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Metformin: update on mechanisms of action and repurposing potential

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Abstract

Currently, metformin is the first-line medication to treat type 2 diabetes mellitus (T2DM) in most guidelines and is used daily by >200 million patients. Surprisingly, the mechanisms underlying its therapeutic action are complex and are still not fully understood. Early evidence highlighted the liver as the major organ involved in the effect of metformin on reducing blood levels of glucose. However, increasing evidence points towards other sites of action that might also have an important role, including the gastrointestinal tract, the gut microbial communities and the tissue-resident immune cells. At the molecular level, it seems that the mechanisms of action vary depending on the dose of metformin used and duration of treatment. Initial studies have shown that metformin targets hepatic mitochondria; however, the identification of a novel target at low concentrations of metformin at the lysosome surface might reveal a new mechanism of action. Based on the efficacy and safety records in T2DM, attention has been given to the repurposing of metformin as part of adjunct therapy for the treatment of cancer, age-related diseases, inflammatory diseases and COVID-19. In this Review, we highlight the latest advances in our understanding of the mechanisms of action of metformin and discuss potential emerging novel therapeutic uses.

Sections

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Key points

- The liver and gut are the main target organs for metformin.

- Mitochondria and lysosomes are the organelle targets in the glucose-lowering effect of metformin.

- Host–gut microbiota interactions contribute to metformin's therapeutic effects.

- Metformin has anti-inflammatory and immunomodulatory properties in various immune-related diseases through AMPK-dependent and AMPK-independent mechanisms involving both the innate and adaptive immune systems.

- Metformin therapy in patients with type 2 diabetes mellitus enhances the release of GDF15, which might facilitate weight loss but is not required for the effect in reducing blood levels of glucose.

Introduction

For the past 60 years, metformin (1,1-dimethylbiguanide hydrochloride) has been the most commonly used glucose-lowering agent and has become the first-line medication for individuals newly diagnosed with type 2 diabetes mellitus (T2DM) in many clinical guidelines[1,2]. Metformin was first synthesized in 1922, and the first report of it being used to lower blood levels of glucose (in rabbits) was published in 1929 (refs. 3,4). In 1949, metformin (called flumamine at that time) was used for the treatment of an epidemic influenza outbreak in the Philippines and was noted to lower blood levels of glucose in some of the patients with influenza[5]. Interestingly, in the past few years, the use of metformin has been explored for the management of patients with T2DM and obesity who contract influenza[6] or COVID-19 (refs. 7–9) (Box 1). In 1957, the French physician Jean Sterne introduced the clinical use of metformin for the treatment of adult-onset diabetes mellitus[3,4]. The benefits of metformin therapy in T2DM have been well documented; it has long-term safety and efficacy data, low risk of hypoglycaemia, cardiovascular benefits, mortality benefits, additive or synergistic effects in combination therapy, low cost and wide availability[1,2,10–12]. In addition, metformin moderately reduces body weight gain, possibly through upregulation of the anorectic cytokine growth differentiation factor 15 (refs. 13,14) (Box 2). Metformin is now used daily by >200 million patients with T2DM worldwide as monotherapy or in combination with sulfonylureas or dipeptidyl peptidase 4 inhibitors[1,2]. Administered orally as an immediate-release or extended-release formulation, the usual dose of metformin (0.5–2.5 g daily) is effective for long-term glycaemic control, with notable changes in HbA_{1c} from baseline[1,2].

It is important to note that metformin is increasingly being used during pregnancy for the management of gestational diabetes mellitus and in those with polycystic ovary syndrome or T2DM[15]. As metformin crosses the placenta and circulates in the developing fetus, the long-term effects of fetal exposure to metformin have raised concerns about the potential risk to growth and development of the fetus, and later, on offspring health[16]. While in utero exposure to metformin was considered safe, some studies have found higher occurrence of small for gestational age birthweight and increased risk of childhood obesity[17]. However, reassuring data were provided in a long-term follow-up study showing similar anthropometrics in children exposed or not to metformin in utero[18].

Metformin is a synthetic biguanide (two coupled molecules of guanidine) that is mainly absorbed in the upper small intestine and exhibits flip–flop pharmacokinetics with limited oral bioavailability[19,20]. Metformin is a basic hydrophilic drug with a pK_a value of 11.5, and exists as an organic cation at physiological pH. The biodistribution and pharmacodynamics of the drug are dependent on the expression of transporters for cationic compounds, such as organic cation transporters (OCTs), plasma membrane monoamine transporter and multidrug and toxin extrusion proteins[21,22]. The half-life of metformin in plasma is short, at 2–6 h, and leads to a steady-state plasma concentration of metformin in patients with T2DM of ~4–15 μM (~0.5–2.0 μg/ml)[23]. Concerns have been expressed regarding the therapeutically appropriate concentrations to establish the cellular actions of metformin in preclinical models[24–27]. It should be noted that metformin accumulates in multiple tissues, including tumours, to concentrations higher than those in plasma, which highlights uncertainty about the relevant metformin concentrations for in vitro studies[19,24,27,28]. Kinetic and biodistribution studies in humans using ^{11}C-metformin PET–CT

Box 1

Metformin and COVID-19

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread throughout the world. Advanced age, obesity and pre-existing type 2 diabetes mellitus were rapidly identified as risk factors for developing severe COVID-19 (refs. 200–203). This increased risk and increased mortality is believed to be partly linked to chronic low-grade inflammation; that is, inflammaging and/or obesity-associated metaflammation[204]. Observational retrospective studies during the first wave of the pandemic suggested that despite increased incidence of lactic acidosis[205], metformin could have both a protective and a therapeutic role against COVID-19 (refs. 7–9), through its anti-ageing[168], antiviral[206,207] and anti-inflammatory properties[208]. However, the beneficial effects of metformin in patients infected with SARS-CoV-2 could actually mostly result from its efficient glycaemic control in people with diabetes mellitus; diabetes mellitus is one of the most important determinants of survival outcome in severe COVID-19 (ref. 203). The TOGETHER trial investigated whether early treatment with metformin could lower the risk of emergency care and hospitalization among high-risk patients with COVID-19 (ref. 209). However, enrolment in this trial was stopped, as the intermediate assessment indicated that metformin treatment had no effect on the outcome parameters[209]. Similarly, the phase III, double-blind, randomized, placebo-controlled trial COVID-OUT, which was conducted in non-hospitalized adults with obesity or overweight, also found that metformin treatment within 3 days of infection did not prevent the occurrence of hypoxaemia, emergency department visit, hospitalization or death[210]. The lack of clinical benefits observed did not support the repurposing of metformin for early treatment of COVID-19. Whether metformin treatment could eventually reduce the incidence of long COVID, as suggested in a preprint article[211], remains to be confirmed.

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showed that the highest metformin uptake is in the gastrointestinal tract, liver and kidneys[29]. However, ^{11}C-metformin PET–CT lacks the resolution to determine the subcellular distribution of metformin and cannot be used to establish whether metformin accumulates within specific organelles.

In 2019, we extensively reviewed the glucoregulatory mechanisms of metformin action in T2DM in *Nature Reviews Endocrinology*[26]. As such, the objective of this Review is to provide an update of our current understanding of the molecular mechanisms of metformin action. Early studies highlighted the liver as the major site of metformin action for the control of hepatic glucose production, through both AMP-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms. However, there is increasing evidence that other sites of action might also be important, including the gastrointestinal tract, the gut microbiota and the tissue-resident immune cells. In addition, owing to its pleiotropic modes of action, with multiple sites of action and signalling pathways, the repurposing of metformin is being expanded to include various pathophysiological conditions[9,30,31]. In this Review, we mostly focus on selected papers published since our previous Review and discuss the interaction of metformin with novel factors, most of which were revealed by studies in preclinical models. We also examine current knowledge on the immune-modulating and anti-inflammatory effects of metformin that underlie its therapeutic benefits as a potential anti-inflammatory and anti-ageing drug. Finally, we discuss the benefits of repurposing metformin in the treatment of local and systemic inflammation, cancer, age-related diseases and coronavirus disease 2019 (COVID-19).

Paradigm changes in glucoregulatory actions
Metformin target organs
Gastrointestinal system. The antihyperglycaemic effects of metformin are traditionally thought to primarily arise through effects in the liver; however, emerging evidence supports the role of extrahepatic mechanisms[26]. In the past few years, clinical studies conducted in individuals with recent-onset T2DM (duration of T2DM less than 50 months) and in non-diabetic control individuals demonstrated a metformin-associated increase in endogenous glucose production, which indicates that the glucose-lowering action of metformin is not entirely governed by a reduction in hepatic gluconeogenesis[32,33]. Over the past few years, the gastrointestinal tract has been the focus of attention as an additional or even alternative site of metformin action in the management of T2DM; multiple mechanisms, not necessarily mutually exclusive, have been proposed, including direct actions of the drug on intestinal cells or alterations in the composition and metabolic profile of the gut microbiota[26]. Specifically, the role of intestinal AMPK has been highlighted in the glucose-lowering and metabolic actions of metformin in rodents[34–37]. High accumulation of metformin in the gut has been reported with concentrations up to 30–300-fold times greater than in plasma and other tissues, suggesting that the gut acts as an important metformin reservoir in both humans and animal models[19,37–39]. The 'sponge' hypothesis has been proposed to explain the slow and dose-dependent absorption of metformin along the gastrointestinal tract[40]. In humans, metformin enters enterocytes via saturable apical transport but its release through the basolateral membrane is inefficient due to the absence of efflux intestinal transporters, which sequester and concentrate the drug within enterocytes. Hence, metformin in the intestinal lumen might undergo a predominantly saturable paracellular transport across the human intestine to access circulating blood[40,41].

