Trends in Pharmacological Sciences

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Review

Striking a gut–liver balance for the antidiabetic effects of metformin

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Metformin is the most prescribed drug for the treatment of type 2 diabetes mellitus (T2DM), but its mechanism of action has not yet been completely elucidated. Classically, the liver has been considered the major site of action of metformin. However, over the past few years, advances have unveiled the gut as an additional important target of metformin, which contributes to its glucose-lowering effect through new mechanisms of action. A better understanding of the mechanistic details of metformin action in the gut and the liver and its relevance in patients remains the challenge of present and future research and may impact drug development for the treatment of T2DM. Here, we offer a critical analysis of the current status of metformin-driven multiorgan glucose-lowering effects.

Highlights

Despite the widespread use of metformin in the treatment of type 2 diabetes mellitus, its mechanism of action remains to be completely elucidated.

Historically, the major site of action of metformin has been thought to be the liver, but recent findings confirm that it also has notable effects in the gut.

Metformin promotes intestinal glucose uptake, establishing a gut–liver crosstalk that inhibits hepatic glucose production, increases the secretion of glucagon-like peptide-1 and the expression of growth differentiation factor 15, and alters the microbiota. All these effects contribute to reducing hyperglycemia.

Since most of the glucose-lowering effects of metformin depend on its action on the intestines, research over the past few years has suggested a switch from the liver to the gut as its primary site of action.

Putting the hepatic and intestinal antidiabetic effects of metformin into perspective

To date, the liver has been considered the major site of action of metformin because its glucose-lowering effects mainly result from the inhibition of **hepatic glucose production** (see Glossary) [1] (Figure 1, Key figure). However, despite decades of research, the precise mechanisms of action by which metformin acts at the molecular level have not yet been completely elucidated. Indeed, some of its effects have only been observed in the context of supratherapeutic concentrations in animal models, which are not achieved in the clinical setting [1,2]. Furthermore, some of its beneficial effects have not been observed consistently. For example, it has been proposed that metformin ameliorates hyperglycemia by the inhibition of mitochondrial glycerol 3-phosphate dehydrogenase [3,4], but this was not confirmed in a recent study [5], and other new mechanisms with potential as drug targets for T2DM have emerged [6]. Similarly, the suggested role for **growth differentiation factor 15 (GDF15)** in the reduction in body weight caused by metformin in humans and mice [7,8] was not confirmed in a recent study [9]. However, a new study reported that GDF15 is required for the antidiabetic effects of metformin in mice [10]. In contrast to these differences in observations, it has become clear that activation of **AMP-activated protein kinase (AMPK)** by metformin is key for the beneficial glucose-lowering effects of this drug [11,12].

One reason that progress in our understanding of the mechanism of action of metformin and its discrepant effects has slowed is that the liver was considered its only site of action [13]. However, recent findings also identified the gut as an important target organ of metformin. In fact, the intestine is the tissue with the highest metformin-induced glucose uptake rate and metformin might reduce hepatic glucose production through the promotion of a gut–liver crosstalk [14]. Moreover, metformin also diminishes hyperglycemia by increasing the secretion of **glucagon-like peptide 1 (GLP1)** [15], by affecting the gut microbiota [16], and increasing the amount of bile acid [17]. Despite these recognized actions of metformin in the gut, their contribution to its glucose-lowering effects in the clinical setting remains uncertain [1]. Thus, it is important and timely to

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Key figure

Schematic showing the AMP-activated protein kinase (AMPK)-dependent hepatic effects of metformin on glucose metabolism

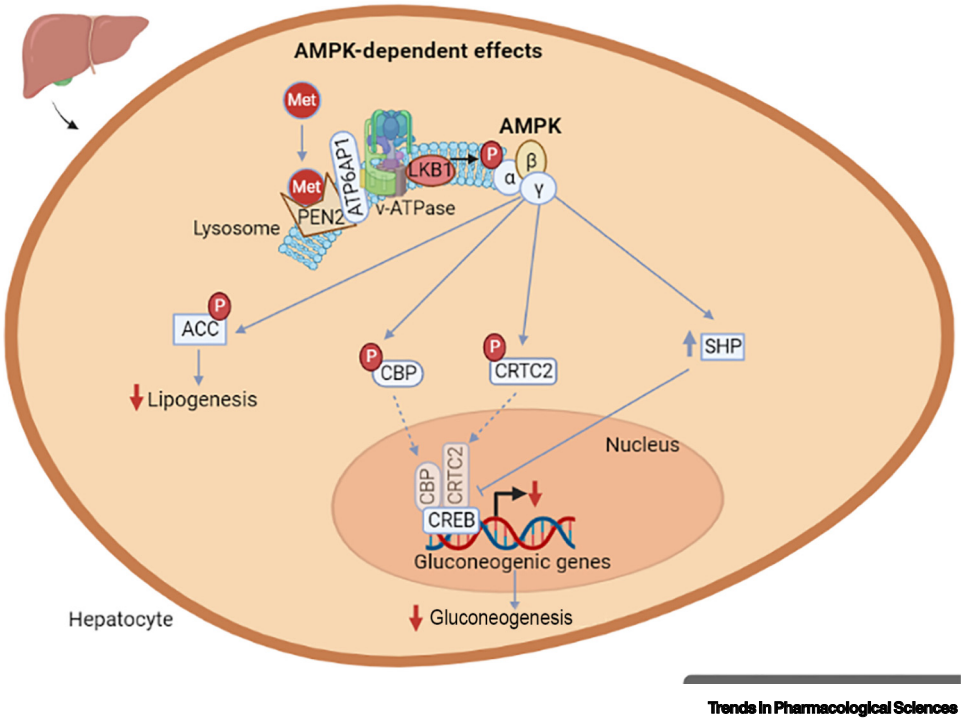

Figure 1. Metformin modulates hepatic glucose metabolism through AMPK-dependent mechanisms. It activates AMPK by increasing the formation of the trimeric complex of this kinase in hepatocytes, thereby promoting the phosphorylation of its α subunit at Thr172 by the upstream kinase liver kinase B1 (LKB1). This is initiated by the binding of metformin to PEN2 in the lysosomal membrane and the subsequent formation of a complex with ATP6AP1, a subunit of the v-ATPase, which leads to inhibition of v-ATPase and activation of AMPK. Once AMPK is activated by metformin, this kinase phosphorylates cAMP-response element element-binding protein (CREB)-binding protein (CBP) and CREB-regulated transcription coactivator 2 (CRTC2), and interferes with their binding to CREB. Metformin also increases the levels of the transcriptional repressor small heterodimer partner (SHP). All these changes reduce the gluconeogenic program, ultimately leading to a reduction in hepatic glucose production. Moreover, metformin phosphorylates and inhibits acetyl-CoA carboxylase (ACC), reducing lipogenesis, and, via direct or indirect mechanisms, helps to ameliorate insulin sensitivity. Therapeutic concentrations of metformin increase complex I activity by stimulating mitochondrial fission via AMPK, thereby contributing to reduce insulin resistance (not shown). Created with BioRender (biorender.com).

review recent progress on the antidiabetic mechanisms of action of metformin in the liver and the gut, and whether they are interdependent, in both preclinical models and the clinic.

