburia, which in turn were found in low concentrations in AD patients, was also demonstrated.[99] In addition, metformin promoted the reduction of the TMAO levels by increasing the strains negatively related to the production of TMAO (Akkermansia and Bifidobacterium) and reducing strains associated with TMAO production, such as Lachnoclostridum and Ruminoclostridium.[215] Therefore, by reducing the plasma TMAO levels and enhancing the SCFAs, metformin would exert an anti-inflammatory action and contribute to preventing the progression of AD.

Amyloid fiber-producing strains are considered a concern for AD. In this context, metformin has an antimicrobial action against amyloid fibers producers, such as the Salmonella, Staphylococcus aureus, and Mycobacterium strains.[241,242] Beyond its action on microbiota, metformin through the AMPK activation is able to reduce the p62 accumulation in microglia (an indicator of the impairment of the autophagy process) leading to an increase in the phagocytosis of the Aβ plaques and tau proteins by microglia, consequently reducing the brain deposits of these proteins that corroborated to an anti-AD effect of metformin.[243]

Furthermore, obesity is a risk factor for the impairment of the cognitive function and for diseases like AD.[237,244] In this context, a study by Ma et al. demonstrated that metformin was able to restore the cognitive impairment related to obesity through the recuperation of neurogenesis by the modulation of microbiota that acted on the attenuation of neuroinflammation.[245] Moreover, this study corroborated the observation that through its action in pathologies at risk for AD, such as diabetes and obesity,[246] where metformin promoted the increase of butyrate-producing strains that were associated with intestinal integrity,[7] It could prevent bacterial products, such as LPS from accessing the host's circulatory system and causing systemic inflammation, which could favor neuroinflammation and the development of AD.[106,247]

Metformin and intestinal ischemia-reperfusion (I/R) injury

Intestinal ischemia reperfusion injury is a surgical complication characterized by the temporary interruption of blood circulation in the intestine, which is a serious complication that could lead to death. During the ischemia, the gastrointestinal barrier is impaired possibly from the release of microorganisms and of inflammatory factors. When the reperfusion occurs, these microorganisms can access the blood circulation and initiate an inflammatory cascade and sepsis.[248,249]

Understanding that the ischemia is an unpredictable complication, adopting strategies during the reperfusion that would reduce the inflammation and cellular damage would be important. In this context, the study of Jia et al., applied metformin before the reperfusion in mice, which resulted in the protection of the gastrointestinal barrier and prevented the ZO-1 and occludin tight junctions' damage. Beyond that, it was observed that metformin reduced (1) inflammatory factors, such as IL-6 and IL-1beta, (2) the activation of NLRP3 inflammasome, (3) cleaved caspase-1, and (4) the levels of gasdermin D (GSDMD). These were important factors for pyroptosis, an inflammatory cell death predominant during the I/R. Thus, metformin proved to be a promising therapeutic tool for intestinal I/R injury; however, its clinical application would be necessary for evaluating metformin's effects on intestinal I/R injury in humans.[250]

Metformin and its role in healthy aging

Aging can be understood as a process that people go through as they get older, which is determined by genetics and influenced by the environment.[251] Associated with the improvement in the quality of life, people are living longer; as a consequence, there is an increasing number of people that are going through aging. The WHO's data has estimated that between 2015–2050, the proportion of people who will reach the age of 60 years or more, in relation to the world population, will increase from 12% to 22%.[252] In addition, it is estimated that between 2020–2050, the number of people aged 80 years or more will triple and comprise more than 426 million people worldwide.[252]

The molecular and cellular characteristics of aging involve (1) genomic instability, (2) telomeric wear, (3) epigenetic changes, (4) loss of proteases, (5) mitochondrial impairment, (6) cellular senescence, and (7) impairment of stem cells and intercellular communication.[253] Nevertheless, studies have pointed to the influence of intestinal microbiota on healthy aging.[1,254]

During aging, the gradual impairment of immunity is common associated with the loss of balance between pro- and anti-inflammatory actions.[255] Thus, aging is marked by a chronic inflammation called inflammaging, an important risk factor for the development of chronic diseases that increase the chance of mortality in these individuals like CVDs, dementia, and diabetes mellitus.[256–259] Thus, the reduction of inflammatory processes enables healthy aging.