Box 2

Metformin, GDF15 and body weight control

Elevated circulating levels of growth differentiation factor 15 (GDF15), a stress-responsive cytokine of the transforming growth factor-β family, has drawn attention as a novel biomarker for the use of metformin[212,213]. This relationship between GDF15 and metformin might provide new clues about the mechanism of action through which metformin produces its beneficial clinical outcomes. Metformin treatment increases the levels of *Gdf15* mRNA in the intestine, kidney, skeletal muscle and the liver of mice fed a high-fat diet[14,214,215]. However, the role of the AMPK signalling pathway in *Gdf15* gene regulation by metformin remains disputed[13,215]. GDF15 release from the gastrointestinal tract in response to metformin was shown in human studies[57]. Interestingly, increased GDF15 expression and circulating levels have been associated with weight loss and reduced appetite in humans and mice[216]. This peptide hormone functions through its receptor, glial cell-derived neurotrophic factor family receptor-α-like (GFRAL), which is only expressed in the hindbrain, to facilitate weight loss[217,218]. Metformin might promote body weight loss in people with or without type 2 diabetes mellitus (T2DM)[219,220]. For instance, elevated circulating levels of GDF15 are associated with metformin-induced weight loss in patients with T2DM; this effect could be mediated by a reduction in food intake as a result of taking metformin[13,14], independently of its glucose-lowering effect[14,214,221]. Supporting the requirement of the GDF15–GFRAL signalling axis, metformin-induced weight loss was abrogated in both GDF15-knockout and GFRAL-knockout mice[13,14]. Nevertheless, studies have challenged this concept by showing that metformin can lower body weight and food intake independently of GDF15–GFRAL signalling in obese mice[214,222]. In addition, the association between metformin-induced increases in circulating levels of GDF15 and changes in body weight was not confirmed in individuals with prediabetes and overweight following metformin administration for up to 13 weeks[214]. These results question the importance of long-term drug exposure and individual responsiveness to the weight-lowering effects of the metformin-induced rise in GDF15 levels and warrant further clarification[223].

Metformin inhibits the intestinal absorption of dietary glucose in rodents and minipigs[42–44] and patients with T2DM[45]. This finding was confirmed by PET–CT imaging showing accumulation of ^{18}F-labelled fluorodeoxyglucose (FDG), a non-metabolizable glucose analogue, in the intestinal lumen of Goto–Kakizaki diabetic rats treated with a single oral dose of metformin[46]. The inhibition of intestinal glucose absorption resulted from the transient decrease in the abundance of sodium–glucose transporter 1 (SGLT1) at the apical membrane of enterocytes in the jejunum. The reduction in postprandial glucose response mediated by a single administration of metformin was abrogated in mice lacking SGLT1, but not in those lacking glucose transporter 2 (GLUT2)[46]. Interestingly, the glucose-lowering efficacy after a single administration of metformin into the jejunum of minipigs was combined with a reduction in intestinal glucose absorption and

an increase in glucagon-like peptide 1 (GLP1) release, suggesting that delayed intestinal glucose absorption and the exposure of glucose to more distal regions of the intestine is sufficient to stimulate GLP1 secretion following oral intake of glucose[46]. In line with this observation, a single administration of metformin into the proximal and distal small intestine markedly attenuated the glycaemic response to oral glucose in patients with T2DM, concomitantly with enhanced GLP1 secretion[47].

Based on observations in patients with T2DM receiving metformin, PET–CT imaging after intravenous administration of ^{18}F-FDG, which enters enterocytes via basolateral GLUT2, showed that increased glucose uptake from the circulation into the gastrointestinal system contributes to the glucose-lowering effect of the drug and the improvement in glycaemic control[37,48,49]. Similarly, ^{18}F-FDG PET–CT imaging in mice fed a high-fat diet (HFD) confirmed that metformin-induced basolateral intestinal glucose uptake is accompanied by an improvement in glucose tolerance, which occurs in a dose-dependent manner[37]. Taking advantage of newly developed ^{18}F-FDG PET–MRI techniques, a dose-dependent metformin-induced accumulation of ^{18}F-FDG was demonstrated in both the intestinal wall and the luminal space of the ileum and the colon of participants with T2DM receiving metformin[50,51], which suggests that metformin also promotes the release of glucose from enterocytes into the intraluminal space. In addition, after acute oral administration of ^{18}F-FDG and metformin in HFD-fed mice, ^{18}F-FDG PET showed reduced transepithelial glucose transport from the proximal small intestine lumen into the circulation[52].

Altogether, these studies suggest that intestinal glucose uptake along the gastrointestinal tract from both the bloodstream and the intestinal lumen is critical to the glucose-lowering capacity of metformin. Therefore, the gut might predominantly act as a glucose sink through the uptake of glucose by enterocytes in response to metformin action. In this context, the contribution of GLUT2 expression at the basolateral surface and at the apical surface of enterocytes that is triggered by metformin[53], possibly via an AMPK-dependent mechanism[37], is consistent with the modified glycaemic response to metformin of patients expressing a particular *GLUT2* variant[54]. Similarly, metformin-induced GLUT1 expression in the colon and ileum, secondary to increased expression of activating transcription factor 4, might also contribute to the metformin-mediated basolateral intestinal glucose uptake in HFD-fed mice[37]. After uptake into enterocytes, glucose anaerobic metabolism resulted in lactate and acetate accumulation in the wall of the small intestine and release into the circulation; this finding is supported by preclinical[37,55,56] (note that ref. 56 is a preprint and has not yet been peer-reviewed) and clinical studies[57]. Intestinal lactate and acetate production establishes a gut–liver crosstalk to blunt hepatic glucose production, possibly through reduction in the activity of the hepatic pyruvate carboxylase by reducing pH in the portal vein (a consequence of increased lactate) and of the hepatic mitochondrial pyruvate carriers 1 and 2 by acetylation (a consequence of increased acetate)[37]. Intestinal lactate production might also participate in an intestinal–liver futile cycle that results in increased energy expenditure during long-term treatment with metformin, which has been reported in HFD-fed mice[58].

Brown adipose tissue. Brown adipose tissue (BAT) is a highly metabolically active organ and is well-recognized for its thermogenic role by dissipating energy to heat. However, a growing number of studies have shown that BAT contributes to the regulation of whole-body glucose homeostasis[59,60] and it has been suggested that BAT could be a therapeutic target in the treatment and prevention of T2DM. Using ^{11}C-metformin PET imaging, uptake of metformin was demonstrated in the interscapular BAT depot of mice[61], which supports the suggestion that BAT could be a metformin target (Box 3).

Metformin target organelles

Since the early 2000s, mitochondria have been considered the classic target organelles for the glucose-lowering actions of metformin, based on matrix enrichment and the specific, mild and reversible inhibition of the mitochondrial respiratory chain complex I[26]. Remarkably, a study published in 2023 combining cryo-electron microscopy and enzyme kinetics identified three possible independent binding or interaction

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Box 3

Targeting brown adipose tissue function

Analysis of metformin biodistribution in mice revealed uptake and accumulation in the interscapular brown adipose tissue (BAT) depot through OCT3-mediated uptake, to levels similar to those in the kidney and intestine[61]. Metformin treatment increased the expression of cellular proliferation and differentiation markers in brown adipocytes, alongside increased BAT mass[224]. Metformin increased the expression of the transcriptional co-regulator PR domain containing 16, a brown adipocyte differentiation marker, and rescued BAT mass and functionality in offspring of obese female mice[225]. Metformin is recognized as directly targeting brown adipocyte metabolism, contributing to a metformin-induced improvement in the blood lipid profile through increasing VLDL triglyceride clearance in mice[226]. By increasing hormone-sensitive lipase and AMP-activated protein kinase (AMPK) activities, metformin promotes intracellular triglyceride lipolysis and mitochondrial fatty acid oxidation in BAT. Metformin also upregulates enzymes involved in fatty acid oxidation and increases expression of genes related to adaptive thermogenesis in BAT in rodents[224,227], but has a limited effect on energy expenditure in most human and animal studies[227–229]. Interestingly, findings have emphasized the importance of an intestinal AMPK–gut microbiota–BAT axis for metformin regulation of BAT function[26]. Evidence supports the suggestion that AMPK in the intestine is required for metformin action on BAT thermogenic activity by modulating the gut microbiota and decreasing circulating levels of the bacterial metabolite methylglyoxal in mice[35]. The therapeutic benefit of metformin has also been examined in the context of BAT meta-inflammation, highlighting its anti-inflammatory effects via the regulation of the interactome between macrophages and brown adipocytes in mice[230]. Metformin alleviated inflammation by promoting HIF1α degradation as a result of a metformin-induced reduction in oxygen consumption in macrophages in BAT of mice. Metformin attenuated macrophage-mediated pro-inflammatory signalling cascades in brown adipocytes and restored the responsiveness of BAT to cold exposure in obese mice[230]. These findings illustrate the protective effects of metformin on BAT function and might be of therapeutic relevance to alleviate BAT dysfunction in metabolic disorders.

