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Review

Metformin: Antidiabetic actions from cells to tissues

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ABSTRACT

Metformin (dimethyl biguanide) is a primary pharmacotherapy to treat hyperglycemia in type 2 diabetes. It counters the effects of insulin resistance, improves glucose homeostasis, assists weight control and avoids overt hypoglycemia via reduced hepatic gluconeogenesis, increased splanchnic glucose turnover and greater peripheral glucose utilization. The underlying cellular actions of metformin differ between tissues and drug exposures. High concentrations of metformin (e.g. millimolar in the intestine) can interrupt the mitochondrial respiratory chain at complex 1, increase cytosolic NADH (favouring pyruvate conversion to lactate), decrease ATP synthesis, raise cytosolic AMP and activate AMP-activated protein kinase (AMPK). Lesser concentrations of metformin in liver can interrupt the respiratory chain at complex 4, which inhibits mitochondrial glycerol-3-phosphate dehydrogenase and impedes the mitochondrial glycerophosphate shuttle. Low concentrations of metformin (e.g., ~10 μM) can activate AMPK by a lysosomal pathway without interrupting oxidative metabolism. While AMPK implements many of the metabolic effects of metformin, other contributing mechanisms include separate effects on metabolic pathways (e.g. inhibiting fructose-1,6-bisphosphatase) and signalling intermediates (e.g. inhibiting phosphatases) to reinforce the actions of insulin. Thus, the antidiabetic effects of metformin reflect diverse concentration-dependent cellular actions on nutrient metabolism and energetics in different tissues. The breadth of cellular actions of metformin encourages investigation of potential opportunities to assist in the management of cardiovascular, inflammatory, neoplastic and neurodegenerative disorders.

1. Introduction

Most accounts of metformin acknowledge that its mode of action is not fully understood – a comment that might reasonably be applied to almost any drug. However, much is known to guide the effective use of metformin in the prevention and management of diabetes, particularly type 2 diabetes [1]. Metformin counters the effects of insulin resistance and improves glycaemic control without causing overt hypoglycemia, while often improving weight control and lipid metabolism and reducing long-term cardiovascular risk [1,2]. Beyond diabetes, metformin has shown benefit in other insulin-resistant conditions such as polycystic ovarian syndrome (PCOS) and hyperglycemia during pregnancy, and it is being evaluated for possible use in the management of chronic inflammation, neurodegenerative disorders, certain cancers and general age-related health [3–5]. The focus of this narrative review is to clarify current understanding of the cellular actions of metformin that account for its glucose-lowering effect in the management of diabetes.

2. Cellular mechanisms of action of metformin

2.1. Overview of antihyperglycemic actions

Several excellent reviews have detailed the intracellular actions of metformin and their impact on glucose metabolism and related metabolic effects [6–8]. To appreciate the therapeutic relevance of these actions it is necessary to consider how they vary between tissues due to differences in drug exposure, expression levels of target molecules and the dwindling response capacity of tissues as the severity of diabetes increases [9–11]. The efficacy of metformin relies upon the collective impact of multiple actions, some of which are dependent and some independent of insulin. However, the blood glucose-lowering effect in diabetes requires a presence of insulin such that metformin cannot be used as a substitute for insulin therapy in type 1 diabetes, although it can reduce the amount of insulin required by reducing insulin resistance. The glucose-lowering efficacy of metformin is diminished as blood glucose concentrations approach normo-glycemia, and the glucose-

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lowering effect can be overridden by counter-regulatory mechanisms at low glucose concentrations so as to prevent hypoglycemia [1]. Accordingly, some studies in non-diabetic fasting animals and humans have not observed a glucose-lowering effect, and metformin is aptly described as an 'antihyperglycemic' rather than a 'hypoglycemic' agent. Also, in vitro studies and animal studies have exposed tissues to much higher concentrations of metformin than achieved with clinical doses, potentially confusing therapeutic and supra-therapeutic effects [12,13]. Therefore, attention is given to such sources of disparity in the present review.

It is appreciated that laboratory rodents require a higher dose of metformin to achieve effects that equate with those in humans. This is because metformin is eliminated unchanged via the kidney, and small laboratory rodents have a higher glomerular filtration rate for their body mass than humans. Thus, whereas the clinical dose range of metformin is 500–2550 mg/day (approximately 10–50 mg/kg), which achieves plasma concentrations of 0.5–2 μg/ml (~10 μmol/L), laboratory rodents often require doses up to 300 mg/kg to generate comparable effects.

The main target tissues for the metabolic effects of metformin are the intestine (very high drug exposure, e.g. >300 μM), liver (moderate drug exposure, e.g. >50–300 μM), muscle and adipose tissue (lesser drug exposures, e.g. ≤50 μM) (Fig. 1) [13–15]. The pharmacokinetic factors that determine this are described in Box 1 [Box 1, Pharmacokinetics of metformin]. Key intracellular targets for metformin are the mitochondria and adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK), which can modify cellular energetics, redox state, gene expression and the cellular signalling pathways of insulin and glucagon. These in turn affect nutrient sensing and metabolism as summarized in Table 1.

2.2. *Metformin and mitochondrial function*

Many of the cellular effects of metformin have been attributed to reduced activity of the mitochondrial respiratory chain, thereby reducing ATP synthesis, raising the AMP:ATP ratio and increasing cytosolic reduced nicotinamide adenine dinucleotide (NADH) [6,25–29]. The commonly stated corollary is that increased AMP then activates AMPK which mediates many of the cellular effects of metformin, while reduced oxidative phosphorylation increases the NADH:NAD+ ratio and drives increased anaerobic glycolysis and lactate formation.

Being positively charged, metformin is considered likely to accumulate at the inner mitochondrial membrane. On theoretical grounds this has been variously estimated at 60–1000 fold greater than concentrations in plasma or the cytosol. However, such high values have not been confirmed in studies with isolated mitochondria in which concentrations of ~50 μM metformin have been observed, although these are still higher than plasma concentrations (~10 μmol/L) [28–32]. Most studies with isolated mitochondria have used metformin concentrations in the millimolar (mM) range and recorded an acute (e.g. 10–30 min) decrease in the activity of the respiratory chain at complex 1 and a decreased rate of oxygen consumption (Fig. 2) [29]. Lower metformin concentrations (e.g. <100 μM) have also reduced oxygen consumption in some cultured cell lines and in vitro tissue preparations, and the involvement of mechanisms other than inhibition of complex 1 has been demonstrated [7,29,33].

Metformin is a weak and reversible inhibitor of complex 1 (NADH dehydrogenase) of the respiratory chain, even at millimolar concentrations (IC50 ~20–60 mM), acting by binding to and holding the conformation of the complex in a deactivated state [27–29]. Based on the millimolar concentration of metformin within the intestinal mucosa, interruption of the mitochondrial respiratory chain at complex 1 appears to be a pharmacologically relevant action of metformin within this tissue and is consistent with the activation of AMPK, increased anaerobic glycolysis and lactate production by the intestine [10,14,34–36]. However, inhibition of complex 1 is considered to be less therapeutically relevant at the metformin concentrations in liver, and negligible within muscle and adipose tissue where metformin can increase oxidative metabolism [13,30,37].