By characterizing the microbiota of older individuals, it was possible to highlight the reduction of strains associated with butyrate production (Table 2).[260–270] The reduction of SCFAs was associated to the AMPK inhibition that promoted the increase of gut permeability and possibly the LPS release in circulation. The LPS was recognized by TLR4 in the macrophages surface resulting in macrophage polarization to the M1 phenotype and in an associated-inflammatory cascade that involved NF-κB activation and secretion of pro-inflammatory cytokines, consequently producing an inflammatory environment during aging and increasing the risk of the development of diseases that could compromise healthy aging.[205,271]

Metformin is associated with long and healthy aging,[272–274] due to its antidiabetic[263,264] cardioprotective,[265,275] anti-tumor,[266,267] neuroprotective action,[276] and through its action on the microbiota.[277,278] Deficiency on SCFA-producing strains in elderly indicates a scenario of high intestinal permeability and inflammation.[279,280] Ahmadi et al. demonstrated these features among older mice that were reduced by metformin by increasing mucin production in the gastrointestinal tract.[281] Metformin increased the abundance of the strains associated with the production of the SCFAs in diabetic patients, such as Bifidobacterium, Prevotella, Blautia, Butyrivibrio, Megasphaera, Akkermansia, Lactobacillus, and Shewanella,[56,167,207] hence reducing the inflammatory environment and contributing to healthy aging (Fig. 3).

In addition, metformin was able to modulate the microbiota to exert its cardioprotective, anti-obesity, antidiabetic, antitumor, and neuroprotective effects, which in turn were considered risk factors for long-term healthy aging.[282–287] In this way, the microbial modulation exerted by metformin led to the effects listed above by reducing the pro-inflammatory and increasing the anti-inflammatory strains and consequently acting on aging (Fig. 3).[56,138,167,215,287]

Table 2. Dysbiosis related to the aging process

Authors	Results
Kim et al.;[263] Wu et al.;[264] Odamaki et al.;[268] Yu et al.;[269] Rizzatti et al.[270]	↑Proteobacteria (gram negative strain and LPS producer related to inflammation)
Biagi et al.[265]	↓Families Bacteroidaceae, Lachnospiraceae and Ruminicoccaceae; ↑Genus Eggerthella, Akkermansia, Anaerotruncus, and Bilophila
Kushugulova et al.[266]	↓Butyricimonas virosa and Anaerostipes butyraticus (Butyrate producers strains related to anti-infammatory properties)
Drago et al.;[267] Wu et al.[264]	↑Clostridia sensu stricto, Methanobrevibacter smithii and Bifidobacterium adolescentes (anti-inflammatory strain); ↓Faecalibacterium prausnitzii*, Dorea longicatena, Eubacterium rectale*, Bacteroides caccae and Fusobacterium mortiferum; * SCFAs strains producers related to anti-inflammatory properties and reduced risk for the development of inflammation-related diseases

LPS, lipopolysaccharide; SCFAs, short chain fatty acids.