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sites for biguanides on various complex I protein subunits[62]. The major inhibitory site is located in the amphipathic region of the quinone-binding channel (Q-channel), adjacent to a mobile structural element in the NDUFS7 subunit; when a biguanide binds to this site, reactivation of the enzymatic deactivated state is prevented[62], as previously reported[63]. These structural data obtained using a synthetic biguanide-like molecule supports a specific effect on complex I. Of note, it is worth mentioning that inhibition of mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) and mitochondrial respiratory chain complex IV have also been suggested as alternative mechanisms of metformin action, according to studies performed in rodents[64,65]. However, the arguments supporting a role for these other putative mitochondrial sites of action of metformin remain highly debated[66–69]. Finally, the identification of metformin-binding proteins associated with isolated lysosomes in human and mouse cells highlights lysosomes as an alternative or additional functional target of metformin[36].

AMPK-dependent and AMPK-independent mechanisms

Initially, it was thought that metformin acts principally through the activation of the LKB1–AMPK signalling pathway[70,71]. Metformin-induced AMPK activation can occur through AMP-dependent and AMP-independent AMPK-activation pathways that are correlated with the concentrations of metformin used and the target organelles (mitochondria or lysosome)[36,72,73]. However, AMPK-independent mechanisms of metformin action have also been documented extensively[26,74,75]. Of note, the acute glucose-lowering effect of metformin is preserved in liver-specific, intestine-specific and skeletal muscle-specific AMPK-knockout mouse models, which indicates that at least some direct effects of metformin are AMPK-independent[56,76,77]. By contrast, intestinal AMPK is required for the therapeutic effects of chronic metformin administration in HFD-fed mice[35,36]. Even though metformin-induced AMPK activation does not directly inhibit hepatic gluconeogenesis[36,76], the effect of chronic metformin administration might indirectly improve the ability of insulin to lower hepatic glucose production as a result of an AMPK-dependent reduction in hepatic lipid-induced insulin resistance. Supporting this suggestion, impairments in the glucose-lowering and lipid-lowering effects of chronic metformin administration in mice have been associated with compromised AMPK signalling[36,78]. Lastly, to determine the phosphorylation events induced by metformin independently of the LKB1–AMPK signalling pathway, a quantitative proteomic approach based on selective enrichment of the phospho-scaffolding protein 14-3-3ζ interactors was conducted in liver-specific LKB1-knockout and AMPK-knockout mice in response to acute metformin administration[79]. Roughly half of the phosphorylation events were independent of LKB1–AMPK signalling, indicating that other stress kinases acutely activated by metformin might participate in the pleiotropic action of the drug[79].

New glucose-lowering mechanisms of metformin

The antidiabetic effect of metformin is mainly mediated through inhibition of hepatic gluconeogenesis[80]; however, emerging evidence suggests that the gastrointestinal tract also has a role in the glucose-lowering action of metformin. It is widely accepted that metformin targets hepatic mitochondria and inhibits mitochondrial respiratory chain complex I; the inhibition is reversible and weak[26,81]. This inhibition leads to a moderate decrease in ATP synthesis by mitochondrial oxidative phosphorylation (OXPHOS) and an increase in the AMP to ATP ratio in hepatocytes. Consequently, because gluconeogenesis is a costly ATP-dependent metabolic pathway, the reduction in cellular

energy charge could be sufficient to account for the decrease in hepatic gluconeogenic flux[76,82]. Furthermore, the metformin-induced mild increase in intracellular levels of AMP leads to inhibition of AMP-regulated enzymes involved in hepatic gluconeogenesis (such as fructose-1,6-bisphosphatase and adenylate cyclase), which contributes to a decrease in hepatic glucose production and activation of the cellular energy sensor AMPK; however, AMPK activation has no direct effect on glucose production[26,74–76] (Fig. 1). The physiological relevance of these mechanisms has been questioned due to the use of suprapharmacological (millimolar) concentrations of metformin in the studies[25,80]. Indeed, studies showed that clinically relevant (micromolar) metformin concentrations suppress glucose production in primary mouse hepatocytes via mechanisms independent of apparent changes in adenine nucleotide levels[36,64,83,84].

Lysosomal PEN2–ATP6AP1 axis

It has been reported that, in primary mouse hepatocytes, low concentrations of metformin activate AMPK in lysosomes through an AMP-independent mechanism involving the recruitment of a complex composed of AXIN and the upstream kinase LKB1 to the surface of lysosomes via docking onto the vacuolar H⁺-ATPase (v-ATPase)–Ragulator complex[72,85]. In this model, low-dose metformin inhibited v-ATPase in lysosomes, which acts as a sensor of low metformin concentrations. In a study published in 2022, the membrane protein presenilin enhancer 2 (PEN2; a subunit of γ-secretase complex) was identified as a partner of metformin[36]. Notably, metformin at concentrations as low as 5 μM could trigger robust activation of AMPK in both primary mouse and human hepatocytes after only 2 h of treatment, which contrasts with other reports that concentrations <40 μM failed to induce AMPK phosphorylation after a 24-h incubation period[84] (Foretz, M., unpublished work). In that 2022 study[36], primary hepatocytes were treated with concentrations of metformin found in the bloodstream (5–10 μM) rather than those measured in the liver (100–200 μM)[19,64], calling into question whether a PEN2-dependent mechanism of action is relevant in in vivo settings.

At the molecular level, the phenylalanine-35 (F35), glutamate-40 (E40) and tyrosine-47 (Y47) residues of PEN2 are critical for binding to metformin, as these residues interact with the biguanide group of the molecule. Metformin-bound PEN2 is then recruited to ATP6AP1, an accessory protein of v-ATPase, leading to the inhibition of v-ATPase and the activation of AMPK at the lysosome surface without altering cellular levels of AMP[36] (Fig. 1). Of note, high-dose metformin (>100 μM) bypassed the requirement of PEN2–ATP6AP1 signalling for AMPK activation at the lysosome surface, because high doses of metformin increase the intracellular levels of AMP[36,76]. In terms of the physiological response, intestine-specific deletion of PEN2 in mice impaired improvements in glucose tolerance associated with GLP1 secretion in response to metformin[36]. However, it remains unclear whether these effects are mediated by PEN2–AMPK signalling in enterocytes that are exposed to metformin concentrations much higher than 5 μM[19,36]. Furthermore, PEN2 ablation in the liver abolished the metformin-induced reduction in hepatic lipid content in HFD-fed mice, supporting the suggestion that metformin might indirectly suppress gluconeogenesis in the long term through an AMPK-dependent reduction in lipid-induced insulin resistance[36,76,78]. However, the relevance of this mechanism in humans is questionable as metformin has no substantial efficacy in the treatment of fatty liver in patients with non-alcoholic fatty liver disease[86,87].