Hepatic actions of metformin

Metformin is used particularly for the treatment of patients with T2DM who are overweight or obese with normal kidney function because of both its robust glucose-lowering effects and its safety profile (Box 1). In these patients, the most important antidiabetic effect of metformin is thought to occur as the result of its hepatic actions. However, some of the reported effects of metformin have only been observed in the context of supratherapeutic concentrations, which are not reached in the clinical setting. Therefore, the concentrations and doses of metformin used in different studies,

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Box 1. Gastrointestinal side effects of metformin

A considerable proportion of patients taking metformin cannot tolerate an adequate dose of this drug due to its associated gastrointestinal side effects. These reversible side effects (diarrhea, nausea, and vomiting are the most common) develop in ~25% of patients treated with metformin, and treatment has to be discontinued in ~ 5% of patients [88]. Use of lower doses of metformin, progressive increase in the dose, or administration of metformin with food can attenuate its side effects [16]. The adverse gastrointestinal effects of metformin might be related to its accumulation and remarkable effects in the gut. In fact, increased exposure of the large intestine to bile acids due to the inhibition of their reabsorption and the changes in gut microbiota caused by metformin might be responsible for the increased incidence of diarrhea in patients taking this drug [76,79]. Of note, patients with T2DM with *OCT1* alleles producing organic cation transporter 1 (OTC1) with reduced activity exhibit an increased risk of metformin intolerance, and this risk is even higher when metformin is associated with other drugs that inhibit OCT1 [88].

Long-term treatment with metformin reduces vitamin B_{12} absorption, although the mechanism involved remains unclear [16]. This reduction in the absorption of vitamin B_{12} has been associated with calcium-dependent ileal membrane antagonism by metformin, an effect that may be reversed with calcium supplementation [89]. In fact, clinical studies have shown that the use of metformin is associated with vitamin B_{12} depletion in 10–30% of patients with T2DM and, thus, supplementation with this vitamin is recommended for these patients [90].

As mentioned above, metformin increases plasma lactate levels in a metformin concentration-dependent manner. Metformin-associated lactic acidosis may occur when plasma metformin concentrations become too high (e.g., in patients with kidney disease) in the setting of a condition that alters lactate production or clearance (e.g., cirrhosis, sepsis, or hypoperfusion), although its incidence in clinical practice is very low (fewer than ten cases per 100 000 patient-years) [91].

especially in *in vitro* studies and animal models, become relevant for understanding the mechanisms of action of this drug [1,2]. The maximal approved dose of metformin per day for the treatment of patients with T2DM is 2.5 g (~35 mg/kg of body weight). After oral administration, the bioavailability of metformin is ~50%. It is absorbed by enterocytes in the duodenum and jejunum by the action of several transporters, such as organic cation transporter (OCT1), plasma membrane monoamine transporter (PMAT), and serotonin transporter (SERT) [13,18]. From the enterocytes, metformin is delivered to the liver via the portal vein, where its concentrations are ~40–70 μM. In hepatocytes, metformin concentrations have been considered to be equilibrated with those of the portal vein [18], while other reports suggest that this drug accumulates in the hepatocytes reaching 3–5-fold higher concentrations in these cells [1] compared with those of the portal vein. Finally, systemic plasma concentrations of metformin drop to 10–40 μM after administration of a single dose (20 mg/kg/day in humans or 250 mg/kg/day in mice) [18]. Metformin is not metabolized and is eliminated unchanged by the kidney, with a half-life in the body of ~5 h.

The glucose-lowering effect of metformin has traditionally been believed to result from the inhibition of hepatic glucose production (Figure 1), which contributes to the fasting hyperglycemia in patients with T2DM owing to their **insulin resistance**. The mechanisms underlying this effect of metformin involve AMPK-dependent and -independent mechanisms.

AMPK-dependent mechanisms

More than two decades ago, a seminal study reported that metformin activated AMPK [19] (Table 1). Mechanistically, metformin directly increases the formation of the AMPK trimeric complex in hepatocytes, thereby promoting the phosphorylation of its α subunit at Thr^{172} by the upstream liver kinase B1 (LKB1) (Figure 1) [18]. This mechanism was confirmed later by a study using mice with a deletion of hepatic LKB1, which did not respond to the glucose-lowering effect of metformin, thus confirming that the LKB1–AMPK axis was required for the glucose-lowering effect of the drug [20]. This direct activation of AMPK occurs at relatively low concentrations of metformin in hepatocytes of animals treated with therapeutic doses of the drug [19]. More recently, it was shown that therapeutic doses of metformin also activate AMPK through the lysosomal pathway. In fact, metformin can directly act on the proton pump vacuolar-ATPase and promotes the translocation of AXIN/LKB1 to the lysosome to form a complex with vacuolar-ATPase-Ragulator, eventually leading to AMPK activation.

Glossary

Acetate: shortest fatty acid derived from the diet or fiber fermentation in the gut, which supports acetyl-coenzyme A metabolism and, thus, lipogenesis and protein acetylation.
AMP-activated protein kinase (AMPK): central regulator of energy homeostasis, which coordinates metabolic pathways and, thus, balances nutrient supply with energy demand. It is a serine/threonine protein kinase complex comprising a catalytic α-subunit (α1 or α2), a scaffolding β-subunit (β1 or β2), and a regulatory γ-subunit (γ1, γ2, or γ3).
Enteroendocrine L-cells: cells secreting GLP1, GLP2, and peptide YY (PYY). These cells are widely distributed in the distal small intestine and colon (mainly in the proximal portion).
Glucagon-like peptide 1 (GLP1): gastrointestinal peptide that is released in response to nutrients, neuronal or hormonal stimuli. The main actions of GLP-1 are stimulating insulin secretion and inhibiting glucagon secretion.
Gluconeogenesis: anabolic process that produces glucose primarily from lactate, pyruvate, amino acids, and glycerol.
Glycolysis: energy-generating process that converts glucose into pyruvate in the presence of oxygen, or into lactate in the absence of oxygen.
Growth differentiation factor 15 (GDF15): divergent member of the transforming growth factor β (TGFβ) superfamily. GDF15 serum levels increase in response to cell stress. The endogenous receptor for GDF15 is glial-derived neurotrophic factor-family receptor α-like (GFRAL), detected selectively in the brain. Binding of GDF15 to GFRAL regulates energy balance by reducing food intake.
Hepatic glucose production: process by which the liver produces and releases glucose into the blood by regulating the two primary glucose production metabolic pathways, glycogenolysis and gluconeogenesis. Excessive hepatic glucose production is a major contributor to the hyperglycemia observed in T2DM.
Insulin resistance: defect in the ability of insulin to drive glucose into its target tissues.
Lactate: major gluconeogenic precursor in the liver.
Pyruvate: major gluconeogenic precursor in the liver.