A separate mechanism through which metformin interacts with the mitochondrial respiratory chain to alter ATP synthesis and redox state is by reducing electron transfer at complex 4 (cytochrome C oxidase) [7,38,39]. This creates an electron tailback in the respiratory chain which decreases the ability of coenzyme Q (CoQ, ubiquinone) to accept more electrons. Because mitochondrial glycerophosphate dehydrogenase-2 (mGPD2) transfers electrons to CoQ (to generate ubiquinol), the activity of mGPD2 is reduced, causing the mitochondrial glycerophosphate shuttle to be reduced and more NADH to remain in the cytosol (Fig. 2) [7,39]. This has been demonstrated at moderate metformin exposure (e.g. 200 μM) and contrasts with an increased NADH:NAD^+ ratio in the mitochondrial matrix by inhibition of complex 1

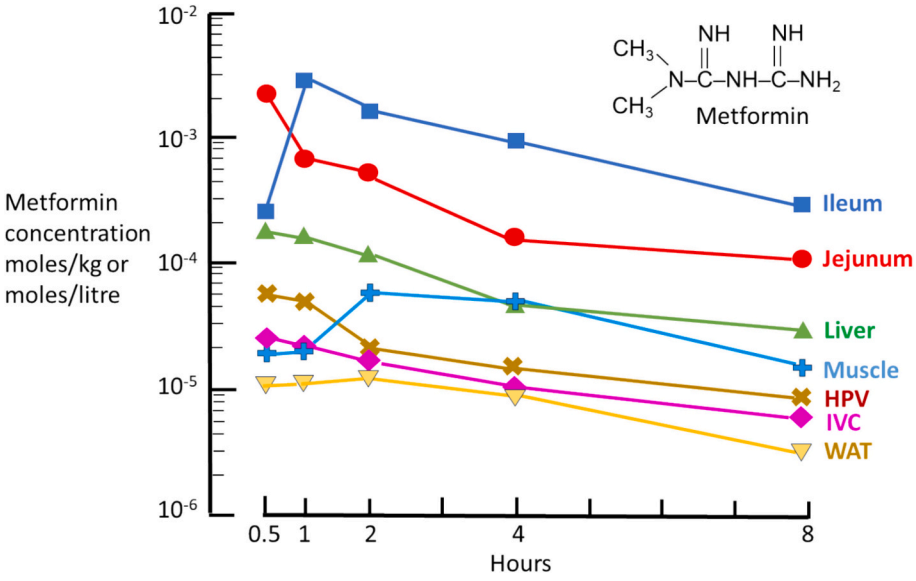

Fig. 1. Exposure of different tissues to metformin. Metformin (50 mg/kg body weight containing ^{14}C-metformin 25 μCi/kg body weight in 5 ml/kg water) was administered orally to 18 h fasted normal mice. Concentrations of metformin were determined at intervals in plasma and tissues. Inset shows chemical structure of metformin. HPV, hepatic portal vein; IVC, inferior vena cava; WAT, white adipose tissue. [Original data in reference [13]].

Box 1
Pharmacokinetics of metformin.

Metformin is available as immediate release (IR) tablets (500, 850, 1000 mg) taken before/with main meals, and extended release (ER, SR, XR) tablets (500, 750, 1000 mg) taken once or twice daily [16,17]. Absorption is mostly in the small intestine with a bioavailability of 50–60% and a plasma Tmax at 0.9–2.6 h for IR tablets and 4–8 h for XR tablets. With a single dose of an IR tablet the plasma Cmax is typically 1–2 μg/ml (~10 μmol/L) declining to <0.5 μg/ml overnight during steady state treatment. The XR tablets may have a slightly lower Cmax but an extended area under the drug concentration curve. Plasma protein binding is negligible and tissue distribution is extensive (volume of distribution variously estimated at 63–276 L). Metformin is recirculated via salivary secretion which may assist re-delivery of drug into the intestine with subsequent meals [15,18–20].

At physiological pH metformin exists mostly as a cation (pKa ~11.5) and is transferred across plasma membranes via organic cation transporters (mainly OCT1, but also OCT2 and OCT3) and the plasma membrane monoamine transporter (PMAT). Additional transmembrane transport of metformin may occur via the serotonin re-uptake transporter in the intestine and the multidrug and toxin extrusion transporters (MATE1/2) in the kidney [21]. Thus, bioavailability, tissue exposure and elimination of metformin are affected by functional variants of these transporters which may alter clinical efficacy and side effects [21,22]. Metformin occurs at very high (millimolar) concentrations within the intestinal lumen, >300 μM in intestinal mucosa, ~50–300 μM in liver and mostly ≤50 μM in muscle and <10 μM in adipose tissue [13–15]. Metformin is not metabolized and is eliminated unchanged in the urine (terminal half-life of ~6–7 h). Renal clearance is >400 ml/min indicating ~20% is by filtration and ~80% is by tubular secretion [15,19]. Thus, metformin is contraindicated if renal function is significantly impaired (e.g. estimated glomerular filtration rate <30 ml/min/1.73m^2) to avoid excess drug accumulation which can cause hyperlactatemia and (very rarely) precipitate lactic acidosis (incidence 0.03–0.1/1000 patient years) [23]. Metformin-associated lactic acidosis is mostly due to severe acute renal failure that increases plasma concentrations of metformin considerably in excess of 5 μg/ml [23,24].

Table 1
Main metabolic effects of metformin that impact glucose homeostasis, weight control and lipid metabolism.

Organ	Glucose homeostasis	Weight control	Lipid metabolism
Intestine	↑ Anaerobic glycolysis (lactate formation)	↑ Splanchnic glucose-lactate-glucose turnover	↓ Chylomicron production
	↑ Glucose utilization	↑ Secretion of GDF-15, GLP-1, PYY	↑ Short-chain fatty acid-producing bacteria
	Altered composition and activities of the microbiome	↑ Lac-Phe	
Liver	↓ Gluconeogenesis ↓ Glycogenolysis ↓ Glucagon action		↑ Fatty acid oxidation ↓ VLDL triglyceride
Muscle	↑ Insulin-mediated peripheral glucose uptake and oxidation ↑ Glycogenesis	↑ Lac-Phe during exercise	↑ Fatty acid oxidation
Adipose	↑ Insulin-mediated peripheral glucose uptake and oxidation	↑ Fatty acid oxidation ↓ Lipogenesis	↑ Uptake of fatty acids
		↑ Mitochondrial uncoupling (e.g. UCP-1)	↑ Fatty acid oxidation

↑, increase; ↓, decrease. GDF-15, growth differentiation factor-15; GLP-1, glucagon-like peptide-1; Lac-Phe, N-lactoyl phenylalanine; PYY, peptide tyrosine tyrosine; UCP-1, uncoupling protein-1; VLDL, very low density lipoprotein. Based on reference [1].

through high (e.g. millimolar) metformin exposure. Inhibition of complex 1 reduces NAD^+ formation within the mitochondria and could increase the cytosolic NADH:NAD^+ ratio though suppression of the malate-aspartate shuttle. An increased cytosolic NADH:NAD^+ ratio affects the activity of lactate dehydrogenase and reduces (or reverses) the conversion of lactate to pyruvate, so reducing the supply of pyruvate for the Krebs cycle, reducing gluconeogenesis and contributing to the risk of lactate accumulation. An increased cytosolic NADH:NAD^+ ratio also decreases gluconeogenesis from glycerol by reducing the conversion of glycerol (via glycerol-3-phosphate) to dihydroxyacetone phosphate [7].

Since high concentrations of metformin (typically >300 μM) are required to achieve a significant inhibition of the respiratory chain at complex 1, an effect on complex 4 by lower concentrations of metformin (e.g. ≤300 μM) offers a more realistic pharmacological mechanism for liver tissue [7,8,39,40]. However, this is certainly not the only mechanism of action for a moderate or low metformin concentration, because metformin can reduce hepatic gluconeogenesis and increase insulin-mediated glucose uptake and metabolism by muscle without measurable changes in the cytosolic redox state or AMP:ATP ratio [7,8,33,41].