Interestingly, in response to low concentrations of metformin, the PEN2–ATP6AP1 axis leads to activation of the AMPK pool in lysosomes
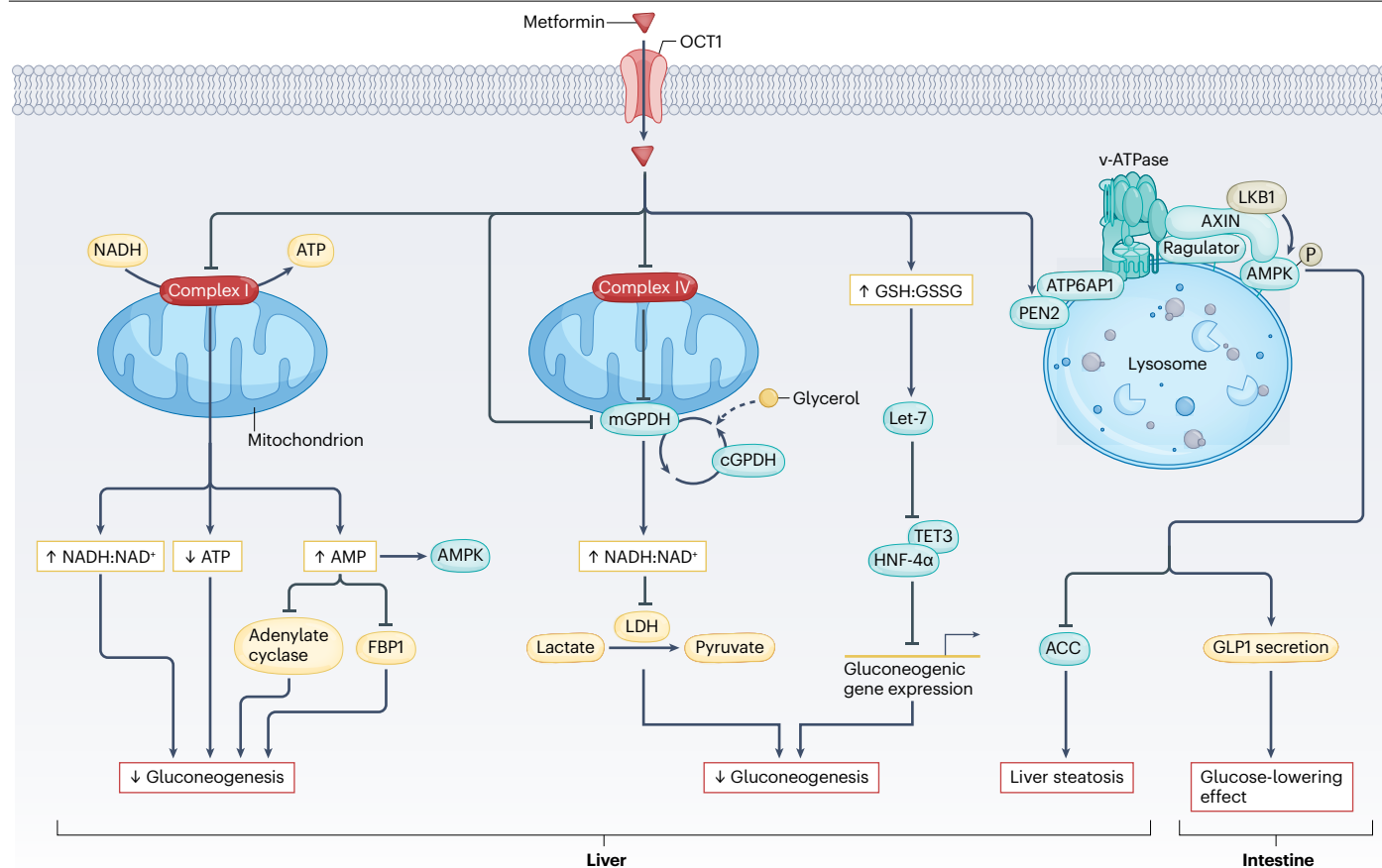
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Fig. 1 | Proposed mechanisms for metformin-induced reductions in blood levels of glucose. Left: Complex I inhibition-dependent mechanisms. In the liver, metformin induces a mild inhibition of mitochondrial respiratory chain complex I, leading to a moderate decrease in ATP synthesis and a concomitant increase in cellular levels of AMP. The metformin-induced decrease in hepatic gluconeogenic flux, an ATP-dependent metabolic process, could result from this reduction in ATP levels. In addition, increased AMP levels lead to inhibition of the activity of enzymes that are regulated by AMP and are involved in gluconeogenesis, such as adenylate cyclase and fructose-1-6-bisphosphatase (FBP1), which contributes to decreased glucose output. Of note, the metformin-induced increase in the AMP to ATP ratio also activates AMP-activated protein kinase (AMPK), but this has no direct effect on the regulation of glucose production. The inhibition of complex I by metformin is also accompanied by an increase in cellular redox potential (NADH:NAD⁺). Middle: Mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH)-dependent and complex IV inhibition-dependent mechanisms. Metformin directly inhibits mGPDH, resulting in an increased cytosolic redox state (NADH:NAD⁺), reduced gluconeogenesis from lactate and reduced activity of the glycerol–phosphate shuttle (which transfers NADH from the cytosol to mitochondria). In addition, metformin raises the hepatic redox state through an increase in the glutathione to oxidized glutathione ratio (GSH:GSSG), leading to inhibition of genes encoding enzymes involved in gluconeogenesis through a let-7–TET3–HNF-4α pathway. Finally, metformin inhibits mitochondrial respiratory chain complex IV, which can also result in an indirect inhibition of mGPDH activity. Right: AMPK activation-dependent mechanisms in lysosomes. Metformin at low concentrations binds presenilin enhancer 2 (PEN2), which is recruited to ATPase H⁺ transporting accessory protein 1 (ATP6AP1) independent of changes in AMP levels, leading to inhibition of v-ATPase and phosphorylation and/or activation of AMPK in lysosomes through the formation of a supercomplex containing the v-ATPase, Ragulator, AXIN, liver kinase B1 (LKB1) and AMPK. Thereafter, metformin-activated AMPK from lysosomes reduces lipid accumulation in the liver via acetyl-CoA carboxylase (ACC) inhibition and increases glucagon-like peptide 1 (GLP1) secretion in the gut, inducing reductions in blood levels of glucose. cGPDH, cytosolic glycerol-3-phosphate dehydrogenase; HNF-4α, hepatocyte nuclear factor 4α; LDH, lactate dehydrogenase; OCT1, organic transporter 1; TET3, Tet methylcytosine dioxygenase 3.

in primary hepatocytes without affecting other AMPK pools, such as those of the endoplasmic reticulum (ER) and mitochondria[36]. Of note, it is unclear how AMPK anchored at the lysosome surface can phosphorylate its lipogenic target acetyl-CoA carboxylase and inhibit lipogenesis, which takes place in the cytoplasm and ER, to ultimately reduce hepatic lipid content. Lastly, knockdown of PEN2 or ATP6AP1 in *Caenorhabditis elegans* abrogated the metformin-induced extension of lifespan[36]. However, these experiments were conducted using extremely high concentrations of metformin (50 mM) that are 10,000 times higher than those used in vitro (5 μM)[36]. Additionally, the PEN2 residues involved in metformin binding in mice and humans (F35, E40 and Y47) are not conserved in *C. elegans* (F35, D40 and N47)[88], which questions the real contribution of PEN2 in this metformin effect. The lysosomal metformin–PEN2 model raises other essential questions about the mechanism of action of metformin. Notably, this model suggests that the glucose-lowering effects of metformin occur through

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AMPK in the intestine but does not explain how low concentrations of metformin directly inhibit acute hepatic glucose production, which is clearly AMPK-independent[64,76,89,90].

Mitochondrial glycerol-3-phosphate dehydrogenase

Other mechanisms involving changes in hepatic redox state, but independent of changes in adenine nucleotide levels and AMPK activation, have been proposed to explain the inhibition of hepatic glucose production in response to low concentrations of metformin. The cytosolic reducing equivalents (NADH) produced by intermediate metabolism are transferred from the cytosol to mitochondria through NADH shuttle systems to be oxidized by the mitochondrial electron transport chain (ETC) to generate ATP. The malate–aspartate shuttle and the glycerol–phosphate shuttle are the two major redox shuttle systems maintaining the redox balance between cytosolic and mitochondrial compartments. Clinically relevant concentrations of metformin increase the hepatic cytosolic NADH to NAD^+ ratio (lactate to pyruvate ratio) independently of changes in intracellular levels of ATP, which results in inhibition of glucose production from reduced gluconeogenic substrates (lactate and glycerol) but not from oxidized substrates (alanine and pyruvate)[64,91]. The increase in cytosolic redox state induced by metformin was postulated to be mediated by direct inhibition of mGPDH activity (Fig. 1). mGPDH is located on the outer side of the inner membrane and, with its cytosolic partner cGPDH, constitutes a glycerol–phosphate shuttle.

However, this model of metformin-induced mGPDH inhibition raises some issues. The malate–aspartate shuttle is the main NADH shuttle in the liver and inhibition of the glycerol–phosphate shuttle might be insufficient to reduce gluconeogenesis[92]. Indeed, mice lacking the glycerol–phosphate shuttle display normal fasting blood levels of glucose, whereas mice lacking the malate–aspartate shuttle have reduced blood levels of glucose and an increased cytosolic NADH to NAD^+ ratio[93]. Although many studies have demonstrated that low doses of metformin cause an increase in the cytosolic NADH to NAD^+ ratio, several studies did not find a decrease in glucose production from lactate or a direct inhibition of mGPDH activity in response to metformin[66,83,94]. As such, a body of evidence questions whether mGPDH is a direct molecular target for metformin[68,81,95].

Mitochondrial respiratory chain complex IV

In an attempt to address the controversies described in the previous sections, an alternative interpretation was proposed in 2022, in which clinically relevant concentrations of metformin inhibit complex IV activity, resulting in inhibition of mGPDH activity, an increased cytosolic redox state and a reduction in hepatic gluconeogenesis[65]. In this newly proposed mechanism, it has been postulated that the inhibition of complex IV by metformin blocks the ETC, leading to indirect inhibition of mGPDH activity through a decrease in the ubiquinone pool, which is the electron acceptor of mGPDH (Fig. 1). The interaction between metformin and complex IV could be driven by the ability of biguanides to bind metal ions, such as iron and copper[96], which are both present in complex IV. However, all complexes of the ETC contain iron and/or copper ions, which are essential for the transfer of electrons, therefore making it unlikely that metformin specifically targets complex IV in this manner. Altogether, the fundamental concern with the hypothesis of metformin-induced complex IV inhibition is that it postulates an increase in the redox potential without impaired cellular energy charge, which goes against the OXPHOS bioenergetic process. Indeed, disruption of the ETC through the inhibition of complex IV by

metformin would presumably, like inhibition of complex I, impact redox potential-dependent ATP synthesis, leading to a decrease in cellular levels of ATP. Of note, early studies that suggested that metformin induces complex I inhibition simultaneously showed an increase in the AMP to ATP and the NADH to NAD^+ ratios[82,97].