Table 1. Mechanisms of action of metformin in the liver and gut proposed to contribute to its glucose-lowering effects

Mechanism of action	Organ	Concentration/dose	Model	Refs	Validated in patients
Direct AMPK activation that results in inhibition of hepatic glucose production	Liver	Therapeutic	Rat primary hepatocytes	[18]	AMPK has been reported to be activated by metformin in skeletal muscle of patients with T2DM [52,91]
Pen2-mediated AMPK activation that contributes to inhibition of hepatic glucose production	Liver, intestine	Therapeutic	Mouse primary hepatocytes; mice	[11]	
AMPK-mediated activation of mitochondrial respiration	Liver	Therapeutic	Mouse primary hepatocytes; mice	[26]	[28,29]
Inhibition of mitochondrial respiration that leads to AMPK activation	Liver	Supratherapeutic	Mainly hepatocytes in culture	[2]	
Inhibition of gluconeogenesis through AMPK-independent reduction in hepatic energy state that decreases levels of ATP required for glucose synthesis	Liver	Supratherapeutic	Mouse primary hepatocytes; mice	[31]	
Inhibition of glucagon-dependent production of glucose by hepatocytes via reduction of protein kinase A activity	Liver	Therapeutic and supratherapeutic	Mouse primary hepatocytes; mice	[32]	
Inhibition of mitochondrial glycerol 3-phosphate dehydrogenase, which attenuates conversion of lactate and glycerol to glucose	Liver	Therapeutic	Rats	[3]	
Upregulation of miRNA let-7, which in turn inhibits hepatic glucose production by targeting TET3-HNF4α pathway	Liver	Therapeutic	Mouse primary hepatocytes; mice	[6]	
Stimulation of glycolysis	Liver	Therapeutic and supratherapeutic	Rat and mouse primary hepatocytes	[33]	
Increase of glucose uptake and utilization (glycolysis) in human small intestine and colon	Intestine	Therapeutic and supratherapeutic	Patients; mice; Caco-2 cells; Huh7 cells	[13]	[13,39–41]
Gut–liver crosstalk: increase in glucose uptake in enterocytes caused by metformin is paralleled by stimulation of glycolytic pathway that converts glucose into lactate and acetate, which are released into portal vein and reach the liver, where they ultimately inhibit hepatic glucose production	Intestine–liver	Therapeutic and supratherapeutic	Patients; mice; Caco-2 cells; Huh7 cells	[13]	Metformin increases lactate in portal vein by stimulating intestinal glycolysis [14]
Increased [^{18}F]fluorodeoxyglucose accumulation in intraluminal space of intestine, suggesting that metformin promotes release of glucose into stools	Intestine	Therapeutic	Patients	[53]	[53]
Enhanced GLP1 levels	Intestine	Therapeutic	Animal and human studies	[39]	[14,39,56,57]
Gut–brain–liver neuronal axis	Intestine–brain–liver	Therapeutic	Rats	[58]	
Increased GDF15 circulating levels	Liver, intestine	Therapeutic and supratherapeutic	Animal and human studies	[7–10,66]	Increased circulating levels of GDF15 in patients treated with metformin [7–9,66]
Regulation of gut microbiota	Intestine	Therapeutic	Patients	[72,74–76]	[72,74–76]
Inhibition of bile acid reabsorption	Intestine	Therapeutic	Patients	[77]	[77]
Alterations in bile acid composition	Intestine	Therapeutic	Mice; mouse primary hepatocytes; patients	[16,83]	[83]

This complex also inactivates mTORC1, which controls anabolic pathways. Together, these effects of metformin mimic a frugal nutrient supply or fasting condition [21]. At clinical concentrations, metformin binds PEN2, a subunit of γ-secretase, and subsequently forms a complex with ATP6AP1, a subunit of the vacuolar-ATPase, causing its inhibition and ultimately leading to the activation of AMPK [11] (Figure 1). Interestingly, in *Pen2* liver-specific knockout mice, there is no metformin-mediated reduction of hepatic fat content, while in *Pen2* intestine-specific knockout mice, the metformin glucose-lowering effect is attenuated [11]. Once AMPK is activated by metformin, different molecular pathways are stimulated that ultimately lead to a reduction in the expression of genes implicated in hepatic **gluconeogenesis**, a key process that ensures hepatic glucose production (Figure 1). AMPK activation leads to the phosphorylation of the histone acetyltransferase cAMP-response element-binding protein (CREB)-binding protein (CBP) at Ser^{436}. This triggers disassembly of the CREB-CBP-CREB-regulated transcription coactivator 2 (CRTC2) complex and subsequent inhibition of gluconeogenic gene expression [22]. Moreover, AMPK further inhibits the gluconeogenic program by directly phosphorylating CRTC2, which lowers its nuclear translocation and, thus, gluconeogenic gene expression [23]. Metformin additionally suppresses hepatic glucose production through an AMPK-dependent mechanism upregulating small heterodimer partner (SHP) [24]. This transcriptional co-repressor interacts with, and represses the activity of, key transcriptional factors, such as hepatocyte nuclear factor 4α (HNF4α), forkhead box protein O1 (FoxO1), and FoxA2, which regulate gluconeogenic genes. Moreover, ACC inhibition by AMPK is required for the antidiabetic effect of metformin [25]. In fact, metformin fails to suppress high-fat diet (HFD)-induced hyperglycemia in mice with mutations in acetyl-CoA carboxylases (ACC) 1 and 2, downstream targets of AMPK. The contribution of ACC inhibition to the glucose-lowering effects of metformin includes a reduction in hepatic *de novo* lipogenesis and steatosis, thereby reducing insulin resistance and improving insulin sensitivity. Overall, these findings indicate that therapeutic doses of metformin suppress hepatic glucose production and lipogenesis in the liver through AMPK activation.

Mitochondrial dysfunction is implicated in the development of T2DM and, in fact, patients with this disease have decreased mitochondrial number and cellular respiratory activity [26]. Metformin shows a biphasic effect on hepatocyte mitochondrial respiration, with therapeutic concentrations activating mitochondrial respiration, while supratherapeutic concentrations inhibit this process. At therapeutic concentrations, metformin increases mitochondrial respiration and ATP levels in hepatocytes and augments total mitochondrial complex I (NADH:ubiquinone oxidoreductase) activity in the liver of HFD-fed mice [27]. The increase in mitochondrial respiratory activity caused by metformin is AMPK dependent, and is the result of the stimulation of mitochondrial fission, a process associated with increased mitochondrial respiration and nutrient oxidation [28]. Supporting this mechanism, metformin increases mitochondrial respiratory chain activity in humans [29,30]. However, supratherapeutic concentrations of metformin applied to cultured cells (10–100 times higher than maximal concentrations found in the blood of patients with T2DM) inhibit mitochondrial respiration and activate AMPK [2]. Under these conditions, metformin inhibits the mitochondrial complex I *in vitro*, reducing ATP synthesis, and leading to an increase in the AMP/ATP ratio, which ultimately activates AMPK. The need for supratherapeutic concentrations of metformin to observe this inhibition reflects the weak inhibitory effect of metformin on complex I [31], which therefore is unlikely to occur in patients. The high metformin doses and concentrations used in some animal and *in vitro* studies, respectively, are not relevant for human studies and are an important parameter to consider in metformin investigations, since they might mask some AMPK-mediated effects, which are achieved at lower concentrations [2].

AMPK-independent mechanisms

The inhibition of hepatic glucose production by metformin has also been associated with AMPK-independent mechanisms. The first study reporting this alternative mechanism found that the

drug inhibits hepatic gluconeogenesis in AMPK-deficient mice [32]. The inhibition in gluconeogenesis caused by supratherapeutic metformin doses in these animals is mediated by a reduction in the hepatic energy state due to the decrease in the levels of ATP required for glucose synthesis. Likewise, metformin increases AMP levels in hepatocytes independently of AMPK, which, in turn, inhibits adenylate cyclase. This causes a reduction in cyclic AMP and in the activity of protein kinase A, eventually leading to the inhibition of glucagon-dependent production of glucose by hepatocytes [33] (Figure 2). In addition, metformin can lower blood glucose by a redox mechanism [4] through the inhibition of mitochondrial glycerol 3-phosphate dehydrogenase [3], thereby attenuating the conversion of **lactate** and glycerol to glucose. However, this inhibition was not observed in a more recent study [5]. Interestingly, another recent study reported how modulation of cellular redox balance by metformin may result in a reduction of hepatic glucose production via a new mechanism [6]. By modulating hepatic redox, clinically relevant doses of metformin induce the expression of the miRNA let-7 in the hepatocyte nuclei, which in turn inhibits hepatic glucose production by lowering the activity of the Tet methylcytosine dioxygenase 3 (TET3)-hepatocyte nuclear factor 4α (HNF4α)-P2 promoter pathway (Figure 2). Moreover, liver-specific let-7 miRNA inhibition abolishes the beneficial effects of metformin on glucose homeostasis and let-7 miRNA