Effects of metformin on mitochondrial function may impact more than immediate bioenergetics. For example, a reduced proton gradient consequent to reduced activity of complex 1 or complex 4 could favour mitochondrial membrane depolarisation, alter the generation of reactive oxygen species and apoptotic signals, and modify mitochondrial DNA copy number [39,42]. As mentioned, metformin might affect the mitochondrial malate–aspartate shuttle, particularly if this shuttle compensates for a reduced glycerophosphate shuttle [33,43]. Metformin is known to increase mitophagy, possibly via activation of AMPK, and the removal of damaged mitochondria is anticipated to confer general cellular benefits against oxidative stress, inflammation and programmed cell death [44–46].

2.3. Metformin and AMPK

As noted above, AMPK is an important mediator of many effects of metformin (Fig. 3). Interruption of the mitochondrial respiratory chain by high concentrations of metformin (typically >300 μM) in the intestinal mucosa and more moderate concentrations of metformin (e.g. ≤300 μM) in liver can raise cytosolic AMP (relative to ATP) and activate AMPK [7,8]. AMP binds to the γ subunit of AMPK which causes a conformational change firstly to the γ subunit and then to the α subunit. This exposes the catalytic domain of the α subunit which can then be phosphorylated by liver kinase B1 (LKB1) to activate AMPK or prolong activation by various calcium/calmodulin-dependent protein kinases [6,47–49].

In addition to the mitochondria-AMP-AMPK pathway, metformin has long been known to exert some of its biological effects without measurable changes in mitochondrial function or raised AMP, and an AMP-independent pathway through which metformin activates AMPK has more recently been identified [12,41]. Low intracellular metformin concentrations (e.g. <50 μM) can bind to the presenilin enhancer-2 (PEN2) protein in the lysosome membrane. This enables PEN2 to bind with a protein encoded by ATP6AP1, which is an accessory protein of the vacuolar-type ATPase (v-ATPase) complex. Interaction of the ATP6AP1 protein with v-ATPase deactivates the v-ATPase complex, allowing the scaffold protein AXIN to co-translocate with LKB1 to the

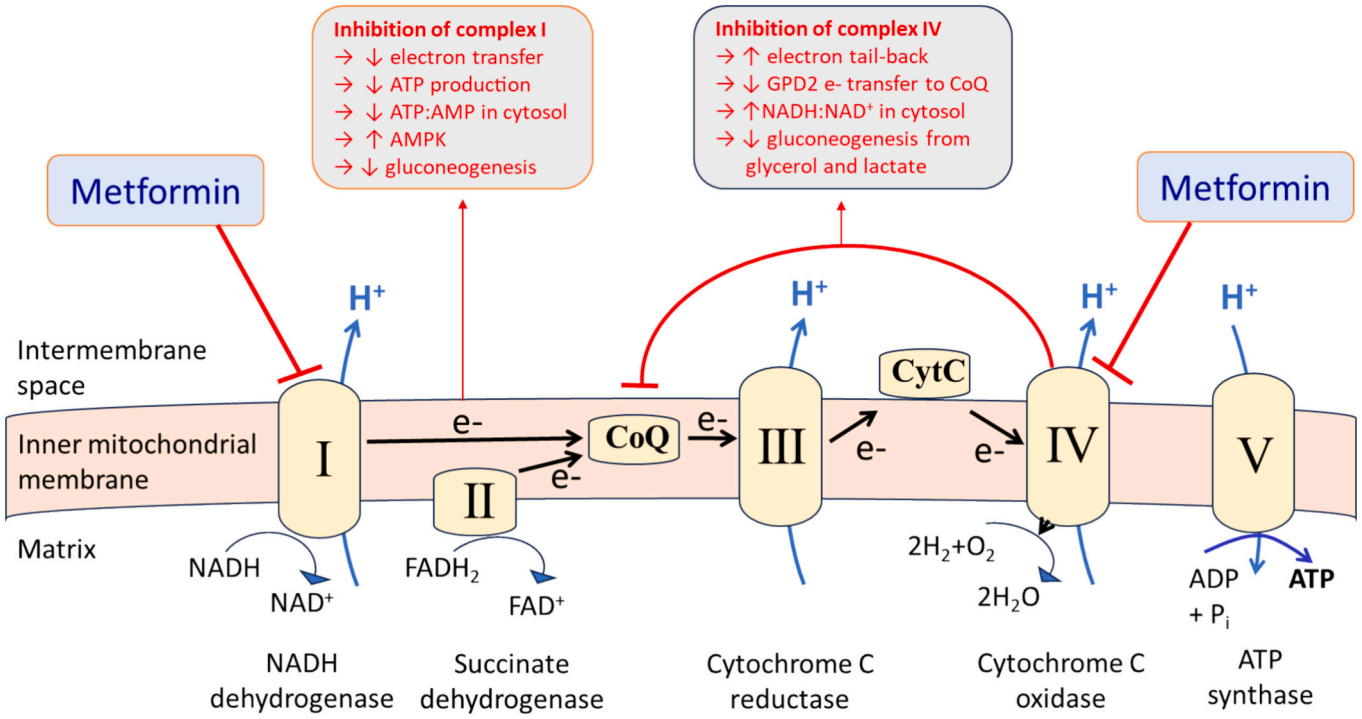

Fig. 2. Metformin can reduce activity of the mitochondrial respiratory chain. High concentrations of metformin (e.g. >300 μM) can bind weakly and reversibly reduce (IC50 ~20–60 mM) the activity of complex 1 of the respiratory chain. This reduces use of NADH which increases the cytosolic NADH:NAD^+ ratio. Reduced activity of complex 1 also decreases electron transfer into the respiratory chain, reducing oxygen consumption and reducing ATP synthesis. Lower concentrations of metformin (e.g. ≤300 μM) can inhibit complex 4 of the respiratory chain. This creates an electron tail-back along the chain and over-loads the capacity for coenzyme Q (CoQ, ubiquinone) to accept electrons. Because mitochondrial glycerophosphate dehydrogenase-2 (mGPD2) transfers electrons to CoQ the activity of mGPD2 is reduced. This interrupts the mitochondrial glycerophosphate shuttle which reduces the use of NADH, providing another mechanism to increase the cytosolic NADH:NAD^+ ratio. Raised NADH reduces the conversion of glycerol to glucose. Raised NADH also increases pyruvate conversion to lactate, further limiting the availability of pyruvate for the Krebs cycle, reducing gluconeogenesis and potentially increasing the risk of lactate accumulation. Reduced ATP synthesis through interruption to the respiratory chain at complex 1 or 4 will increase the cytosolic AMP:ATP ratio. Raised AMP will activate AMP-activated protein kinase (AMPK) which is responsible for many of the effects of metformin. ADP, adenosine diphosphate; ATP, adenosine triphosphate; CoQ, co-enzyme Q (ubiquinone); CytC, cytochrome C; e-, electron; FAD, flavin adenine dinucleotide; $FADH_2$, reduced form of flavin adenine dinucleotide; H, hydrogen; NAD, nicotinamide adenine dinucleotide; NADH, reduced form of nicotinamide adenine dinucleotide; Pi, inorganic phosphate.

surface of the lysosome to facilitate phosphorylation and activation of lysosomal AMPK [50–52].