It is worth noting that the concept of linking complex I inhibition by metformin to suppression of energy-demanding gluconeogenic flux has been discredited because low concentrations of the drug repress glucose production in hepatocytes independently of any detectable changes in the AMP to ATP ratio[64,83,84]. However, the measurement of small and transient physiological changes in adenine nucleotide levels might sometimes be impeded by technical limitations. Notably, the use of AMPK as a sensitive probe for assessing subtle changes in the AMP to ATP ratio revealed metformin-dependent changes in cellular energy charge that are not detectable with other methods[98]. Thus, a reduction in energy charge dependent on complex I inhibition cannot be ruled out as a cause of the inhibition of hepatic gluconeogenesis in response to low concentrations of metformin.

Let-7 microRNA

In line with the redox effects of metformin, a study suggested that clinically relevant concentrations of metformin could inhibit hepatic gluconeogenesis through redox-dependent transcriptional regulation[99]. Mechanistically, metformin induces let-7 microRNA expression in a redox-dependent manner through an increase in the reduced glutathione to oxidized glutathione (GSH to GSSG) ratio. In turn, let-7 downregulates TET3 and changes in the ratio of HNF-4α isoforms, leading to inhibition of the expression of genes encoding enzymes involved in gluconeogenesis (Fig. 1). However, this mechanism cannot explain the acute reduction in gluconeogenic flux induced by metformin, in which the drug can inhibit hepatic glucose production in the absence of transcriptional changes in the gluconeogenic programme[76].

Metformin and microbiota interactions
Modulation of microbial communities

In the past few years, the relationship between metformin and the gut microbiota has attracted much attention[26]. Metagenomics studies indicate that metformin induces alterations in the overall structure and functions of gut microbial communities, leading to amelioration of dysbiosis associated with T2DM and subsequently of host metabolism[100–107]. Of note, the effect of metformin on the gut microbiota in people with T2DM could differ with ethnicity and shows high variability[102]. Nevertheless, a signature of metformin treatment across different ethnicities has been established, with the enrichment of two operational taxonomic units from *Bacteroides* and reduced abundance of one operational taxonomic unit from *Faecalibacterium*[108]. In healthy people, metformin also affects the composition of the gut microbiota, validating the concept that the interaction between metformin and the gut microbiota is independent of the dysbiosis induced by T2DM or prevailing blood levels of glucose[109,110]. The gut microbial signature following metformin treatment is associated with increased levels of *Escherichia* spp. and decreased levels of *Intestinibacter* spp. in both healthy normoglycaemic people and those with T2DM[104,106,110]. In addition to its interactions with intestinal microbial communities, metformin treatment in patients with T2DM and periodontitis was associated with changes in oral microbiota composition[111]. However, it remains unclear whether the oral microbiota is directly targeted by metformin or by the influence of systemic glycaemic changes on the oral environment.

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Similarly, alterations in the gut microbiota related to metformin treatment were reported in rats and mice fed a regular diet[112] or a HFD[113–115], as well as in various animal models of obesity and diabetes mellitus, in parallel to improvements in glucose tolerance[107]. Of interest, sex-related differences were reported for metformin-induced changes in gut microbiota composition and function in HFD-fed mice, indicating the relevance of interactions between sex hormones and the microbiota[116]. Importantly, metformin-induced changes in the diversity of the gut microbiota are linked to oral, but not intraperitoneal, metformin delivery in mice[117]. Metformin-induced changes in gut microbiota diversity of HFD-fed mice were associated with reduced microbiota encroachment into the mucus layer, which is a driver of low-grade inflammation[118]. Improvements in the metabolic parameters in HFD-fed mice recipients of faecal microbiota transplants from metformin-treated donors have given support to the overall beneficial metabolic effect of metformin-induced changes in gut microbiota[104,114]. In addition, depletion of the gut microbiota by antibiotic treatment abrogated the glucose-lowering action of metformin in HFD-fed mice[113]. However, some studies demonstrated the ability of metformin to reduce glucose levels in HFD-fed mice under antibiotic treatment[118], making the contribution of gut microbiota to the glucose-lowering action of metformin still a matter of debate[119].

Most of the clinical evidence concerning the action of metformin on the microbiota was established from the observed changes in the faecal microbiota that relate mainly to the large intestine, which is where the highest concentrations of metformin have been reported[19,37,38]. However, metformin can also accumulate and reach high concentrations in the small intestine[19,39], triggering specific changes in the local microbiota of the duodenum, jejunum and ileum in rodents[114,120]. Alteration of the microbiota composition in the small intestine is accompanied by modifications in the expression of genes related to intestinal glucose and fatty acid uptake that contribute to the metabolic effects of metformin[114,120,121]. The distinctive role of the different microbiotas along the gastrointestinal tract was demonstrated by metformin-treated microbiota transplants to the upper small intestine that resulted in the restoration of glucose-sensing mechanisms[114]. In addition, the glucose-lowering efficacy of a delayed-release formulation of metformin that was delivered to the distal small intestine also favours compartmentalized gut-based mechanisms of metformin action in humans[122].

The influence of metformin on gut microbiota composition can result from direct effects on bacterial growth and changes in the intestinal environment. Metformin treatment alters gut microbiota composition, which is dependent on AMPK in the intestine and probably results from the modulation of expression of antimicrobial peptides, including regenerating islet-derived protein 3γ[35]. In addition, accumulating evidence shows that metformin enhances the protection of the intestinal mucosal barrier function by increasing the relative abundance of *Akkermansia muciniphila* and the number of goblet cells, leading to thickening of the mucus layer[113,123–125]. Furthermore, metformin also promotes the expression of the tight-junction protein occludin[113,124–126], which reduces the translocation of lipopolysaccharide, colonic inflammation and dysfunctional gut permeability. Using in vitro gut microbiota culture models, metformin was shown to directly alter gut microbiota growth curves and compositional profiles[104,127,128]. By combining data from metatranscriptomics and metaproteomics analyses, metformin–microbiota interactions were found to be associated with changes in microbiota functional activities linked to the modification of multiple metabolic pathways[104–106,127,128],

including the production of short-chain fatty acids (SCFAs) and glucose metabolism (glycolysis, aerobic oxidation and pentose phosphate pathways). Metformin use was linked to the enrichment of bacterial genes with products involved in carbohydrate, lipid, amino acid and nitrogen metabolism, drug resistance and lipopolysaccharide biosynthesis[104,105,127]. By selectively altering the metabolic profile of the human gut microbiota, metformin provides a competitive advantage and promotes specific bacterial populations, including *Escherichia coli* and *A. muciniphila*[102–105,127,129].

Metformin–microbiota relationship

Previous studies have shown that metformin impairs folate and methionine production in the microbiota of the nematode *C. elegans* (using worms fed with live *E. coli*), leading to altered host methionine metabolism and healthspan extension[130]. In subsequent studies, a high-throughput host–microbiota–drug–nutrient screen approach identified a bacterial signalling pathway that integrates metformin and nutrient signals to alter metabolite production by the microbiota[131]. In support of metformin–microbiota interactions, an in silico human microbiota metabolic modelling study showed a predicted increase in agmatine production capacity by *E. coli* in metformin-treated patients with T2DM[131]. In addition, using systems biology methodologies, a competing behaviour of prevalent gut microbiota from metformin-treated patients with T2DM was linked to their capacity to utilize and produce SCFAs and amino acids[129]. Similarly, using global metabolomic approaches, the metabolism of branched-chain amino acids was correlated to changes in the gut microbiota and the hypoglycaemic effect associated with metformin administration in healthy individuals[132].

The crosstalk between metformin and gut microbiota metabolism was further emphasized by the causal relationship reported between the long-term durability of the glycaemic response to metformin monotherapy in T2DM and different gut microbiota compositions that were characterized by unique microbial metabolic pathways[133]. For instance, pathways involved in thiamine biosynthesis could be involved in ensuring the sustained durability of the glycaemic response to metformin monotherapy[133]. Another well-documented metformin–gut microbiota interaction is the shift towards SCFA-producing bacteria and increased faecal concentrations of both butyrate and propionate in patients with T2DM and obesity[102–104,106]. SCFAs exhibit beneficial effects on glucose metabolism via multiple pathways, including stimulation of the release of the incretin hormone GLP1 and peptide YY from enteroendocrine L cells and modulation of intestinal gluconeogenesis, which enhances glucose management[134,135]. Metformin use is also associated with modulation of the bile acid pool by decreasing the abundance of *Bacteroides fragilis* and its bile salt hydrolase activity[121]. This effect is accompanied by increased levels of the bile acid glycoursodeoxycholic acid, which improves glucose metabolism homeostasis through inhibition of intestinal farnesoid X receptor signalling via an AMPK-independent mechanism that is associated with increased GLP1 levels[121,136]. These findings are consistent with the therapeutic effect of metformin-induced selective alterations in intestinal microbial composition and metabolism on host targets, which improve host metabolism[102,104,134,137].