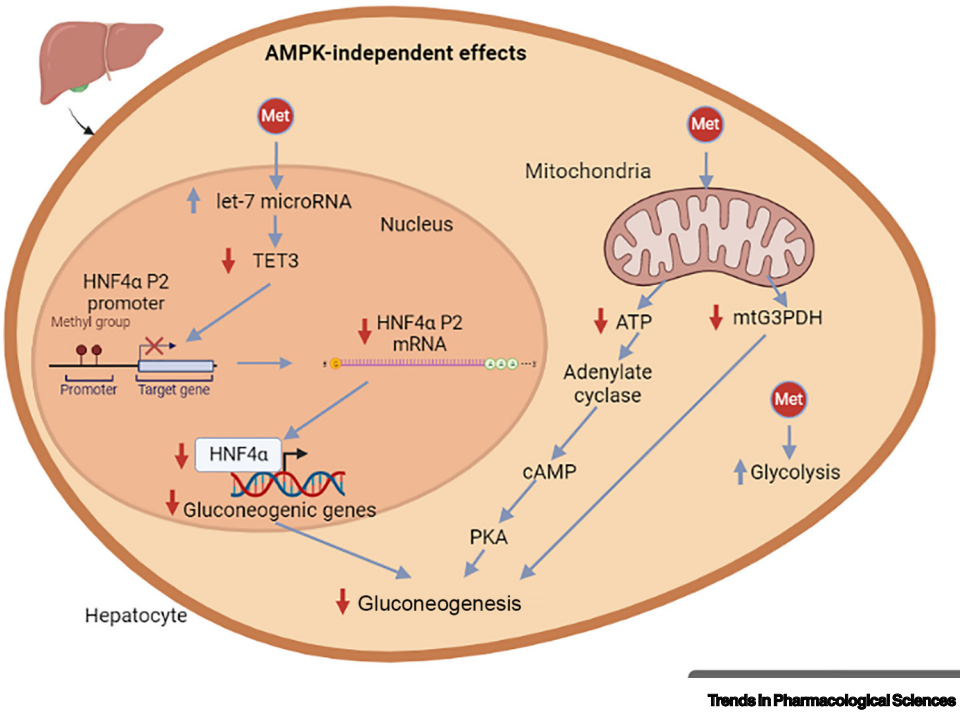

Figure 2. Schematic showing the AMP-activated protein kinase (AMPK)-independent hepatic effects of metformin on glucose metabolism. Metformin upregulates miRNA let-7, which, in turn, reduces expression of the DNA demethylase Tet methylcytosine dioxygenase 3 (TET3). This leads to a subsequent demethylation of the hepatocyte nuclear factor 4α (HNF4α) P2 promoter, decreasing its activity and the mRNA and protein levels of the key gluconeogenic transcription factor HNF4α. As a result, there is less binding of HNF4α to the promoter of the gluconeogenic genes and gluconeogenesis is reduced. Moreover, metformin decreases ATP levels and induces a state of reduced energy, thereby attenuating glucose synthesis. Likewise, metformin increases AMP levels in hepatocytes independently of AMPK, which, in turn, inhibits adenylate cyclase. This reduces cAMP and the activity of protein kinase A (PKA), eventually leading to inhibition of the glucagon-dependent production of glucose by hepatocytes. Metformin also inhibits mitochondrial glycerol 3-phosphate dehydrogenase (mtG3PDH) activity via an AMPK-independent mechanism, which helps to attenuate gluconeogenesis. However, this mechanism was not confirmed in more recent studies. Finally, metformin also increases glycolysis. Created with BioRender (biorender.com).

overexpression decreases glucose production in primary hepatocytes from obese humans. These findings open the possibility of activating let-7 miRNA expression, which is repressed in diabetic states, as a new therapeutic strategy for the treatment of T2DM [6].

The stimulation of **glycolysis** by metformin is an additional mechanism by which this drug can contribute to its glucose-lowering effect [34]. Metformin stimulates glycolysis by an AMPK-independent mechanism through changes in allosteric effectors of phosphofructokinase-1 and fructose bisphosphatase-1, including AMP, Pi, and glycerol 3-phosphate [34].

Metformin effects in the gut

Although the liver has been considered its primary site of action for decades [1,35], several observations suggest that metformin acts not only on the liver, but also in the gut, where it also has antidiabetic effects [13]. First, ~50% of metformin doses do not reach the systemic circulation but accumulate in the gut, attaining concentrations 30–300 times greater than in the blood [36,37], and suggesting the gut as an important target of the glucose-lowering effect of metformin. Second, oral administration of metformin is more effective compared with intravenous administration [38], indicating that the gut might be required to achieve the maximal glucose-lowering effect of the drug. Third, a metformin delayed-release formulation, which results in low systemic drug concentrations, primarily targets the ileum and has glucose-lowering efficacy similar to a standard metformin formulation (metformin immediate-release) [39].

Metformin increases intestinal glucose uptake and utilization and establishes a potent gut–liver crosstalk

Metformin increases glucose uptake and utilization in the human small intestine and colon [40–42] (Table 1). This is a well-known process observed by radiologists during clinical cancer diagnosis when they examine the uptake of [^{18}F]-fluoro-2-deoxy-D-glucose (FDG), a non-metabolized glucose analog, using positron emission tomography (PET) in patients under metformin treatment. Metformin diffusively increases FDG uptake along the large bowel [40,42,43] and may lead to false positive cancer detection. Therefore, metformin treatment is discontinued 48 h before a FDG-PET analysis. However, the relevance of this glucose uptake effect on the glucose-lowering action of metformin is unknown. In fact, it has been argued that, since normalization of FDG in the intestine takes several days after metformin withdrawal, the enhanced uptake caused by metformin might be a prolonged secondary effect [43]. In addition, the increased glucose uptake in the gut of patients might be the result of the inhibition of mitochondrial respiration by high concentrations of metformin [44]. This inhibition would reduce ATP synthesis, thereby stimulating glucose uptake and glycolysis to synthesize ATP for cellular energy needs.

The metformin-mediated increase in FDG uptake throughout the intestine includes the jejunum, ileum, and colon in patients with T2DM compared with patients not treated with this drug or control subjects [14]. In patients treated with metformin, the intestine exhibits a higher rate of glucose uptake compared with the liver, heart, or muscle [14]. In addition, the mechanism whereby metformin increases glucose uptake by enterocytes and glycolysis involves upregulation of the glucose transporters GLUT1 and GLUT2 via activating transcription factor 4 (ATF4) and AMPK, respectively [14] (Figure 3). The involvement of AMPK in the effects of metformin in the gut is consistent with a recent finding showing that intestinal epithelium-specific AMPKα1-knockout mice fail to respond to metformin and display metabolic derangements secondary to alterations in the gut microbiome [45,46]. Although the net effect of metformin results in an increase in intestinal glucose absorption, one recent study reported that the drug can also reduce the apical density of intestinal sodium glucose cotransporter 1 (SGLT1) in the enterocyte [47]. This leads to an acute and transient reduction in the intestinal absorption of intraluminal glucose and a decrease in the