Once activated, AMPK modifies genetic targets and more immediate signalling steps in pathways regulating metabolic and other cellular processes [6,49]. In liver for example, metformin concentrations ≤1 mM can promote AMPK-mediated inhibition of various transcription factors notably cAMP response element binding protein (CREB)-regulated transcription co-activator-2 (CRTC2) and hepatocyte nuclear factor-4 (HNF4). These normally enhance the expression of key gluconeogenic enzymes, namely phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [7,43,53]. An example of a more rapid effect on a metabolic pathway is the AMPK-mediated serine phosphorylation of the isoforms of acetyl-CoA carboxylase (ACC). This enables AMPK to decrease formation of malonyl-CoA which in turn reduces lipogenesis and facilitates fatty acid transfer into mitochondria for oxidation. The extent to which clinical doses of metformin mediate these mechanisms remains to be established, and it is reminded that metformin can exert anti-gluconeogenic effects through actions that are independent of AMPK [7,8]. Although metformin activates AMPK, and AMPK increases translocation of the facilitative glucose transporter GLUT4 into the plasma membrane to promote glucose uptake in pre-adipocytes and muscle, it is not confirmed that metformin activates this process at clinical doses [6,49,53].

It is possible that metformin could enhance AMPK-mediated epigenetic effects involving interaction with histone deacetylases. For example, by increasing the production of NAD^+, AMPK facilitates activation of the histone deacetylase sirtuin-1 (silent mating type information regulation 2 homologue-1; SIRT1) which activates (phosphorylates) the forkhead box (FOXO1) transcription factor which is then excluded from the nucleus resulting in reduced expression of the gluconeogenic enzyme glucose-6-phosphatase (an effect also mediated via insulin) [53]. Increased expression of GLUT4 and mitochondrial biogenesis are also mediated in part via an epigenetic effect of AMPK [49,54,55]. Whether these epigenetic mechanisms are triggered with clinical doses of metformin is not established. It is noted that some epigenetic effects of AMPK have been determined after activation with AMP mimetic agents which may interact with targets additional to AMPK. Accordingly, it is not given that metformin could replicate all of these effects ascribed to AMPK. Proteomic screening studies leave open the prospect that metformin may exert further effects on AMPK independently of AMP and independently of currently recognised kinase activators that act directly on downstream targets of AMPK [56].

2.4. *Metformin and insulin signalling*

Although metformin reduces the effects of insulin resistance, its interaction with insulin signalling pathways is often overlooked. Early studies indicated that therapeutically relevant concentrations of metformin could increase the number of insulin receptors at the cell membrane, prolong receptor signalling and increase post-receptor signal conduction in hepatocytes isolated after in vivo treatment and in cultured muscle cells, adipocytes and granulosa cells [57–61]. Studies in *Xenopus* oocytes have noted that clinical concentrations of metformin can reduce the activity of protein-tyrosine phosphatase-1B (PTP1B), and

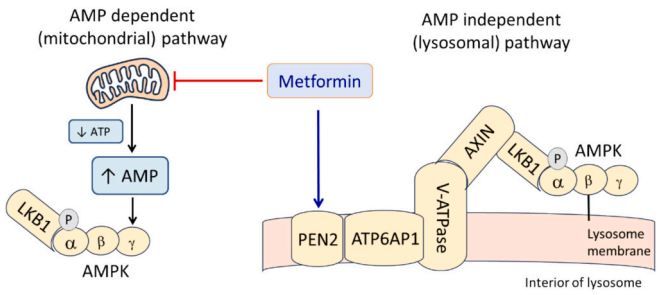

Fig. 3. Metformin exerts multiple metabolic effects via activation of AMPK. Metformin is known to activate AMP (adenosine monophosphate)-activated protein kinase (AMPK) via an AMP-dependent and an AMP-independent pathway. High metformin concentrations (>300 μM) in intestinal tissue (and possibly to a much lesser extent in liver) can interfere with the mitochondrial respiratory chain, reduce ATP synthesis, and cause an elevation of cytosolic AMP. The AMP binds to the γ subunit of non-lysosomal AMPK causing a conformational change in the γ subunit which then alters the conformation of the α subunit to expose the catalytic region of the α subunit. This enables phosphorylation (Thr 172) of the α subunit by liver kinase B1 (LKB1) and possibly certain other kinases thereby activating and/or prolonging the activity of AMPK. Exposure to a clinically relevant concentration of metformin appears to be sufficient to activate AMPK by an AMP-independent lysosomal pathway. This involves binding of metformin to the presenilin enhancer-2 (PEN2) protein in the lysosome membrane which then activates ATP6AP1 which is an accessory protein of the vacuole-type ATPase (v-ATPase) complex. ATP6AP1 deactivates the v-ATPase complex which allows the scaffold protein AXIN to co-translocate with LKB1 to the surface of the lysosome for the LKB1 to phosphorylate the α subunit of lysosomal AMPK. AMP-dependent or AMP-independent activation of AMPK can then give rise to the multiple metabolic effects and other effects associated with this kinase. These include the transcriptional effects mediated by inhibition of the cyclic-AMP response element binding protein (CREB)-regulated transcription co-activator-2 (CRTC2) reducing gene expression of key gluconeogenic enzymes. Other adjustment of metabolic pathways occurs by serine phosphorylation of the isoforms of acetyl-CoA carboxylase which decreases formation of malonyl-CoA which in turn reduces lipogenesis and favours fatty acid oxidation. Additionally, AMPK can enhance translocation of the facilitative glucose transporter GLUT4 into the plasma membrane to promote glucose uptake.

suggested this could prolong insulin receptor phosphorylation and signalling [62]. Also, clinical concentrations of metformin are reported to increase phosphorylation of insulin receptor substrates and signalling into the mitogen-activated protein kinase (MAPK) pathways in cultured hepatocytes and muscle cells [60,63]. Activity of Src homology-2 domain-containing inositol-5-phosphatase-2 (SHIP2) is inhibited by binding of metformin at <10 μM concentration, and suppression of SHIP2 has been shown to increase insulin post-receptor signalling through the phosphatidylinositol 3-kinase (PI3K) pathway to Akt in cultured muscle cells [64–66]. Several further studies have noted that therapeutic concentrations of metformin increase insulin-stimulated Akt-mediated GLUT4 translocation into the plasma membrane of skeletal muscle and adipose tissue, associated with increased glucose uptake [66]. Enhanced insulin signalling in liver cells after metformin exposure in vivo and in vitro has increased Akt and reduced the transcriptional effects of FOXO1 and CREB, resulting in decreased production of the gluconeogenic enzymes PEPCK and G6Pase [66–69].

Although not all of the effects of metformin on cellular insulin signalling have been demonstrated in tissue of metformin-treated patients with type 2 diabetes, it is appreciated that metformin does not achieve a clinically significant blood glucose-lowering effect in the absence of insulin in type 1 diabetes. Moreover, increased insulin-mediated glucose disposal (albeit sometimes very modest) has been observed consistently in type 2 diabetes patients during insulin clamp studies [66,70,71]. It is well established that reducing the glucotoxic effects of persistent hyperglycemia will improve insulin sensitivity as measured by glucose disposal during insulin clamp studies and improve cellular pathways of insulin signalling [72]. Therefore, it is anticipated that improved insulin action during the therapeutic use of metformin in type 2 diabetes will reflect in part the reduction in hyperglycemia achieved through reduced hepatic glucose production [1,71].

2.5. Further cellular mechanisms of metformin

Although many metabolic effects of metformin can be attributed to a combination of altered redox state, activation of AMPK and improved insulin signalling, other mechanisms play a complementary role. Of particular note, clinically relevant metformin concentrations contribute to the suppression of gluconeogenesis by inhibiting fructose-1,6-bisphosphatase (F16BPase) through an increased cytosolic AMP, which is an allosteric inhibitor of F16BPase [6,40,73]. Raised AMP will also reduce adenylate cyclase activity which will impair the signalling activity of glucagon receptors, thereby providing a further mechanism through which metformin could contribute to reductions in hepatic gluconeogenesis and glycogenolysis [74,75].