Dysbiosis is associated with pathologies (including cancer and cardiovascular, metabolic and inflammatory bowel diseases). Thus, therapeutic strategies could be developed that are based on metformin-induced modulation of the microbiome and/or metabolite production independently of the antidiabetic effect of metformin. Metformin reduces circulating levels of trimethylamine *N*-oxide, a microbiota–host co-metabolite synthesized from choline that is

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associated with an increased risk of cardiovascular disease in mice fed a high-choline diet[138,139]. In addition, metformin treatment is correlated with the remodelling of the gut microbiota, with increased relative abundance of *Bifidobacterium* and *Akkermansia* and decreased abundance of *cutC* (which encodes a protein involved in choline metabolism in bacteria)[138]. In addition, metformin has shown potential as an intervention for preventing cognitive decline related to dysbiosis caused by obesity and ageing in mice[125,140,141].

Metformin-induced alterations in the gut microbiota also contribute to the antitumour effects of metformin. Orally ingested metformin, but not intraperitoneally injected metformin, suppresses tumour growth in HFD-fed mice[117]. Furthermore, faecal transplants of the microbiota from metformin-treated donor mice were sufficient to recapitulate the metformin-induced reduction in tumour growth and were associated with increased abundance of SCFA-producing bacteria and reduced expression of genes with protein products critical for regulating cholesterol synthesis in the tumour[117]. In addition, in a mouse model of colorectal cancer induced by *Fusobacterium nucleatum*, which is enriched in colorectal carcinogenesis, metformin decreases tumour size and the abundance of *F. nucleatum* in tumour tissue[142]. Additionally, the use of metformin in the treatment of inflammatory bowel disease demonstrated positive effects through its action on inflammatory pathways, intestinal barrier integrity and gut microbiota[143]. Metformin increased the relative abundance of *Lactobacillus* and *Akkermansia* species and alleviated gut dysbiosis, colonic inflammation and mucus barrier disruption induced by experimental colitis[123,144].

Available data suggest that metformin and the gut microbiota work synergistically to produce beneficial therapeutic effects. Furthermore, adding gut microbiota modulators, such as probiotics or prebiotics, to metformin therapy results in improved T2DM outcomes in animal and human studies[145]. However, the metabolites produced by the gut microbiota can also influence the efficacy of metformin treatment and contribute to the interindividual variability in response to the drug. For example, metabolomic analysis of plasma from people treated with metformin who had high blood levels of glucose and from those with low blood levels of glucose identified a microbial metabolite, imidazole propionate, as a negative regulator of metformin action in humans[146]. Imidazole propionate reduced the acute glucose-lowering effect of metformin in mice fed a western diet by inducing inhibition of AMPK signalling in the liver through a p38γ-dependent mechanism[146]. Interestingly, inhibition of p38γ prevented the inhibitory effect of imidazole propionate on acute metformin action, highlighting potential therapeutic opportunities in patients with T2DM who are not fully responsive to metformin[146].

Metformin intolerance
The gut microbiota is increasingly recognized as a potential mediator of the gastrointestinal adverse effects of metformin[147]. Prevalent gastrointestinal adverse effects after metformin intake have been attributed to gases (such as CO_2 and H_2S) produced by the gut microbiota, seemingly through metformin-induced metabolic modelling of *Escherichia* spp. and *A. muciniphila*[102,110,129]. A study comparing the gut microbiota profile in metformin-tolerant and metformin-intolerant patients with T2DM established that a shift in the composition of the gut microbiota on the introduction of metformin is responsible for the drug intolerance[148]. The use of microbiota modulators, including probiotic, prebiotic and symbiotic supplementation, might be a viable approach to achieving improved tolerability of metformin[145]. Altogether, metformin-induced changes in individual gut microbiotas and

a subsequent shift in bacterial metabolite production could contribute to its therapeutic efficacy, but also to its adverse gastrointestinal effects in the host[102,104,109,128,131,149].

Metformin and the immune system
Dampening of (meta)inflammation
The immune system has a central role in the initiation and progression of many pathologies. For cardiometabolic diseases, the contribution of chronic low-grade inflammation to metabolic dysfunction and the development of atherosclerosis is now well documented[150,151]. Obesity induces various degrees of inflammation in adipose tissues, pancreatic islets and the liver, which contributes to hepatic steatosis, systemic insulin resistance and progression towards T2DM, non-alcoholic steatohepatitis (NASH) and cardiovascular diseases[152–154]. This so-called metaflammation also predisposes the individual to co-morbidities, such as *Mycobacterium tuberculosis* infection[155] or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (Box 1). Although incompletely understood, the crosstalk between parenchymal and tissue-resident immune cells within local niches in metabolic organs is central in these inflammatory processes[153,156]. Remarkably, metformin had some anti-inflammatory effects through both AMPK-dependent and AMPK-independent mechanisms in cross-sectional studies in patients with T2DM, various interventional studies in rodent models of obesity and T2DM, and in in vitro and ex vivo experiments in several immune cell types[26,157] (Fig. 2a).

Metformin treatment also improves mitochondrial functions in peripheral blood mononuclear cells (PBMCs) from patients with T2DM, which is associated with increased AMPK phosphorylation and mitophagy and reduced levels of reactive oxygen species (ROS) and serum levels of the pro-inflammatory cytokines TNF and IL-6 (refs. 158,159). In addition, in a placebo-controlled trial in patients with prediabetes, treatment with metformin reduced the concentrations of the neutrophil-derived extracellular trap (NET) components elastase, proteinase 3, histones and double-strand DNA, independently of its effect on normalizing glucose levels[160]. This finding suggests that metformin can dampen NETosis in activated neutrophils, which is important for host defence against pathogens and is involved in inflammatory-mediated tissue damage and thrombosis. Metformin also induces a dose-dependent inhibition of NETosis induced by phorbol 12-myristate 13-acetate and ionomycin in vitro at concentrations within the therapeutic range[160]. This effect was associated with reduced PKC-βII membrane translocation, which is consistent with a direct effect of the drug on the NETosis machinery[161]. However, further studies are required to decipher the exact mechanism by which neutrophil functions are regulated, directly or indirectly, by metformin.