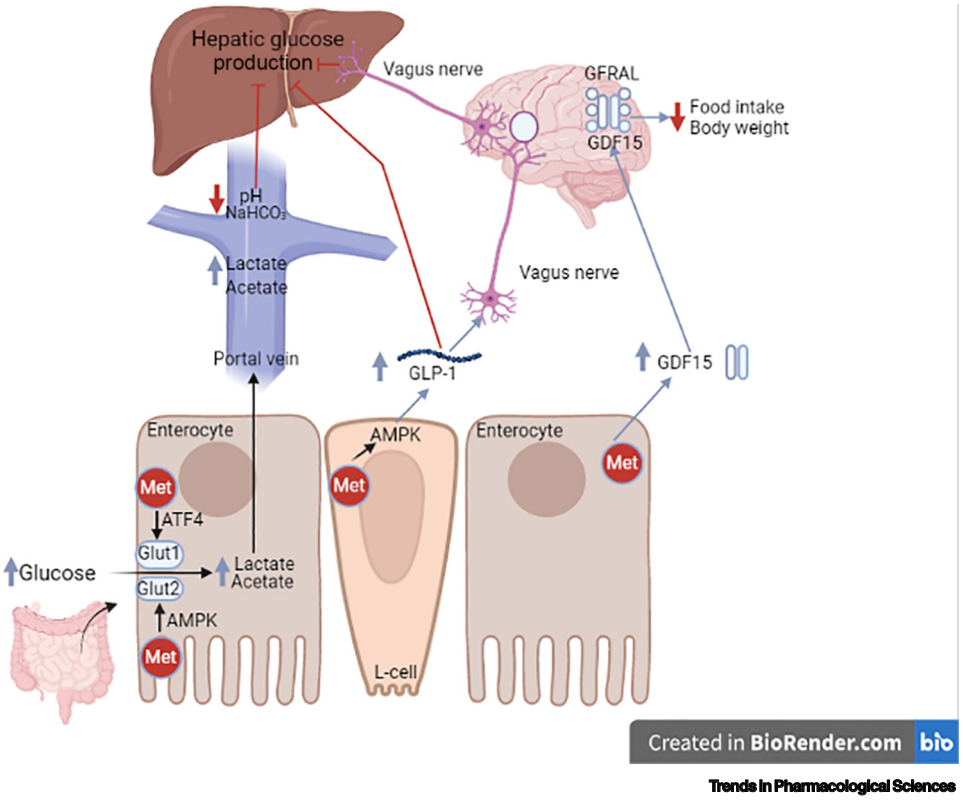

Figure 3. Scheme showing the activation by metformin of a crosstalk between the gut and other organs. Metformin inhibits hepatic glucose production by promoting crosstalk between the gut and the liver. It augments the uptake of glucose by enterocytes by increasing the levels of glucose transporter (GLUT)-1 and GLUT2 via activating transcription factor 4 (ATF4) and AMP-activated protein kinase (AMPK), respectively. In enterocytes, glucose is metabolized to lactate and acetate. The increase in lactate reduces the pH and sodium bicarbonate ($NaHCO_3$) in the portal vein, thereby inhibiting gluconeogenesis in hepatocytes and, therefore, hepatic glucose production. Acetate accumulation also inhibits gluconeogenesis via several additional mechanisms. Metformin also activates AMPK in the gut and induces the secretion of glucagon-like peptide 1 (GLP1) by enteroendocrine L-cells. This incretin directly inhibits gluconeogenesis and triggers a gut–brain–liver neuronal network via the vagus nerve, which mitigates hepatic glucose production. Growth differentiation factor 15 (GDF15) is a cellular stress cytokine that binds to its central receptor glial cell line-derived neurotrophic factor (GDNF) family receptor α-like (GFRAL) and reduces food intake and body weight, ultimately reducing glucose plasma levels. Metformin increases the production of GDF15 by the intestine and the kidney (not shown), and this is responsible for the reduction in body weight caused by metformin. However, a recent study claimed that the GDF15-GFRAL pathway is dispensable for the body weight reduction caused by metformin [9]. Created with BioRender (biorender.com).

postprandial glucose response. The authors of this study suggest that this reduced absorption of glucose promotes other beneficial effects of metformin in the gut, such as an increased secretion of GLP1 due to the higher amounts of glucose that reach the lower intestine [47]. Importantly, the regulation by metformin of intestinal glucose trafficking remains the subject of debate [48], fueled by conflicting data regarding the effects of metformin on SGLT1 levels [47]. While the effects of metformin on glucose transporters in the intestine have been extensively explored, it is unknown whether the mechanism responsible for the increase of glycolysis by metformin in hepatocytes [34] also operates in intestinal cells.

The metformin-induced higher uptake of glucose in the enterocytes is paralleled by stimulation of the glycolytic pathway, which converts glucose into lactate and **acetate**, which are released into the portal vein and reach the liver, where they ultimately inhibit hepatic glucose production [14].

Furthermore, it is hypothesized that the increase in lactate would decrease the pH and the level of $NaHCO_3$ in the portal vein [14]. These effects are thought to lower the hepatic activity of the gluconeogenic enzyme pyruvate carboxylase, leading to a decrease in gluconeogenesis. Likewise, increased acetate levels would inhibit mitochondrial pyruvate carrier 1/2 activity by acetylation. The resulting reduction in **pyruvate** transport to the mitochondria would promote its accumulation, thus inhibiting the initial phases of gluconeogenesis. The increase in pyruvate would subsequently inhibit the uptake of extracellular lactate via monocarboxylate transporter 1. Acetate would also acetylate fructose 1,6-bisphosphatase, an important rate-limiting step in gluconeogenesis, thereby reducing its activity. These effects of metformin are observed in hyperglycemic conditions. However, in normoglycemic conditions, the effects of metformin might be the opposite. In fact, paradoxically, metformin induces hepatic glucose production in nondiabetic subjects with normal glycemia [49,50]. In nondiabetic mice, metformin causes a smaller induction of intestinal glucose uptake compared with diabetic mice, which is sufficient to reduce glucose in the portal vein [14]. This hypoglycemia in the portal vein does not cause a decrease in the pH and $NaHCO_3$ and, consequently, does not activate the gut–liver crosstalk that reduces hepatic glucose production. In fact, it is hypothesized that the hypoglycemia in the portal vein through portal glucose sensing stimulates a counter-regulatory mechanism that prevents the reduction in, or even increases, hepatic glucose production [14].

Although these new findings do not rule out a direct effect of metformin in the liver or in other tissues (Box 2), they nevertheless reveal that metabolites increased by the action of metformin in the gut (e.g., lactate and acetate) reach the liver through the portal vein, where they may contribute to the antihyperglycemic effect of the drug by inhibiting hepatic glucose production. Interestingly, it has been reported that increased lactate production by metformin in the intestine of mice activates a futile intestinal–hepatic cycle, in which lactate is converted back to glucose [51]. This

Box 2. Effects of metformin in skeletal muscle

The antihyperglycemic effects of metformin have been extended to organs other than gut and liver, including kidney, adipose tissue, and skeletal muscle. Since skeletal muscle accounts for most of the insulin-stimulated glucose use [92], the effects of metformin in this tissue might have a strong impact on glucose levels. Interestingly, skeletal muscle takes up and accumulates metformin [93], which is consistent with the reported activation of AMPK by metformin in the skeletal muscle of patients with T2DM [94].

The involvement of AMPK in the glucose-lowering effects of metformin in skeletal muscle was demonstrated by a study showing that chronic treatment with this drug increased insulin-stimulated glucose uptake in soleus muscles of wild-type, but not of muscle-specific kinase-dead, AMPKα_2 mice. Furthermore, an acute metformin treatment did not affect glucose uptake in muscle of either genotype [95].