It has been suggested that metformin may directly affect various deacetylases such as SIRT1 independently of AMPK, but it is unclear whether this mechanism is triggered by clinically relevant metformin concentrations [76,77]. Very high (e.g. millimolar) concentrations of metformin can chelate copper and other transition metal ions which could (theoretically) contribute to interference with the respiratory chain and other cellular actions, although the therapeutic relevance of such an effect is unclear [78]. Reduced oxidative stress is regarded as a general benefit of metformin to improve the cellular environment: this is attributed largely to interference with the mitochondrial respiratory chain and activation of AMPK-mediated metabolic effects. However, most experimental demonstrations have required millimolar concentrations of metformin to show reduced production of reactive oxygen species, leaving the clinical relevance unclear [79,80]. Doubtless, further mechanisms of action of metformin will emerge in the fullness of time, but the foregoing provides a current view of the main intracellular routes through which metformin improves glucose homeostasis in type 2 diabetes.

3. Effects of metformin in target tissues

As noted, the cellular actions of metformin operate to different extents in different tissues as determined by drug exposure, expression of the drug targets and disease severity. The following sections describe how different tissues respond to metformin to generate the overall metabolic effects in non-insulin dependent hyperglycemic conditions (Fig. 4).

3.1. Intestine

The lumen and mucosa of the intestine, which are exposed to high concentrations of metformin, are important sites of action of metformin.

3.1.1. Microbiome

A 1000 mg oral dose of metformin generates millimolar drug concentrations within the human gut lumen (possibly up to 10 mM) which have been reported to alter the composition of the microbiome and the activity of individual taxonomic groups [10,81–83]. For example, metformin has been associated with an increased abundance of *Akkermansia*, *Escherichia* and other genera, alongside a reduced abundance of *Intestinibacter*, *Actinobacteria*, *Crenarchaeota* and *Spirochaetota* genera, but a consistent pattern has yet to emerge [84,85]. The inconsistencies are likely due in part to extensive variations in the microbiome between individuals, particularly relating to ethnicity and diet, and to analyses based on metagenomic sequencing of single spot stool samples [85,86]. Nevertheless, decimation of the microbiome with antibiotics has impeded the glucose-lowering effect of metformin in high-fat fed mice, indicating that effects on the microbiome are likely to contribute to the

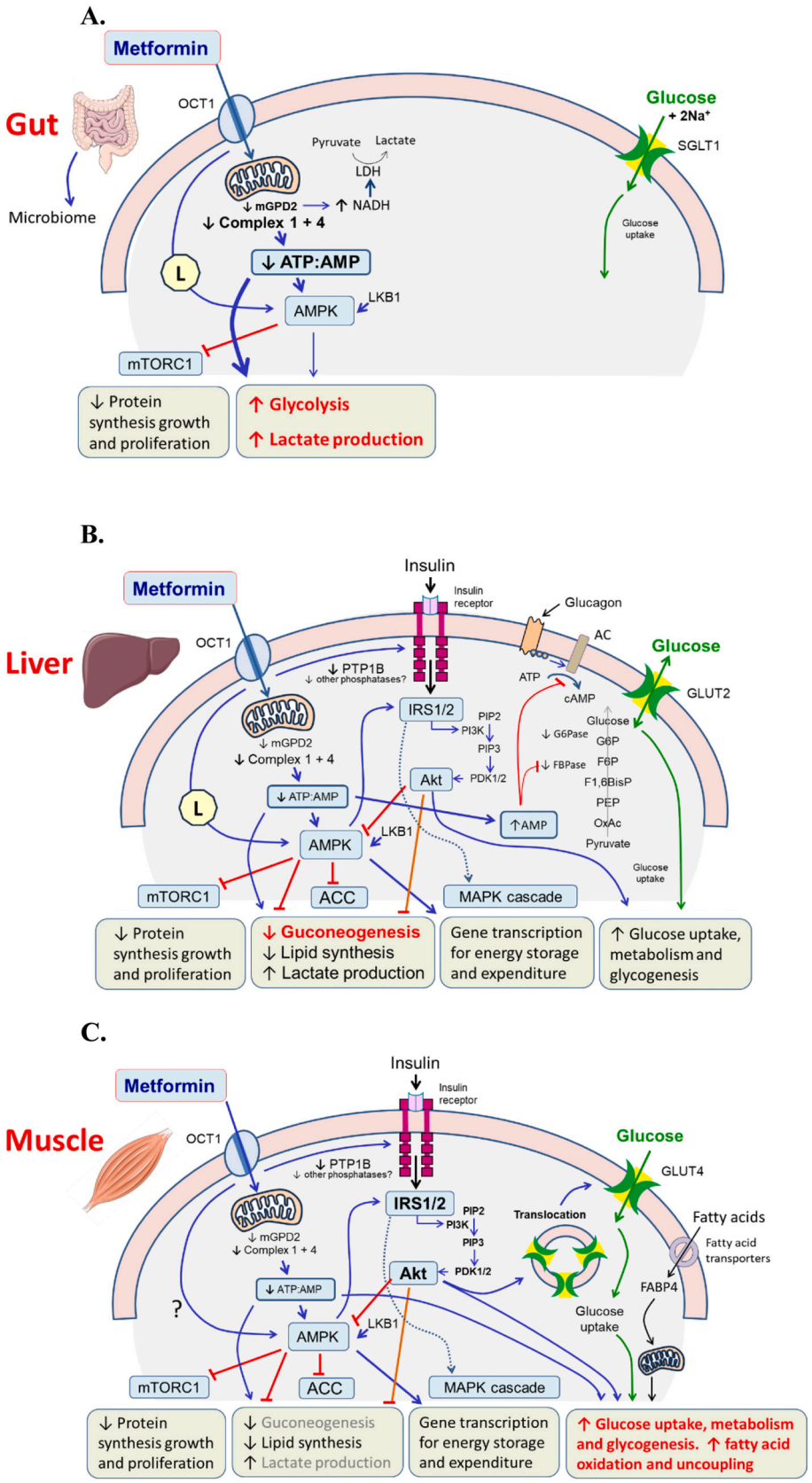

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Fig. 4. Metformin effects vary between tissues according to drug exposure and metabolic capabilities of the tissues: A, gut; B, liver; C, muscle. The main intracellular targets for metformin, notably the mitochondrial respiratory chain, AMP-activated protein kinase and insulin signalling intermediates are affected to different extents in different tissues. Intestinal enterocytes are exposed to high concentrations (>300 μM) of metformin which result in increased anaerobic glycolysis and lactate production. Liver cells are exposed to lesser concentrations (e.g. 50–300 μM) of metformin which reduce gluconeogenesis and potentially glycogenolysis. Muscle cells are exposed to lower concentrations (≤50 μM) of metformin which enhance glucose uptake and metabolism. At this time it is not established if metformin activates lysosomal AMPK in muscle. Akt, protein kinase B; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; AC, adenylate cyclase; ACC, acetyl-CoA carboxylase; FABP4, fatty acid binding protein 4 (aP2); FBPase, fructose-1,6-bisphosphatase; G6Pase, glucose-6-phosphatase; GLUT, glucose transporter isoform; OCT1, organic cation transporter 1; IRS, insulin receptor substrate; L, lysosome; LKB1, serine/threonine–protein kinase STK11; MAPK, mitogen-activated protein kinase; mGPD, mitochondrial glycerol-3-phosphate dehydrogenase; mTORC1, mammalian target of rapamycin (sirolimus) complex 1; PDK, 3-phosphoinositide-dependent protein kinase; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol-3,4-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PTP1B, protein tyrosine phosphatase-1B. ↑, increase; ↓, decrease; → reaction leading to. Solid blue lines with arrows indicate positive and usually direct effects. Solid red lines with blocks indicate negative and usually direct effects. Solid green lines with arrows indicate pathways of glucose metabolism. Solid black lines with arrows indicate pathways of fatty acid metabolism. The broken black line with an arrow indicates a multi-step signalling pathway. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

therapeutic effect [87].