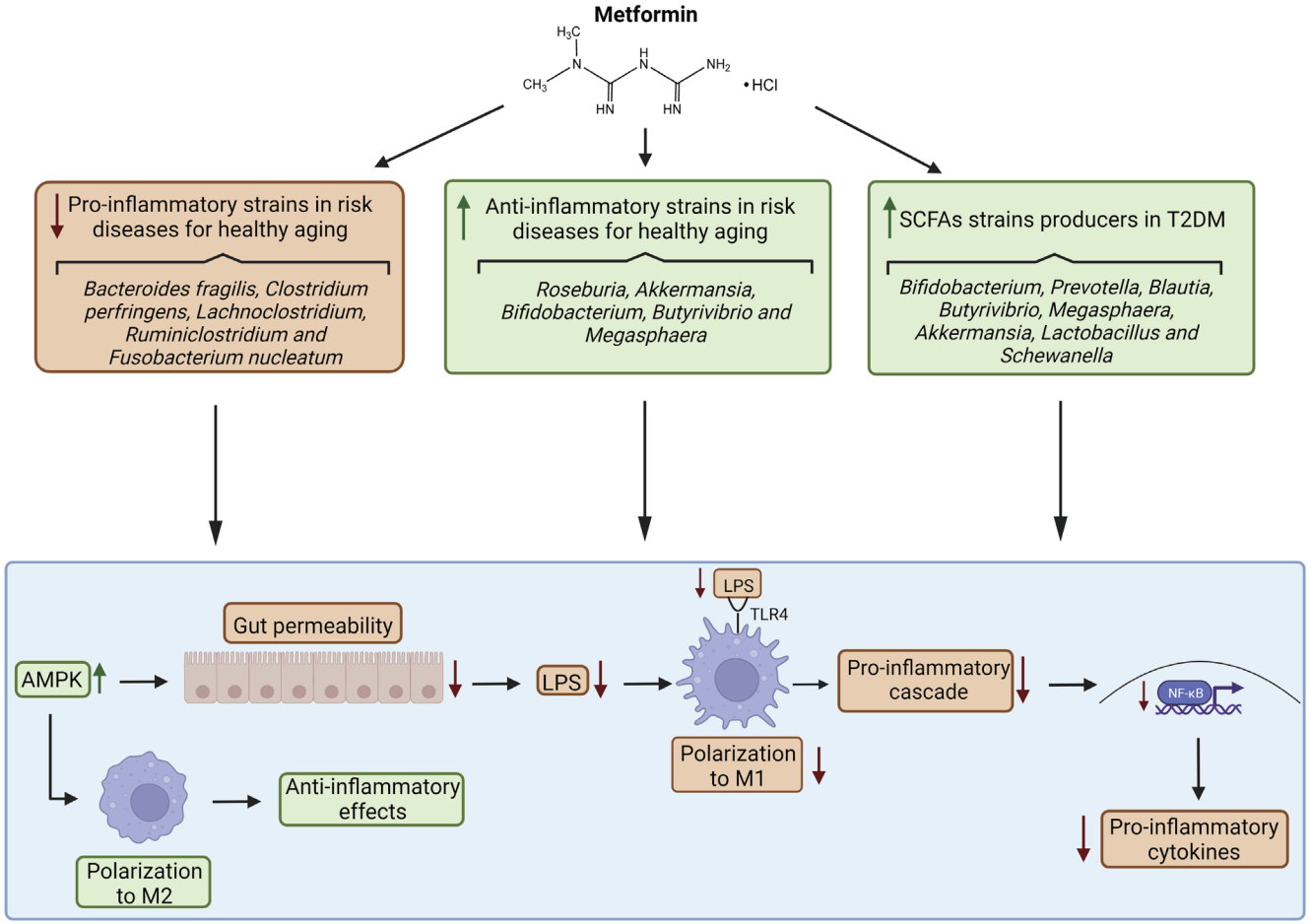

Fig. 3. Metformin and the anti-aging effects through microbiota modulation. Metformin enhanced the SCFAs strains (Bifidobacterium, Prevotella, Blautia, Butyrivibrio, Megasphaera, Akkermansia, Lactobacillus, and Schewanella) in T2DM patients. Through its action against cancer, CVDs, obesity, AD, and diabetes (risk factors for healthy aging), metformin reduces pro-inflammatory strains (Bacteroides fragilis, Clostridium perfringes, Lachnoclostridium, Ruminiclostridium, and Fusobacterium nucleatum) and enhances anti-inflammatory strains (Roseburia, Akkermansia, Bifidobacterium, Butyrivibrio, and Megasphaera). Thus, through microbiota modulation, metformin promoted the activation of AMPK resulting in the reduction of gut permeability, reducing LPS in circulation and the activation of macrophage (M1 phenotype) through LPS recognition by TLR4 by avoiding (1) a pro-inflammatory cascade, (2) the activation of NF-κB, and (3) the production of pro-inflammatory cytokines. The AMPK activation was also related to macrophage polarization to the M2 phenotype, which had anti-inflammatory properties. Therefore, metformin reduced inflammation and the development of inflammation-related diseases contributing to its anti-aging effect. AD, Alzheimer disease; AMPK, AMP-activated protein kinase; CVDs, cardiovascular diseases; LPS, lipopolysaccharide; NF-κB, nuclear factor kappa B; SCFAs, short chain fatty acid; T2DM, type 2 diabetes mellitus; TLR4, toll-like receptor 4. Created with www.biorender.com.