Additional pathways activated by metformin in skeletal muscle have been proposed to contribute to the glucose-lowering effect of metformin. For instance, it has been reported that, in skeletal muscle, metformin binds and inhibits Src homology 2 domain-containing inositol-5-phosphatase 2 (SHIP2), a phosphatase that suppresses phosphatidylinositol 3-kinase-mediated insulin signaling by hydrolyzing phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) to phosphatidylinositol (4,5)-biphosphate (PIP_2) [96]. Likewise, metformin ameliorates skeletal muscle insulin resistance by inhibiting miR-21 expression in rats fed a HFD, which results in attenuation of the expression of transforming growth factor β1/mothers against decapentaplegic homolog 3 (smad3) [97]. It has also been reported that the antidiabetic effects of chronic metformin administration require the presence of GDF15 [10]. It was observed that metformin increased GDF15 levels, ameliorated glucose intolerance, and activated AMPK in the liver and skeletal muscle of wild-type mice, but not in *Gdf15*$^{-/-}$ mice fed a HFD. Interestingly, metformin increased GDF15 in muscle cells via AMPK activation, and this cytokine promoted the activation of AMPK through a positive feedback loop.

Overall, these findings indicate that chronic treatment with metformin promotes glucose uptake via AMPK and additional mechanisms in skeletal muscle. However, conflicting results preclude establishing whether acute metformin treatment affects glucose uptake in skeletal muscle and the mechanism involved. It is likely that differences in results of acute treatments might be caused by the slow accumulation of metformin in skeletal muscle, which requires chronic treatment to clearly observe metformin effects. Further studies are needed to uncover the contribution of skeletal muscle to the glucose-lowering effects of metformin compared with the effects reported in the intestines.

cycle is a highly energy-consuming process and, according to the authors of this study, might contribute to the attenuation of body weight gain by metformin. Metformin causes lactate accumulation in the intestine and portal vein but not in peripheral blood or the liver [51]. This might explain why no changes have been observed in plasma concentration levels of this metabolite in patients treated with metformin [52], while oral metformin administration has been reported to increase lactate production in biopsies of human jejunal mucosa [53]. Similarly, a recent study in humans showed that a single oral dose of metformin increased lactate in the portal vein by stimulating intestinal glycolysis [15]. Further studies are necessary to confirm the presence of this potential gut–liver crosstalk mediated by lactate and acetate and its clinical relevance in patients, but these findings pave the way to a better understanding of the action of metformin in which the gut contributes to its glucose-lowering effects.

Metformin helps to reduce the amount of glucose in the plasma via additional effects. By using a newly developed imaging method, it was shown that metformin treatment increased the accumulation of FDG in the intraluminal space of the intestine, suggesting that the drug promotes the release of glucose into stools [54]. However, other authors claim that this does not imply that metformin stimulates the removal of glucose from the body, since the accumulation of FDG in the intraluminal space might be caused by changes in the gut microbiota produced by the drug [43]. Collectively, all these findings suggest that the gut participates in the antihyperglycemic effect of metformin, although its clinical relevance is still unclear in many ways.

Metformin enhances GLP1 levels

GLP1 is an incretin hormone secreted in response to nutrient intake by **enteroendocrine L-cells**, which are distributed throughout the intestinal mucosa. This gut peptide promotes glucose-dependent insulin secretion, inhibits glucagon secretion and hepatic glucose production, slows gastric emptying, and reduces appetite [55]. In both rodent and human studies, metformin increases plasma GLP1 levels [40] (Table 1). Metformin might increase GLP1 secretion by L-cells and/or inhibit the activity of GLP1-degrading dipeptidyl peptidase 4 (DPP4). Current knowledge supports the former process as being mainly responsible for the metformin-dependent increase in GLP1 [40] (Figure 3). However, the mechanisms responsible for the increased secretion of GLP1 by metformin remain controversial. *In vitro* studies using isolated human intestinal biopsies reported that metformin stimulates GLP1 secretion from L-cells by both direct and indirect mechanisms [56]. *In vivo* administration of a single therapeutic dose of metformin (1000 mg) to the proximal (duodenum) or distal (ileum) small intestine, followed 1 h later by an oral glucose load, demonstrated that metformin does not directly stimulate the secretion of GLP1 from L-cells in patients with T2DM, but enhances GLP1 secretion in response to the glucose load via indirect mechanisms [57]. However, a more recent study reported that administration of a single oral dose of metformin in humans increased GLP1 levels in the portal vein and arterialized blood [15]. As mentioned below, metformin can influence GLP1 secretion through its effects on bile acid resorption and gut microbiota modulation. Stimulation of GLP1 secretion by metformin appears to be crucial for its glucose-lowering effect, since co-administration of a GLP1 receptor antagonist abolished this effect [58,59]. Metformin also increases the plasma levels of the L-cell-secreted peptide YY [60], a potent anorectic hormone similar to GLP1, although the contribution of this active peptide to the glucose-lowering effect of metformin has been less studied.

Metformin regulates the gut–brain–liver neuronal axis

The acute glucose-lowering effect of metformin also relies on the modulation of a gut–brain–liver axis, which mediates intestinal nutrient and hormone-mediated inhibition of hepatic glucose production [59]. Intraduodenal infusion of metformin activates duodenal mucosal AMPK and lowers hepatic glucose production. This is a local direct pre-absorptive effect of metformin on the

duodenum and is not seen when metformin is delivered via the portal vein. The ability of preabsorptive metformin to lower hepatic glucose production is attributed to the release of GLP1 by enteroendocrine L-cells, and activation of the GLP1 receptor on the afferent vagus nerve innervating the small intestine to promote a gut–brain–liver axis that eventually inhibits hepatic glucose production [59]. Interestingly, the effect of intraduodenal infusion of metformin on glucose metabolism is abolished by co-infusion with the GLP1 receptor antagonist exendin, as well as in rats with suppressed neurocommunication between the brain and the liver caused by hepatic vagal branch vagotomy. These observations indicate that duodenal metformin activates a vagus nerve-based gut–brain–liver axis by releasing GLP1 to reduce hepatic glucose production [59]. Altogether, these findings indicate that activation of duodenal AMPK leading to increased secretion of GLP1 that activates the afferent and hepatic branches of the vagus substantially contributes to the acute glucose-lowering effect of metformin (Figure 3).

In addition to this gut–brain–liver neuronal axis, it has also been reported that metformin ameliorates the autonomic nervous system imbalance in obese rats [61]. Metformin treatment normalized the hypervagal response in obese rats, which was associated with a reduction in the protein levels of the M3 muscarinic acetylcholine receptor in pancreatic β cells. However, the mechanism responsible for this effect was not explored and neither was the gut reported to be involved. Overall, these findings indicate that metformin affects the autonomic nervous system, but they need to be validated in patients treated with the drug.