Through its actions on the microbiome, metformin has been reported to increase the production of short-chain fatty acids (SCFAs), but the identity of responsible genera awaits clarification [85]. SCFAs can increase the secretion of glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) from L-cells which are abundant in the colon, and thereby assist in the control of blood glucose, appetite and weight [82]. SCFAs can also suppress inflammation via interaction with fatty acid receptors such as GPR41 and GPR43, and promote browning of white adipose tissue which enhances energy expenditure and weight control [41,88]. Additionally, SCFAs may mediate epigenetic effects on metabolic pathways by altering the activity of histone deacetylases (HDACs) [86].

3.1.2. Enterocytes

The high concentration of metformin in the intestinal mucosa (e.g. up to ~500 μg/g [~3–4 mmol/kg] in human jejunum) compared with venous plasma (~1–2 μg/ml [~10 μmol/L]) is sufficient to interfere with the respiratory chain at complex 1 and increase anaerobic glucose metabolism, making the intestine the main source of increased lactate production by metformin [14,89–92]. Thus, after an intestinal glucose load the amount of glucose emerging across the basolateral membrane is less than the amount absorbed across the apical (luminal facing) membrane due to the diversion of some of that glucose to lactate. This explains the interpretation that metformin can reduce intestinal glucose absorption. Under normal physiological conditions the lactate is converted back to glucose elsewhere in the body, creating an energy-consuming process ('futile cycle') that likely assists metformin's containment of weight gain [90].

The anaerobic effect of high concentrations of metformin in enterocytes is mostly attributed to an inhibitory effect on the mitochondrial respiratory chain described above. The resulting increase in cytosolic NADH favours lactate dehydrogenase (LDH) to convert pyruvate to lactate, and the increase in the cytosolic AMP:ATP ratio increases the activity of AMPK. In the intestine this appears to activate a vago-vagal link to the liver to suppress gluconeogenesis [35]. AMPK also increases secretion of growth-differentiation factor-15 (GDF-15) from the intestine (and kidney): GDF-15 acts centrally to provide one of several mechanisms through which metformin curbs appetite and assists body weight control [93]. Another effect of AMPK is to inhibit the mammalian target of rapamycin (mTORC1), providing pathways that assist infection control and pathways that supress cell growth and division which could contribute to a possible anti-cancer effect of metformin in the colon [6,49]. Additionally, AMPK increases the activity of intestinal goblet cells, thereby enhancing the mucosal barrier and supporting immune protection [94].

Studies assessing the effect of therapeutically relevant concentrations of metformin on the apical sodium-glucose co-transporter-1 (SGLT1) and basolateral bidirectional facilitative glucose transporters GLUT1 and GLUT2 have been inconsistent. This may reflect differences along the intestine because metformin appears to displace glucose absorption and anaerobic metabolism distally along the intestine [10,95–100]. It is noted that increased glucose metabolism by metformin may increase the uptake of glucose across the basolateral membrane when the circulating glucose concentration is high and the glucose concentration within the enterocytes or gut lumen is low [91,101]. This is illustrated by enterocyte uptake of 18F-fluorodeoxyglucose (FDG) during diagnostic positron emission tomography-computed tomography, and by reports of glucose passing from the vascular compartment into the gut lumen during treatment with metformin [101,102]. The passage of glucose across the basolateral membrane appears to involve increased expression and membrane localization of GLUT1 through reduced expression of the thioredoxin-interacting protein (TXNIP), and arrival of the glucose into the gut lumen has been linked to increased production of SCFAs by the microbiome [103,104].

3.1.3. Bile salts and enteroendocrine effects

Metformin at therapeutically relevant concentrations inhibits the apical sodium-dependent bile acid transporter (ASBT) in the ileum, reducing uptake of bile sats from the gut lumen, increasing loss of bile salts in the faeces, decreasing circulating bile salts and so reducing the enterohepatic circulation of bile salts [10,97,105–107]. Reduced hepatic supply of bile salts is anticipated to increase hepatic conversion of cholesterol to bile salts which likely contributes to the reduction in circulating cholesterol often seen with initiation of metformin therapy in hypercholesterolemic patients [108]. Increased bile acids within the distal ileum and colon are also expected to affect the microbiome and stool consistency and may increase intestinal secretion of fibroblast growth factor-19 (FGF19) [10,81,109].

The small increase in secretion of GLP-1 and PYY from enteroendocrine L-cells during metformin therapy, which may be mediated through bacterial SCFAs, may also be promoted through increased AMPK and via increased down-stream effects of luminal bile acids [10,110,111]. Metformin can also prolong active circulating GLP-1 and PYY by decreasing plasma dipeptidyl peptidase-4 activity [112,113]. These effects enable metformin to support the 'incretin effect' that enhances control of prandial glucose excursions, and assist satiety and weight control via GDF-15 [93,114]. Consequent to increased anaerobic metabolism by the gut mucosa, metformin increases production of the metabolite N-lactoyl-phenylalanine (Lac-Phe) which is released into the circulation and provides a further satiety effect [115].

3.2. Liver

Metformin concentrations in the liver (typically 50–200 μM after a 50 mg/kg oral dose in rodents) are much less than in the intestine but still higher than in peripheral plasma [13,30]. The main therapeutic effect of metformin in liver is suppression of gluconeogenesis from lactate/glycerol. This has been observed in many studies with isolated hepatocytes exposed to metformin concentrations ≤100 μM [7,8,43]. It is also a consistent finding in clinical studies of type 2 diabetes (mostly using metformin doses of 1500–2000 mg/day) where suppression of

gluconeogenesis contributes particularly to reduced basal glycemia [116,117]. Metformin has also reduced hepatic glycogenolysis in cultured hepatocytes and in vivo animal studies but this has not been a consistent finding in clinical studies [7,8,116–118]. The suppression of glucose output is partial (typically by 10–20% and rarely >30%), which accounts for the very low risk of overt hypoglycemia. Indeed, the effect is minimal or absent at low glucose concentrations and is overridden by counter-regulatory mechanisms. Accordingly, there is little or no glucose-lowering effect in normo-glycemic individuals [43,116,118,119].

As noted above, the intracellular pathways through which moderate concentrations of metformin (e.g. ≤300 μM) can reduce hepatic gluconeogenesis include suppression of the respiratory chain to enable AMP-activation of AMPK, increased redox state, and AMP-independent activation of AMPK. Many studies have reported that AMPK then inhibits key transcription factors controlling expression of the key gluconeogenic enzymes PEPCK and G6Pase (noted in Section 2.3), [6,8,43,53]. However, it is recognised that these transcriptional effects have not been observed in all studies, suggesting differences between cell models, their energy status and the time frames involved. In addition to transcriptionally-mediated effects, rapid non-transcriptional effects have been reported and await further clarification [43,120]. Also, raised AMP directly reduces the activity of F16BPase and reduces adenylate cyclase activity which impairs glucagon signalling and further contributes to reduced hepatic glucose output [74,75]. In vitro studies indicate that although metformin concentrations in the liver can suppress gluconeogenesis independently of insulin, low metformin concentrations (≤100 μM) accentuate the anti-gluconeogenic action of physiological concentrations of insulin without significant changes to the cytosolic AMP or ATP concentrations, illustrating the subtle interaction of metformin with insulin signalling in this tissue [121].