Changes in intrinsic metabolism of tissue-resident and newly recruited macrophages resulting from alterations in the local microenvironment within metabolic organs are thought to shape their functions and be among the main drivers of obesity-associated metaflammation[162]. Interestingly, metformin reduces pro-inflammatory activation of macrophages in vitro by interfering with the cellular metabolic reprogramming that underpins inflammation[163]. At the molecular level, although a contribution of AMPK activation could not be excluded, metformin reduced fatty acid synthesis mediated by fatty acid synthase (FASN) and suppressed FASN-dependent palmitoylation of AKT in lipopolysaccharide-stimulated mouse bone marrow-derived macrophages, leading to reduced NF-κB activation and reduced expression of pro-inflammatory cytokines[163] (Fig. 2a). A novel pathway by which metformin could dampen inflammation independently of
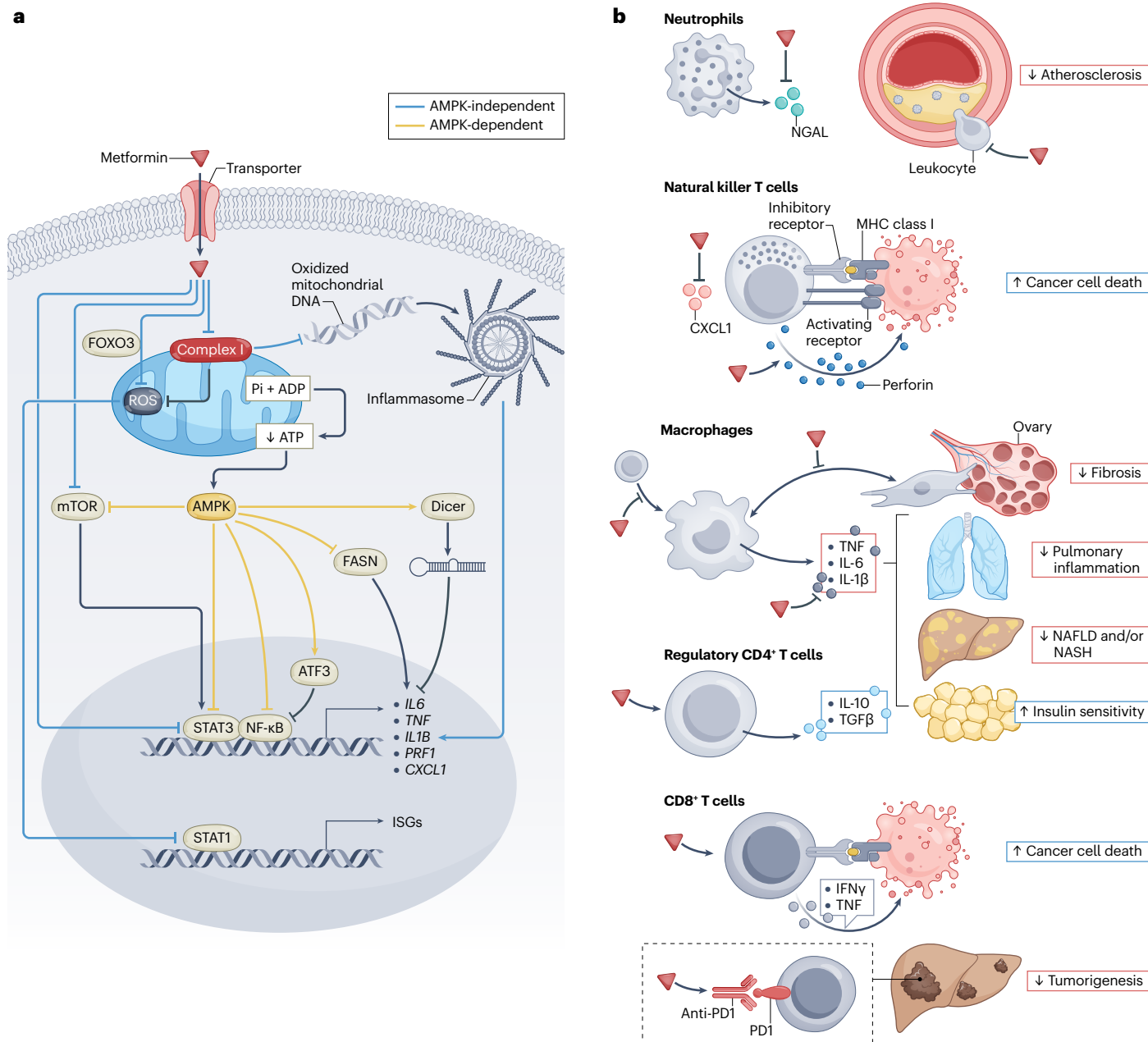
Fig. 2 | Anti-inflammatory and immunomodulatory effects of metformin.
a, Following putative transporter-mediated internalization in various immune cell subsets, metformin inhibits the mitochondrial respiratory chain complex I and can modulate cell-specific inflammatory processes by both AMP-activated protein kinase (AMPK)-independent and AMPK-dependent mechanisms.
b, Metformin can modulate the immune system, which could have beneficial effects in various pathological conditions (such as certain cancers, infections and hyperinflammatory diseases). These effects have been reported to involve various innate and adaptive immune cells, leading to modulation of several cell–cell interactions in local niches, as well as in immunometabolic and cellular processes. ATF3, activating transcription factor 3; CXCL1, chemokine (C-X-C motif) ligand 1; FASN, fatty acid synthase; FOXO3, forkhead box O3; IFNγ, interferon-γ; ISGs, interferon-stimulated genes; mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor-κB; NGAL, neutrophil gelatinase-associated lipocalin; PD1, programmed cell death protein 1; Pi, inorganic phosphate; PRF1, perforin 1; ROS, reactive oxygen species; STAT1 and STAT3, signal transducer and activator of transcription 1 and 3; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor.

the AMPK–NF-κB axis has also been uncovered in mice. This pathway involves direct inhibition of the mitochondrial respiratory chain complex I and downstream inhibition of inflammasomes in alveolar macrophages[164]. Metformin inhibited lipopolysaccharide-induced mitochondrial ATP synthesis and subsequent generation and cytosolic release of mtDNA, a potent NLRP3 ligand, ultimately leading to

reduction in inflammasome activation and IL-1β production in mouse bone marrow-derived macrophages[164] (Fig. 2a).

Remarkably, metformin treatment in mouse models of acute respiratory distress syndrome blunts lipopolysaccharide-induced and SARS-CoV-2-induced pulmonary inflammation, an effect mimicked by myeloid-specific ablation of cytidine monophosphate kinase 2, a rate-limiting enzyme in mtDNA synthesis[164]. By contrast, the inhibitory action of metformin on mitochondrial complex I was also shown to prevent pro-inflammatory activation of alveolar macrophages by urban particulate matter air pollution, notably through reducing levels of complex III-derived ROS and the subsequent decrease in calcium-mediated IL-6 release[165] (Fig. 2a). Of note, a considerable number of in vitro and ex vivo mechanistic studies used concentrations of metformin exceeding the therapeutic range and should therefore be considered with caution.

Prevention of inflammaging

Like cardiometabolic diseases, ageing and age-related disorders are often associated with chronic low-grade inflammation (also called inflammaging) and remodelling of the immune system[166,167]. Metformin has been considered as a treatment for attenuating detrimental consequences of ageing, notably for its possible beneficial effects on immune senescence[168]. Immune senescence is characterized by inflammatory activation of immune cells, a decline in their functions and reduced immune response efficacy, leading to increased vulnerability to infectious diseases, diminished responses to vaccination and increased susceptibility to metaflammation and age-related inflammatory diseases[167]. Interestingly, metformin treatment reduced the frequencies of pro-inflammatory B cell subsets in blood from older patients (>70 years old) with T2DM and increased influenza vaccine-specific antibody responses after vaccination[169]. These results were associated with reduced expression of senescence-associated secretory phenotype markers and reduced secretion of pathogenic autoimmune IgG antibodies by B cells isolated from these patients, supporting an anti-ageing effect of metformin on humoral immunity[169].

Ovarian fibrosis is associated with ageing and an increased risk of developing ovarian cancer, notably in people with T2DM[170]. In a study using single-cell RNA sequencing, metformin treatment modulated the composition of ovarian fibroblastic and immune cells and prevented age-associated ovarian fibrosis in female mice[171]. The underlying mechanism remains unclear and its translation to humans is still questionable. However, this study identifies a unique macrophage subset induced by metformin in the ovary that might contribute to the clearance of senescence-associated secretory phenotype-producing fibroblasts and parallel expansion of myofibroblasts involved in extracellular matrix remodelling, resulting in maintenance of tissue homeostasis during ageing[171] (Fig. 2b). Considering the use of metformin as a potential anti-inflammaging therapeutic strategy was also strengthened with the demonstration that metformin can alleviate ageing-associated T cell inflammation by preventing the age-induced appearance of a T helper 17 inflammaging profile through maintenance of CD4+ T cell autophagy and mitochondrial OXPHOS[172].

Action on immunomodulation

Tuberculosis. A variety of other immunomodulatory properties of metformin have been reported, including enhanced immunosuppressive capacity in several autoimmune hyperinflammatory diseases[173–176], improved immune response to infection[177] and potentiation of anti-tumour immunity[178]. Metformin can reduce *M. tuberculosis* infection,

lower progression to active tuberculosis and decrease mortality in both mice and humans[30,179]. Two studies have shown that part of this beneficial effect can be mediated by enhanced host antimycobacterial immune responses[180,181]. Indeed, although performed using supratherapeutic concentrations, ex vivo treatment of PBMCs from healthy donors with metformin induces mTORC1 inhibition and immune cell metabolic reprogramming, leading to decreased pro-inflammatory cytokine production and increased phagocytosis activity in response to stimulation with *M. tuberculosis* lysate[180] (Fig. 2a). Furthermore, PBMCs from metformin-treated healthy donors display increased AMPK activation, downregulation of genes with protein products involved in the type 1 interferon response and altered cellular composition, with a shift in myeloid cells from classic to non-classic monocytes[180]. On the adaptive side of the immune response, metformin treatment expanded a population of memory-like CD8+CXCR3+ T cells in spleen and lungs, and enhanced immunogenicity and protective efficacy against *M. tuberculosis* challenge in mice vaccinated with Bacillus Calmette–Guérin[181]. Also observed in healthy individuals and patients with T2DM treated with metformin, this metformin-induced CD8+ T cell subset has features of metabolic reprogramming, such as increased mitochondrial OXPHOS and fatty acid oxidation, survival capacity and antimycobacterial properties[181] (Fig. 2b). Collectively, these results might support the use of metformin as a new therapeutic option for patients with tuberculosis and/or as an adjunct drug to the tuberculosis vaccine.

Cancer. Metformin has also been reported to promote anticancer immunity through modulation of the tumour immune microenvironment, notably by increasing levels of tumour-infiltrating cytotoxic CD8+ T cells and decreasing levels of tumour-promoting CD163+ macrophages in human oesophageal squamous cell carcinoma[182]. At the molecular level, metformin triggers AMPK activation and STAT3 inactivation, leading to altered production of the effector cytokines TNF (increase) and IL-10 (decrease) by immune cells[182]. Metformin treatment also increased natural killer cell infiltration and cytotoxicity in head and neck squamous cell carcinoma in patients by promoting perforin release secondary to an AMPK-independent and mTORC1–STAT1-mediated downregulation of CXCL1 (ref. 183) (Fig. 2b).