Metformin regulates GDF15 levels

GDF15 is a cytokine that regulates energy balance by reducing food intake [62]. The anorectic effect of GDF15 is mediated by binding to its cognate receptor glial cell line-derived neurotrophic factor (GDNF) family receptor α-like (GFRAL) [63–66]. This receptor is expressed solely in neurons of the area postrema and solitary tract nucleus, which are brain regions involved in appetite and weight regulation. Of note, a clinical trial in 2017 revealed that metformin increases the serum levels of GDF15 [67]. More recently, two studies reported that metformin augmented the circulating levels of GDF15 in both men and mice and that this increase was responsible for the reduction in body weight caused by the drug [7,8] (Table 1). In fact, these two studies found statistically significant associations between the increase in GDF15 serum levels caused by metformin and the reduction in body weight in nondiabetic subjects and in patients with T2DM. Using primary mouse hepatocytes, both studies demonstrated that metformin exposure increased the release of GDF15. However, one of them reported that oral metformin increased plasma GDF15 levels, with *Gdf15* expression increasing predominantly in the distal intestine and the kidney, but not in the liver [7] (Figure 3). Consistent with the role of GDF15 in the reduction of body weight caused by metformin, treatment with the drug prevented the increase in weight gain in wild-type mice fed a HFD, but not in mice deficient in GDF15 or GFRAL. Although metformin provoked GDF15-independent effects on glucose metabolism, the reduction in body weight caused by GDF15 in metformin-treated mice was likely to have contributed to the improvement in insulin sensitivity. A more recent study confirmed that metformin increases circulating GDF15 levels in humans and mice, which is likely due to increased expression of *Gdf15* in the intestines and the kidney [9]. However, in contrast to previous findings, the reduction in body weight caused by metformin was independent of GDF15 and GFRAL in obese mice. Furthermore, the ability of metformin to acutely lower circulating levels of glucose was preserved in the absence of GDF15 [9]. Likewise, the increase in GDF15 induced by metformin was not associated with body weight changes in overweight individuals with prediabetes. In contrast to these studies mostly evaluating the acute effects of metformin, it was reported that chronic administration of metformin ameliorates glucose intolerance in wild-type mice fed a HFD, but fails to produce a glucose-lowering effect in *Gdf15*-null mice, suggesting that this cytokine is required for the antidiabetic effects of

metformin [10]. In this study, the administration of metformin at a dose of 100 mg/kg/day to wild-type mice did not affect cumulative food intake, suggesting that GFRAL was not involved in the observed effects.

The reasons for the discrepancies among the different studies evaluating the contribution of GDF15 upregulation following metformin treatment are unknown, but several factors may contribute, including the differences in the doses of metformin used (ranging from 100 mg/kg/day to 600 mg/kg/day), the length of the treatments (acute vs. chronic), the type of HFD feeding that influences the effect of metformin on plasma GDF15 levels, the time point of the light phase selected for the administration of the drug, and, given the short-life of GDF15, the time elapsed between final drug administration and the analysis of plasma GDF15 [68]. In addition, the relative contribution of the different organs, the liver [8], intestine [7] and kidney [69], to the increase in GDF15 circulating levels following metformin treatment deserves further attention.

Another factor to be considered when evaluating whether the effects of metformin are mediated by the GDF15-GFRAL pathway is that obesity can induce a state of resistance to the anorexigenic effects of GDF15. This would be consistent with the presence of elevated circulating GDF15 levels in obese subjects and mice and with the fact that only administration of supraphysiological levels of exogenous GDF15 in animal models of obesity-induced T2DM has been shown to ameliorate the metabolic alterations [70]. The development of resistance to GDF15 in obesity may involve metalloproteinase 14 (MT1-MMP/MMP14), which, when activated in obese rodents, proteolytically inactivates GFRAL, thereby suppressing GDF15-GFRAL signaling [71]. Therefore, the use of different animal models of obesity, with different degrees of resistance to GDF15, might generate different outcomes in response to metformin. Additional studies are needed to unveil the relationship between metformin, GDF15, and glucose metabolism and energy balance. It is especially necessary to evaluate the role of the gut-derived increase in GDF15 on the glucose-lowering effects of metformin in chronic treatments since acute antihyperglycemic effects of metformin appear to be mediated by the stimulation of intestinal glucose uptake.

Metformin regulates the gut microbiota

Patients with T2DM show differences in their gut microbiota compared with healthy subjects, and dysbiosis participates in the development of T2DM by affecting the integrity of the intestinal barrier, the production of short-chain fatty acids (SCFAs), and the metabolism of bile acids [72]. In addition, HFD feeding causes a two- or threefold increase in plasma levels of lipopolysaccharide (LPS) [73], the major component of the outer membrane of Gram-negative bacteria that causes inflammatory responses and insulin resistance. Treatment of naive patients with T2DM with metformin has a strong impact on the gut microbiome, and transplantation of fecal samples from metformin-treated donors to germ-free mice lowers serum LPS levels, reduces inflammation, and improves glucose tolerance (Figure 4 and Table 1) [74]. Anaerobic gut bacteria produce SCFAs (such as lactate, butyrate, and propionate) through fermentation of unabsorbable carbohydrates, thereby stimulating mucin production and ultimately leading to a reduction in epithelial permeability, a decrease in inflammation, and a reduction in glucose levels [75]. A study enrolling metformin-untreated patients with T2DM, metformin-treated and nondiabetic subjects showed a reduction in SCFA-producing bacteria in the metformin-untreated patients, while treatment with metformin increased the production of butyrate and propionate [76]. Metformin also shifted the gut microbiota composition toward the enrichment of mucin-degrading *Akkermansia muciniphila* [77], which promotes mucus secretion and reduces epithelial permeability as mentioned above. In addition, feeding a HFD reduces glucose sensing and intestinal SGLT1 in the upper small intestine, while metformin restores SGLT1 levels and glucose sensing partly by increasing the abundance of *Lactobacillus* [78]. Collectively, these studies are consistent with a line of evidence