Another intriguing feature of the anti-gluconeogenic effect of metformin is that clinically relevant concentrations impede glucose production from lactate and glycerol, but not from alanine or pyruvate, indicating dependence on a more reduced cytosolic NADH/NAD^+ ratio [122]. This is consistent with metformin concentrations ≤300 μM acting via the inhibition of mitochondrial complex 4 to interrupt the glycerophosphate shuttle, impede the activity of GPD, raise cytosolic NADH and in consequence suppress gluconeogenic steps requiring NAD^+. This further illustrates the importance of different cellular routes through which metformin can suppress gluconeogenesis acutely, without altering enzyme expression or significantly modifying ATP:AMP balance, depending upon the metformin concentration [43,122]. It also illustrates that the reduction in gluconeogenesis is substrate specific and partial, such that clinical use of metformin does not precipitate overt hypoglycemia.

AMPK inhibits lipid synthesis though inhibition of ACC which then reduces the formation of malonyl-CoA, so reducing fatty acid synthesis and the intracellular accumulation of lipids that would otherwise reduce insulin sensitivity through lipotoxic effects [6,53]. Hepatic insulin sensitivity may be improved by metformin inhibition of phosphotyrosine phosphatases (e.g. PTP1B) that normally deactivate the insulin receptor and its receptor substrates, and long-term insulin action is likely to be improved by reductions in glucotoxicity and lipotoxicity [59,63,72].

3.3. Muscle

The relatively low metformin concentrations in skeletal muscle (e.g. ≤50 μM) are still sufficient to assist in countering insulin resistance by increasing insulin-stimulated glucose uptake, glycogenesis and metabolism without significantly interfering with mitochondrial oxidative phosphorylation and without excess lactate production [6,37,41,116]. Most hyperinsulinemic glucose clamp studies in people with type 2 diabetes have confirmed that the effect of metformin in skeletal muscle is modest (e.g. 5–15% increase in glucose uptake at realistic insulin concentrations) and is more evident in obese than non-obese individuals [66,123–125]. Nevertheless, the body's large muscle mass can still make this a clinically significant impact on whole body glucose homeostasis. Increased glucose uptake is ascribed in part to an AMPK-mediated increase in expression and translocation of GLUT4 glucose transporters, and may also involve enhanced or prolonged insulin signalling as described above [60,64,66,126]. Metformin concentrations <50 μM have been shown to activate AMPK via the AMP-independent PEN2 pathway in hepatocytes, but a study of the effect of metformin on this pathway has not been reported for muscle [52].

It is well established that reducing the glucotoxic effects of persistent hyperglycemia will improve insulin sensitivity as measured by glucose disposal during insulin clamp studies and improve cellular pathways of insulin signalling [72]. Therefore, it is anticipated that improved insulin action during the therapeutic use of metformin in type 2 diabetes will reflect in part the reduction in hyperglycemia achieved through reduced hepatic glucose production. Although metformin does not increase lactate production by muscle during basal metabolism it may increase production of Lac-Phe during exercise [115].

3.4. Adipose tissue

In white adipose tissue the metformin concentration is generally similar or lower than in venous plasma (~10 μM). Nevertheless, metformin has the capacity to increase glucose uptake by this tissue, an effect attributed in part to an AMPK-mediated increase in GLUT4 transporters and insulin signalling similar to that described for muscle [127]. Although this may seem contrary to the ability of metformin to reduce adiposity there are several other relevant effects of metformin in adipose tissue. By raising AMPK in adipocytes, therapeutic concentrations of metformin have been reported to increase mitochondrial biogenesis (via activation of the transcription co-activator PGC-1) and increase the activity of the uncoupling protein UCP-1, thereby increasing thermogenesis and energy expenditure [128]. Additionally, AMPK-mediated phosphorylation of ACC will inhibit malonyl-CoA and reduce lipogenesis, and this is complemented by enhanced lipolysis via activation of hormone-sensitive lipase (HSL) and increased transfer of fatty acids into mitochondria for oxidation [6,12,129]. Some (but not all) rodent studies have noted enhanced mitochondrial biogenesis and an increased thermogenic response to cold exposure by brown adipose tissue during metformin administration, but such an effect has not been evident in man [8,130–134].

4. Diverse effects of metformin beyond diabetes

Metformin has been associated with many effects that are beyond gluco-regulation and outside the remit of this review: the more prominent are summarized in Table 2. Of particular relevance to the complications of diabetes, it is noted that metformin exerts various cardioprotective, anti-atherogenic and anti-coagulant effects as well as reducing markers of chronic inflammation and oxidative stress [4,135,136]. Possible use in the management of metabolic dysfunction-associated steatotic liver disease (MASLD) and lipodystrophy in HIV-AIDS has also been suggested [5]. The value of metformin to reduce hyperinsulinism and hyperandrogenism in PCOS and facilitate ovulation and conception is now widely acknowledged [2,5,17,61,137]. The anti-inflammatory and anti-viral effects of metformin as seen during the SARS-CoV-2 epidemic have been linked to the AMPK-mediated inhibition of mTORC-stimulated cytokine production via nuclear factor-κB (NF-κB) [138,139]. Potentially beneficial effects of metformin to reduce risk or suppress progression of some cancers and some neurological diseases are receiving investigation in clinical trials, and anti-ageing effects of metformin in animal studies are receiving evaluation as a possible approach to increase health span in humans [5,140–142].

Table 2
Examples of the range of effects of metformin beyond nutrient metabolism and cellular energetics.

Target tissue or physiological system	Effect	Mode of action
Cardiovascular		
Endothelium	↑ vasodilation	↑ nitric oxide via AMPK-eNOS pathway ↓ insulin resistance
	↓ monocyte diapedesis	↓ production of cell adhesion molecules ICAM and VCAM via AMPK-NF-κB pathway
Myocardium	Improve parameters of cardiac function	Reports of improved cardiac metabolism e.g. ↑ myocardial energetics, ↑ fatty acid oxidation, ↓ oxidative stress.
Lipid profile	↓ LDL cholesterol	↓ cholesterol synthesis via AMPK-HMG-CoA reductase pathway and ↓ bile salt recirculation
Anti-coagulation	↓ platelet aggregation ↑ thrombolysis	↓ mtDNA release ↓ PAI-1
Chronic inflammation		
Various immune cells Adipocytes	↓ proinflammatory cytokines	↓ IL-1β, IL-6 and TNF-α, ↑IL-10 via AMPK-NF-κB, AMPK-mTORC1 and Dicer-miRNA pathways
Infection		
Viral	↓ Influenza infection	? ↓ viral polymerases and lipid rafts via AMPK-NF-κB pathway
	↓ SARS-CoV-2 infection	↓ ACE2 receptor expression
Cancer risk		
Cancer stem cells	↓ cell growth	↓ insulin, ↓ IGF-1, ↓ protein synthesis via AMPK-mTORC1 pathway
Cancerous cells	↓ tumour proliferation	↑ sensitivity to some cancer treatments ↓ HIF-1α-induced genes ↑ M1 macrophage phenotype (via AMPK?)
PCOS		
Thecal cells	↓ hyperandrogenism	↓ expression of StAR ↓ activity of CYP17 and HSD3B2 ↑ insulin sensitivity ↑ ovulation, conception
Neurodegeneration		
Various neuronal	Slow progression of some neurodegenerative diseases	↓ Tau phosphorylation, ↓ oxidative stress, ↓ amyloid deposition, ↓ inflammation
Ageing		
Various cell types	↑ health span	Overall impact of pleiotropic actions on diverse genetic, epigenetic and enzymatic modulation of cellular functions

↑, increase; ↓, decrease. ACE, angiotensin-converting enzyme; AMPK, AMP-activated protein kinase; CYP17, 17-alpha-hydroxylase; Dicer, endoribonuclease encoded by *DICER1* gene; eNOS, endothelial nitric oxide synthase; HIF-1α, hypoxia-inducible factor 1 alpha; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HSD3B2, 3β-hydroxysteroid dehydrogenase; ICAM, intercellular adhesion molecule; IGF, insulin-like growth factor; IL, interleukin; miRNA, micro-ribonucleic acid, mtDNA, mitochondrial deoxyribose nucleic acid; mTORC1, mammalian target of rapamycin complex-1; NF-κB, nuclear factor κB; PAI-1, plasminogen activator inhibitor-1; PCOS, polycystic ovarian syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; StAR, steroidogenic acute regulatory protein; Tau, tubulin associated unit proteins; TNF, tumour necrosis factor; VCAM, vascular cell adhesion molecule.