Metformin pharmacogenomics and the response to the drug

Metformin is a hydrophilic drug and a cation at physiological pH, so it is unable to cross the plasma membrane passively and requires transporters to enter for its excretion.[288] Several transporters are involved in the transporting of metformin that are tissue-specific, but those that play an important role in defining a response to metformin are (1) organic cation transporters 1–3 (OCTs), (2) the plasma membrane monoamine transporter (PMAT), (3) the protein of multidrug and toxin extrusion 1- 2 (MATE), (4) the serotonin transporter (SERT), and (5) the high-affinity choline transporter (CHT).[31] Despite the variety of transporters, the main ones are the OCTs, PMATs, and MATEs that play an important role in defining a response to metformin.[31,289]

In addition, entry by metformin into the gastrointestinal (GI) tract is crucial for its action. The transporting of metformin to the enterocytes can be mediated by different transporters, such as OCT1, OCT3, PMAT, SERT, and CHT; however, in vitro and in vivo studies have shown that the main mediators of transporting metformin to the enterocytes are OCT1, PMAT, and SERT.[31,288,289] Subsequently, metformin enters the bloodstream and OCT1 mediates the liver uptake, where it would play its main mechanisms of action. Moreover, the excretion of metformin involves two steps: (1) Its transport from the blood to the kidneys via OCT2 and (2) renal extrusion mediated by the MATE1 and 2 transporters, thus enabling its elimination in the urine.[290]

Despite its transporters, about 30% of administered metformin is not absorbed from the GI tract and is eliminated in the feces.[291] Therefore, its uptake by the enterocytes is considered the limiting step to determine its bioavailability, which could vary from 20–70% between individuals.[292–294] In this context, studies have indicated that gene polymorphisms of the metformin transporters could compromise their efficiency, thereby resulting in a lower uptake and response to metformin.[268,290,295,296]

OCT1 (SLC22A1) polymorphisms

The SLC22A1 gene responsible for encoding the OCT1 transporter is highly susceptible to polymorphisms that compromise its function.[291] Several studies have already identified different polymorphisms in this gene in different populations, which is an observation of concern given that OCT1 is considered the central transporter for metformin activity and impacts its antihyperglycemic action.[269,270]

The study by Seitz et al. covered 52 countries and identified 85 OCT1 variants in different populations.[297] The main polymorphisms observed were (1) rs628031 (A<G) that is considered a very common variant and associated with the reduced expression of OCT1 in enterocytes, and promotes a lower uptake of metformin and its accumulation,[298] (2) rs122083571, a polymorphism associated with lower OCT1 activity,[299–301] (3) rs72552763, associated with a reduced uptake of metformin (>60%),[297,302] and (4) rs34059508, associated with compromised OCT1 localization and its inactivation.[302]

In addition to the importance of OCT1 for the antihyperglycemic action of metformin, the study by Sam et al. demonstrated the role of OCT1 in the anti-obesity action of metformin. In this study, it was observed that variations in the SLC22A1 gene would be associated with a lower uptake of metformin by the adipose tissue and the consequent lower inhibition of lipid accumulation by metformin.[270] Thus, the polymorphisms observed in the SLC22A1 gene could explain why 35% of T2DM patients did not respond to metformin, and indicating the importance of OCT1 for the response to this drug.[268]

OCT2 (SLC22A2)

The SLC22A2 gene responsible for encoding the OCT2 transporter plays an important role in the renal excretion of metformin; thus, variations in this gene have been related to impaired renal excretion of this drug.[294] More than 500 variations of SLC22A2 have already been identified. Among the variations found, the following stand out: (1) p.165M>I, p.199T>I, p.201T>M, and p.400R>C, which are associated with lower OCT2 activity[303,304] and (2) p.270A>S (c.808G>T), which has been identified in different populations and is associated with a 40% reduction in metformin excretion in patients with the homozygous genotype.[305]

Impaired renal excretion of metformin is also associated with variations of metformin concentration in plasma between patients and adverse effects, such as elevated hypoglycemia.[294]

MATE1 (SLC47A1)/MATE2 (SLC47A2)