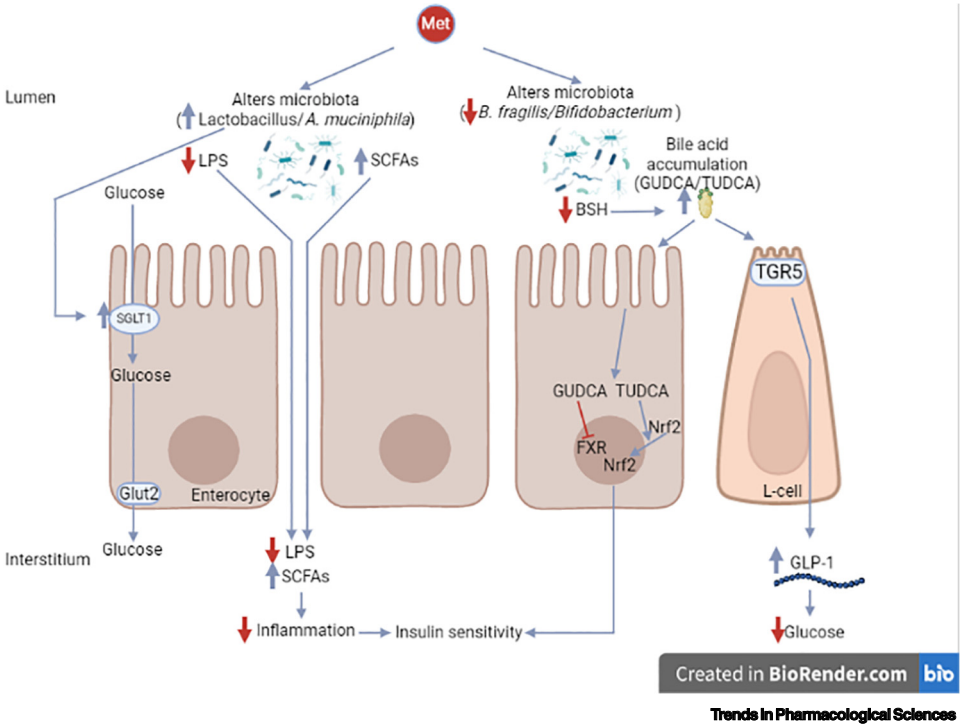

Figure 4. Scheme showing the effects of metformin on gut microbiota and bile acid reabsorption. Metformin alters the gut microbiota, reduces lipopolysaccharide (LPS) levels, and increases short-chain saturated fatty acid (SCFA) production, thereby attenuating inflammation and improving glucose tolerance. Metformin enriches *Akkermansia muciniphila* and *Lactobacillus* in the gut microbiota. The former promote mucus secretion and reduce epithelial permeability, whereas the second increase intestinal sodium glucose cotransporter 1 (SGLT1). Metformin inhibits bile acid reabsorption through direct and indirect mechanisms. It directly inhibits the apical sodium-dependent bile acid transporter in enterocytes. The subsequent increase in bile acids in the intestinal lumen stimulates the secretion of GLP1 by L-cells via Takeda G protein-coupled receptor 5 (TGR5) receptors. Metformin indirectly reduces the abundance of *Bacteroides fragilis* and *Bifidobacterium*, which leads to a decrease in bile salt hydrolase (BSH), and a subsequent increase in the amounts of bile acids, including glycine-ursodeoxycholic acid (GUDCA), an inhibitor of the farnesoid X receptor (FXR) pathway, and tauroursodeoxycholic acid (TUDCA), which activates the antioxidant nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. As a result of the effects of bile acids on these pathways, an improvement in insulin sensitivity is observed. Created with BioRender (biorender.com).

indicating that alterations in the gut microbiota composition contribute to the glucose-lowering effect of metformin.

Metformin inhibits bile acid reabsorption

In addition to the well-established function of bile acids in fat digestion and absorption, they also regulate glucose metabolism. Metformin, through a direct effect on enterocytes, reduces the active reabsorption of bile acids in the terminal ileum via inhibition of the apical sodium-dependent bile acid transporter [79] (Table 1). This effect is mediated by metformin-dependent inhibition of the farnesoid X receptor (FXR), a bile acid sensor involved in ileal absorption of bile acids and the synthesis and secretion of bile acids from the liver. The inhibition of FXR caused by metformin is mediated by AMPK, since this kinase binds and represses FXR activity through phosphorylation, which ultimately results in the reduction in bile acid reabsorption [80]. The inhibition of bile acid reabsorption results in the subsequent exposure of the distal gut to bile acids, which stimulates the secretion by enteroendocrine L-cells of GLP1 via stimulation of the Takeda G-protein-coupled receptor 5 (TGR-5), thereby promoting a reduction in glucose levels [79,81]. Indeed, oral metformin administration in combination with intravenous cholecystokinin to induce bile release from the

gallbladder caused a higher stimulation of GLP1 secretion compared with cholecystokinin or metformin alone in patients with T2DM [82]. However, metformin alone did not increase plasma GLP1 concentrations compared with placebo. Moreover, caution should be taken when interpreting the findings of this study because they might have been influenced by both interindividual differences [83] and cholecystokinin-induced alterations in the rates of gastric emptying, a factor that impacts GLP1 secretion [84].

As stated above, metformin modulates the gut microbiota, leading to alterations in bile acid composition. In fact, metformin reduces the abundance of *Bacteroides fragilis* in the gut, resulting in a decrease in the enzyme bile salt hydrolase (BSH) [85]. This effect induces a subsequent increase in the bile acid glycine-ursodeoxycholic acid (GUDCA), an intestinal FXR antagonist that improves insulin sensitivity. Interestingly, stool and serum from patients with diabetes treated with metformin show increased levels of GUDCA, which is not commonly present in humans. Fecal transplantation of samples from these metformin-treated patients to diabetic mice improved insulin sensitivity, while samples from untreated patients worsened it. Surprisingly, inhibition of the FXR pathway produced by metformin secondary to changes in the gut microbiota is independent of AMPK. Following the same line of thought, metformin treatment is associated with a reduction in the abundance of *Bifidobacterium*, which also produces BSH, leading to the accumulation of another bile acid, tauroursodeoxycholic acid (TUDCA), in the intestine of HFD-fed mice [17]. The increase in TUDCA caused by metformin ameliorates insulin resistance and alleviates oxidative stress and intestinal inflammation in *ob/ob* mice [17]. Mechanistically, TUDCA promotes these actions by blocking the binding of Kelch-like ECH-associated protein 1 (KEAP1) with nuclear factor (erythroid-derived 2)-like 2 (Nrf2), resulting in Nrf2 translocation into the nucleus, which stimulates the transcription of antioxidant genes, and eventually reduces intracellular reactive oxygen species (ROS) accumulation and improves insulin signaling.

In addition, bile acids are bitter substances that can stimulate bitter taste receptors, which are distributed in the gastrointestinal tract and are regulators of glucose metabolism. Given that enteroendocrine L-cells express bitter taste receptors [86], it is likely that activation of these receptors by bile acids may also contribute to the increase in GLP1 secretion induced by metformin.

Concluding remarks and future perspectives

Although the liver strongly contributes to the antidiabetic effects of metformin [1], a growing body of evidence suggests that several of the glucose-lowering effects of this drug originate from its pleiotropic actions in the gut. However, the extent to which each of these effects contributes to the glucose-reducing effects of metformin remains to be elucidated (see Outstanding questions). Moreover, although significant advances in the understanding of metformin have been made in recent years, several questions remain. The discovery that the glucose-lowering effects of metformin rely on communication between the gut and the liver, which modulates hepatic glucose production in opposite directions depending on blood glucose levels, may help to explain the paradoxical effects of this drug in patients with and without diabetes. Likewise, the effects of metformin on body weight, energy balance, and glucose metabolism mediated by GDF15-GFRAL signaling indicate a new pathway to help decipher the mechanisms of action of the drug, and it is likely that this pathway will not be the last metformin target discovered. However, according to the most recent studies, the role of this pathway remains controversial and warrants new research to clearly establish its real contribution to the glucose-lowering effect of metformin. Another issue when evaluating the antidiabetic effects of metformin in the liver and the gut emerges when extrapolating the findings in animal studies to the clinical setting. In fact, some of the animal studies used doses or concentrations higher than the maximally allowed achievable therapeutic concentrations found in patients with T2DM [2,48,53]. This can contribute to explain

Outstanding questions

Do the direct hepatic actions of metformin make a significant contribution to its glucose-lowering effects?

What contributions do the different actions of metformin in the gut make to its glucose-lowering effects?

Is intestinal AMPK involved in the antihyperglycemic effects of metformin?

Is skeletal muscle involved in the glucose-lowering effects of chronic metformin treatment?

the differences between animal models and patients. Moreover, a recent study reported that additional factors can hinder the mouse-to-human extrapolation [87]. This study critically analyzed the effects of metformin in different established procedures, animal models, and treatment modalities. The authors suggested that metformin effects in mice depend on three components (acute glucose lowering, weight-dependent glucose lowering, and metformin-induced deterioration of glucose homeostasis), proposing the first of these as the equivalent to clinical action. The net effect of metformin on blood glucose levels would result from the contribution of these three components and is influenced by the protocol applied to mice, thereby explaining different outcomes in rodent studies [87].

Finally, the most frequent side effects caused by metformin are also likely to originate in the gut and might be related to changes in gut microbiota composition and the levels of transporters of this drug. Resolving these outstanding issues has the potential to help optimize the efficacy of, and tolerance to, metformin and might lead to the development of new drugs acting on novel targets for the treatment of T2DM.

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Declaration of interests

None declared by authors.

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