Finally, metformin treatment rescues NASH-associated impairment in CD8+ T cell response to immune PD1–PDL1 checkpoint inhibitor therapy for advanced hepatocellular carcinoma[184]. Mechanistically, metformin induces metabolic reprogramming of hepatic CD8+ T cells, leading to increased motility of these tumour-infiltrating cells and improved efficacy of anti-PD1 antibody therapy against liver tumours in mouse models of NASH and hepatocellular carcinoma[184] (Fig. 2b). Boosting anti-PD1 therapy with metformin-loaded macrophage-derived microparticles targeting M2-like tumour-associated macrophages has been suggested for resetting the polarization state of these cells towards an antitumoural M1-like phenotype[185]. Interestingly, this macrophage-targeted approach results in remodelling of the tumour immune microenvironment. This remodelling increases recruitment of CD8+ T cells, decreases infiltration of both immunosuppressive myeloid-derived suppressor cells and regulatory T cells and enhances tumour penetration and anticancer activity of the anti-PD1 antibody[185].

Repurposing metformin

Metformin has a long history as the first-choice oral antihyperglycaemic drug for treating T2DM, with a high safety profile. In the past decade, repurposing metformin for additional applications has received

increased attention. Indeed, metformin has shown promising effects beyond T2DM that are now being investigated in several planned and active clinical trials.

An anticancer agent

A retrospective observational study published in 2005 showed reduced risk of a cancer diagnosis in people taking metformin[186]. Subsequently, the effect of metformin treatment in preventing the development and progression of various types of cancer has been extensively investigated[11,31,187]. Repurposing an inexpensive licensed drug for the treatment of cancer was also encouraged by results from cellular and preclinical studies demonstrating antineoplastic effects and inhibition of tumour growth by metformin via inhibition of mitochondrial OXPHOS associated with AMPK-dependent and AMPK-independent mechanisms[188]. However, the high concentrations of metformin used in in vitro studies might not be readily translated to effects in preclinical and clinical studies[24,189], which raises questions as to what the appropriate drug exposure to obtain a direct anticancer effect might be. Importantly,

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Box 4

Environmental concerns

Metformin is not metabolized and is eliminated unmodified by the human body. As such, approximately 70% of the therapeutic dosage is excreted through urine and faeces and is discharged to waste waters. The accumulation of metformin in the environment is therefore predicted to be considerable due to the large number of people taking metformin and the daily dosage (0.5–2.5 g). Based on the large volumes of metformin excreted unchanged in urine, metformin can be regarded as a prominent emerging water pollutant. The presence of metformin in the influents of wastewater treatment plants (WWTPs) has been used as an indicator of pharmacological contamination and in wastewater-based epidemiology studies to assess metformin consumption in populations[231,232]. Due to its low removal rate in most conventional WWTPs, metformin is consistently detected with concentrations ranging from nanograms to micrograms per litre in effluents of WWTPs, surface waters and even drinking water[233,234]. The widespread occurrence of this compound and its numerous organic transformation products generated via microbial degradation and photolysis (for example, guanylurea and $C_2N_4H_6O$) in the aquatic environment poses a global threat to environmental health[232,235,236]. The bioaccumulation of these contaminants in aquatic animals and plants has been extensively documented[233,235]. Ecotoxicological studies have demonstrated their detrimental effects on aquatic biota, causing developmental abnormalities, endocrine disruption and reproductive alterations[232,237–240]. Bioremediation of metformin-contaminated environments using microbial mixtures has provided encouraging outcomes, with the complete degradation of metformin and its transformation products[241]. However, risk assessments for human health of lifelong exposure to metformin via drinking water estimated the risk to be negligibly low[232,235]. Investigations on the adverse long-term environmental risks cannot be overlooked because of the expected increase in global consumption and water contamination of metformin and its transformation products.

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supportive evidence for a protective effect of metformin on cancer risk was not always consistent[190,191]. Thus, validation of the advantage of metformin treatment as a single agent or as an adjuvant in cancer therapy, notably for its emerging role in regulating cancer immunity, awaits further clarification.

Inflammatory and immune-mediated diseases

Studies on the anti-inflammatory properties of metformin and studies highlighting its possible immunomodulatory functions in various pathophysiological conditions suggest that it could be used in the context of several infectious, autoimmune and hyperinflammatory diseases. However, although the initiation of an increasing number of clinical trials supported by these new findings are being undertaken, further work is needed to clarify whether modulation of the immune response results from a direct action of metformin on various immune cell subsets and/or is mostly the consequence of indirect effects of the drug on their microenvironment (for example, nutrients, oxygen tension and non-immune cells present in the local niche).

Of note, based on the immune cell transcriptional profiles available from the ImmGen Consortium[192] and the Tabula Sapiens Consortium[193], the expression levels of genes encoding the main metformin transporters OCT1–3 (*SLC22A1–3*), plasma membrane monoamine transporter (*SLC29A4*) and multidrug and toxin extrusion protein 1 (*SLC47A1*) are all very low to undetectable in both mouse splenocytes and human PBMCs. Therefore, it would be important to investigate whether metformin can be taken up and accumulate in specific innate or adaptive immune cell subsets, in both healthy and disease states. In addition, whether clinically relevant concentrations of the drug, which are much lower than the ones generally used in most of the in vitro and ex vivo studies, can directly trigger immunomodulation, notably through immune cell metabolic reprogramming, needs to be determined. In this context, it should be noted that oral metformin did not enter the thymus to activate AMPK and did not reduce the occurrence of T cell acute lymphoblastic leukaemia in mice, presumably due to a lack of appropriate transporters[194]. By contrast, the cell-permeable biguanide phenformin did enter the thymus and activate AMPK, causing a delayed onset of T cell acute lymphoblastic leukaemia[194]. Thus, using phenformin when metformin is not taken up by cancer or immune cells could provide better efficacy than metformin in current clinical trials[195].

Ageing

Metformin has been proposed as an anti-ageing drug as it increased median lifespan and maximum lifespan in studies performed in several species, including *C. elegans*, *Drosophila melanogaster*, rodents and humans[168]. The interplay between the microbiota and the host organism seemed to be crucial to extend the maximum lifespan in both *C. elegans* and *D. melanogaster*[131], which suggests there is evolutionary conservation of microorganism-derived metabolites in response to metformin. Metformin use has been associated with increased overall survival (for example, in relation to the incidence of cancer and cardiovascular disease in patients with T2DM compared with that in matched control individuals without T2DM[196]); however, the ability of metformin to promote health or increase lifespan in healthy populations remains to be clearly determined. In addition, further studies are also needed to directly link metformin-induced increased longevity with alterations in the profile of the human gut microbiota. Interestingly, the benefits of metformin on longevity are restricted to mice treated with metformin from young adulthood, and are not seen when treatment is initiated in

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older animals[197]. This finding suggests that delaying the occurrence of (immune) senescence and related inflammaging might be one of the important underlying mechanisms. Interestingly, in old *C. elegans*, metformin causes a severe metabolic failure that leads to ATP exhaustion and cell death[198]. More insights into the benefits of metformin on healthspan and lifespan in humans will hopefully be provided by the MILES (Metformin in Longevity Study) and TAME (Targeting Aging with Metformin) clinical trials[199].

Conclusions

Metformin is one of the most common medications used worldwide and has been used for >60 years. However, the mechanisms underlying its therapeutic effects remain incompletely understood. The latest advances regarding metformin's glucoregulatory mechanisms of action have identified new potential factors, adding some layers of complexity and confusion that might fuel ongoing controversies. Whether cellular energy charge is altered or not in response to low metformin concentrations in the liver remains to be properly addressed, specifically using sensitive adenine nucleotide quantification approaches. In addition, whether these bioenergetic effects are the unique consequence of metformin's inhibitory action on complex I or on other mitochondrial targets needs to be clarified. Furthermore, considering the lysosome as an additional organelle target provides insights for novel mechanisms of metformin action through the AMPK signalling pathway. Moreover, although it has long been assumed that the antihyperglycaemic effects of metformin are due to its exclusive action in the liver, there is now strong evidence that extrahepatic sites of action, notably the gut and its microbiota, are involved in its various clinical benefits. In addition, metformin has immunomodulatory properties in various pathological contexts (such as cancer, hyperinflammatory diseases and infectious diseases) involving direct or indirect regulation of the host innate and adaptive immune response. Some of these new insights from preclinical studies are currently being explored in clinical trials that are repurposing metformin for a range of diseases beyond T2DM. Lastly, it is worth mentioning that if the therapeutic prescription of metformin is expanded to other indications in the future, resulting in more widespread use, contamination of global waters with the drug might increase, which could raise environmental concerns (Box 4).

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Competing interests

The authors declare no competing interests.

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