5. Clinical precautions

Clinical experience with metformin, which now approaches 70 years, has confirmed a creditable safety profile if prescribing guidance is observed. This section summarises the mechanisms underlying adverse effects of metformin encountered in clinical practice. Prescribers should always consult their country's product label before prescribing metformin.

5.1. Renal dysfunction and conditions associated with low tissue perfusion

The key prescribing precaution for metformin is to avoid use in individuals with impaired renal function (estimated glomerular filtration rate <30 ml/min/1.73^2) and to temporarily discontinue metformin if using contrast media due to risk of contrast-induced nephropathy and subsequent acute renal failure [16,17]. As noted in Box 1, this is because metformin is eliminated unchanged in the urine, and excess drug accumulation can interrupt the mitochondrial respiratory chain and increase anaerobic metabolism with risk to precipitate lactic acidosis (albeit rare: 0.03–0.1/1000 treatment years) [143]. In the absence of other causative morbidities this is usually a type B acidosis (not due to tissue hypoxia), but the additional burden of concurrent tissue hypoxia due to decompensated heart failure, recent myocardial infarction or shock should be appreciated. Acute kidney injury, regardless of cause, poses a greater risk of lactic acidosis than stable chronic kidney dysfunction, implicating dehydration and severe infections as contra-indications [144].

5.2. Mitochondrial disease

Because the mitochondrion is a key therapeutic target for metformin, the possibility of an inherent mitochondrial disease should be considered before prescribing. Underlying mutations in mitochondrial or nuclear DNA are often unrecognised: particular examples are MELAS (typically presenting as stroke-like episodes or seizures), MIDD (diabetes with deafness and macular degeneration), and more rarely MERRF (usually suspected through myoclonus and ataxia), LHON (optic neuropathy causing progressive loss of vision), Kearns-Sayre syndrome (progressive ophthalmoplegia with ptosis) and Leigh syndrome (progressive neurological decline). An estimated 0.5–2.8% of people with type 2 diabetes may have MIDD, while MELAS and MIDD can have adverse effects on the pancreas and other organs that increase the risk of developing a type 2-like form of diabetes [145,146]. The degree of heteroplasmy will determine phenotype and severity of the mitochondrial disease, including basal hyperlactatemia [147,148]. In vitro studies have suggested that cells with mutations of mitochondrial DNA (e.g. with reduced function of complex 1) may be more sensitive to reduced mitochondrial respiration with metformin, and several clinical reports have suggested that metformin could exacerbate the progression of MELAS and MIDD [149–152]. Also, accounts of sudden onset deafness and other neurological conditions occurring with use of metformin have subsequently been linked to pre-existing mitochondrial disease [153–156]. Thus, updated prescribing guidance for metformin in Europe and elsewhere now includes special reference to mitochondrial diseases amongst the risk factors for lactic acidosis: metformin is not recommended in patients with mitochondrial disease.

5.3. Gastrointestinal side-effects

Gastro-intestinal (GI) discomfort or diarrhoea is experienced by some patients initiating metformin, especially if the dose is escalated too quickly and not taken before or with main meals [1]. This is attributed mostly to the high concentrations of drug in the intestine adversely affecting the microbiome and alimentary motility [10]. Reduced absorption of bile salts may also contribute to GI intolerance.

5.4. Excess alcohol and liver disease

Intake of excess alcohol increases the risk of lactic acidosis because the metabolism of alcohol to acetate, which occurs especially in the liver, involves the reduction of NAD^+ to NADH, and the increased cytosolic NADH favours LDH to convert pyruvate to lactate. Hence seriously impaired liver function and alcohol intoxication are contra-indications for metformin.

5.5. Vitamin B12 deficiency

Long-term use of metformin can decrease the absorption of vitamin B12 through interference with the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum [157]. This is not usually sufficient to be a cause of anaemia but can aggravate neuropathic symptoms and is often placated with adequate dairy products or a calcium supplement in the diet.

6. Conclusions

Metformin is an established first-line glucose-lowering therapy for type 2 diabetes that reduces hepatic glucose production, increases splanchnic glucose-lactate cycling and promotes peripheral glucose disposal while tempering appetite, assisting weight control and avoiding overt hypoglycemia [1]. Intracellular actions of metformin vary between tissues depending on the level of drug exposure. Current understanding suggests that high concentrations of metformin (e.g. 3–4 mmol/kg in intestinal mucosa) can interrupt the mitochondrial respiratory chain at complex 1, increase the cytosolic NADH:NAD^+ ratio (which favours lactate production), decrease production of ATP, raise the cytosolic AMP:ATP ratio and activate AMPK. Lower concentrations of metformin in liver (e.g. >50–300 μM) can exert similar but less pronounced effects on the AMP:ATP and NADH:NAD^+ ratios by interrupting the respiratory chain at complex 4. This creates an electron tailback that inhibits mGPD2, impedes the mitochondrial glycerophosphate shuttle which raises the cytosolic NADH:NAD^+ ratio leading to inhibition of glycerol and lactate conversion to glucose. Clinically relevant metformin concentrations can activate AMPK via a lysosomal pathway without significant impact on energy status or redox state such that oxidative metabolism is not impeded. AMPK is a mediator of many of the metabolic effects of metformin including reduced gluconeogenesis, reduced lipogenesis and increased fatty acid oxidation. However, other pathways are also involved, e.g. inhibition of F16BPase and reduced glucagon signalling which further reduce gluconeogenesis. Additionally, direct effects of metformin on the insulin signalling pathway may assist insulin-stimulated glucose uptake and metabolism. Beyond these effects on nutrient metabolism and cellular energetics metformin offers a portfolio of effects that is being evaluated for additional clinical applications, indicating that new opportunities may exist for this adaptable medicine.

CRediT authorship contribution statement

Clifford J. Bailey: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Michael Gwilt:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Kerstin M.G. Brand:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

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Declaration of competing interest

CJB has undertaken consultancy for various pharmaceutical companies involved in the development of medicines for the treatment of diabetes. He has also served on advisory boards, steering committees, safety monitoring boards and manuscript writing groups for clinical trials. He has additionally served as a reviewer and expert witness for regulatory authorities involved in the assessment of diabetes medicines. None of these activities is considered to unbalance his independence or objectivity in the preparation of this review.

MG has provided editorial consultancy services to companies involved in the development of medicines for the treatment of diabetes including Merck Healthcare KGaA, a pharmaceutical sponsor of metformin. This activity is not considered to unbalance his independence or objectivity in the preparation of this review.

KMGB is a full-time employee of Merck Healthcare KGaA, Darmstadr, Germany.

Data availability

This article uses only data extracted from sources in the published public domain.

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