The multidrug and toxin extrusion protein 1 (MATE1) is encoded by the SLC47A1 gene. It is highly expressed in the liver and kidney, and has metformin, among other drugs as a substrate. MATE 1 may excrete metformin from the hepatocytes,[306] and along with MATE 2 in the kidneys, is responsible for the extrusion of metformin into urine. In vitro studies have shown that some genomic variants of this gene induced a complete loss of function for the transportation of metformin among other drugs, while others significantly altered the transport function in a substrate dependent way.[307] These alterations in expression of MATE1 could lead to increased systemic metformin and lactic acidosis induced by metformin.[308,309]

Furthermore, the SLC47A2 gene responsible for encoding the MATE2 transporter is highly expressed in renal cells and acts in the renal excretion of metformin together with MATE1 and OCT2. MATE2 pharmacogenomics demonstrate that responses to metformin transport capabilities resulting from different genetic alterations, where some polymorphisms have been thought to impact negatively metformin extrusion,[310] while others impact positively, as the most common promoter variant of MATE2, g.-130G>A, when in homozygosis leads to a significant enhancement of metformin extrusion and renal clearance.[311]

LKB1 (STK11)

In addition to the transporters for the response to metformin, another important factor would be serine -threonine kinase 11(LKB1) (STK11 gene), which participates in the activation of AMPK, through which metformin exerts most of its mechanisms of action.[312] In the study by Li et al., it was observed that the rs2075604 variation was associated with an improvement in the response to metformin being able to promote the reduction of HbA11c and FBG (fasting blood glucose) more efficiently, which was important for the action of metformin in T2DM.

Future Directions

In the past years, many studies about the relationship of metformin, microbiota, and diseases have been performed and bringing important discoveries and updates. Through these studies, ir was possible to observe the important role of the microbiota in the development, progression, and treatment response of many diseases, in addition to the importance of metformin as a microbiota regulator. Understanding the importance of a regulated microbiota for health, its use as a therapeutic tool through a fecal microbiota transplan-

tation (FMT) has been growing. Hu et al. demonstrated that the FMT in a myocarditis mouse model improved the symptoms of the disease.[313] Likewise, in the study of Wang et al., it showed a reduction of hyperglycemia, glycated hemoglobin, and inflammation beyond the improvement of insulinemia and insulin sensitivity after a FMT of a healthy mouse to a T2DM mouse model.[314] Additionally, Broadfield et al. were the first to shown a FMT from a metformin-treated healthy mouse to a CRC mouse model that was responsible for reducing the tumor volume and cholesterol metabolism, in addition to the enhancement of the SCFAs producers,[315] a transplant methodology that would be beneficial for individuals with reduced response to metformin due to metformin's transporters polymorphisms. This would indicate that the FMT would be a promising therapeutic tool, and its use combined with metformin could promote better results.

Conclusions

The importance of the microbiota for the health of the host and its involvement with several pathologies have been studied with different perspectives. It was found that most of them converged to the central role of dysbiosis in the development of several pathologies, and highlighted the importance of therapeutic agents capable of regulating the host microbiota by exploiting microbiota's potential role in prophylaxis and treatment. The role of metformin in the regulation of the microbiota also has a strong impact on the prevention and progression of pathologies, such as obesity, T2DM, colorectal cancer, HIV, CVDs and in the healthy aging process. Pharmacogenomic data on metformin absorption and excretion added an important tool to predict patients' responses to the presence of metformin, and how this could impact microbiota. The role of probiotics and prebiotics as adjuvants to the antidiabetic action of metformin through the modulation of the intestinal microbiota has been widely studied, while its impact on HIV infection[316] and neurodegenerative diseases[317] is still under debate, thus more studies would be necessary. The understanding of the interplay of so many factors on patients' outcome for several diseases is highly complex, and more correlations should be explored.

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Conflict of interest

Dr. Janaina Fernandes has been an editorial board member of Journal of Exploratory Research in Pharmacology since November 2021. The authors have no other conflicts of interest to declare.

Author contributions

Contributed to the study concept and design (GSP and JF), acquisition of the data (GSP and JF), assay performance and data analysis (GSP and JF), drafting of the manuscript (GSP), critical revision of the manuscript (JF), and supervision (JF).

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