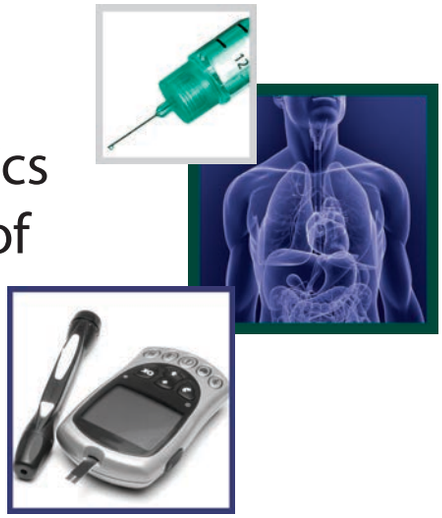
REVIEW

Molecular action and pharmacogenetics of metformin: current understanding of an old drug



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Practice Points

- Metformin uptake and efflux is mediated by a variety of transporters.
- High expression of organic cation transporter 1 and other transporters on hepatocytes ensures efficient delivery of the drug to this key target cell type.
- The key clinical hallmark of metformin in Type 2 diabetes treatment, inhibition of hepatic gluconeogenesis, appears to be mainly mediated indirectly through inhibition of mitochondrial respiration.
- The role of AMPK, a key energy-regulating enzyme that is activated by metformin, in inhibiting gluconeogenesis and lipid accumulation in the liver is uncertain.
- The superior clinical performance of metformin over other Type 2 diabetes drugs is unlikely to be explicable on the basis of glycemic control alone, yet the molecular mechanisms underlying putative glucose-independent therapeutic effects of metformin are poorly understood at present.
- The role for variation in the genes encoding organic cation transporters is inconsistent.
- One locus close to the *ATM* gene has been identified and replicated in multiple cohorts, but this accounts for only 2.5% of the interindividual variability in responses.
- Our understanding of metformin pharmacokinetics, pharmacogenetics and molecular action has improved greatly in recent years.
- Further investigation of new targets for this old drug may trigger the rapid generation of a newer generation of drugs to support metformin therapy.

SUMMARY Metformin is the most commonly used diabetes therapy, with over 100 million patients prescribed this drug per year globally. This popularity stems from a number of factors: it is weight neutral, or in some studies associated with weight loss; and the UK Prospective Diabetes Study (UKPDS) established its cardiovascular benefit and improved mortality compared with nonintensive treatment. Metformin is a very safe drug with over 50 years of clinical use; indeed, metformin has recently been shown to have beneficial off-

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target effects including a reduction in cancer incidence. Metformin, however, remains an intriguing drug with multiple physiological and molecular effects that remain incompletely understood. In this review we address what is known and unknown about the mechanisms of action of metformin, and how an individual's genotype may be expected to alter the efficacy of action or the severity of side effects of this drug.

Metformin, a biguanide, is the most commonly used diabetes therapy, with over 100 million patients prescribed this drug per year globally. The popularity of metformin stems from a number of factors:

- It is weight neutral, or in some studies associated with weight loss;
- The UK Prospective Diabetes Study (UKPDS) established its cardiovascular benefit and improved mortality compared with nonintensive treatment [1] (an effect that was not seen with sulfonylurea treatment or insulin; however, the direct cardiovascular benefit of metformin remains debated);
- Despite the initial safety concerns largely driven by the high rates of lactic acidosis seen with phenformin, metformin is a very safe drug with over 50 years of clinical use; indeed, metformin has recently been reported to have beneficial off-target effects including a reduction in cancer incidence in some [2–4], but not all studies [5];
- In addition, metformin is used in nondiabetic women with polycystic ovarian syndrome to improve fertility [6].

Metformin, however, remains an intriguing drug with multiple physiological and molecular effects that are incompletely understood. In this review we address what is known and unknown about the mechanisms of action of metformin, and how an individual's genotype may be expected to alter the efficacy of action or the severity of side effects of this drug.

Brief history: how plant-derived compounds became a most widely used antidiabetes drug

Structurally, biguanides such as metformin each possess two molecules of guanidine, joined together with the elimination of one molecule of ammonia. The development of guanidine-derived structures in medicine has been traced all the way back to therapeutic use of *Galega officinalis,* which is rich in guanidine and was used as a herbal medicine in medieval Europe [7]. Hypoglycemic properties of guanidine itself were reported in 1918 [8], but it proved too toxic for clinical use, and other guanidine-based compounds were then investigated. Effects of synthetic biguanides on blood glucose were first reported in 1929 by Slotta, his student Tscheche and colleagues [9–11]. At the time, their discovery was overshadowed somewhat by the earlier discovery of insulin [12], and this was exacerbated because biguanides were neither the first nor the most potent noninsulin hypoglycemic agents to have been discovered. Of particular note, diguanides, known as synthalins [13] and structurally related to biguanides, had already entered clinical use when the first paper on metformin's action on blood glucose was published. With war approaching in Europe, Slotta emigrated to Brazil shortly afterwards, dedicating his exceptional talent for much of the rest of his career towards ground-breaking work on snake venom rather than diabetes [11].

By the 1950s, important developments in the field meant that the outlook for metformin had brightened considerably. By then, the proposal that diabetes could be divided into insulin-dependent (Type 1) and noninsulin-dependent (Type 2 diabetes [T2D]) forms [14] had become widely accepted, providing renewed momentum for the search for noninsulin treatments. In addition, many of the competing noninsulin antihyperglycemic diabetes treatments, including the synthalins, had fallen out of favor because of unacceptable toxicity. Thus, in a particularly fertile period during the late 1950s, there was a systematic re-examination of the biguanides [15], Jean Sterne reported the first clinical use of metformin [16], and two other biguanides, phenformin and buformin, both entered clinical use at about the same time. In the 1970s, evaluation of case reports established that these two other drugs are both much more likely than metformin to induce lactic acidosis, which is often fatal [17]. Combined with other concerns, this led to the rapid withdrawal of both phenformin and buformin in most countries where they were in clinical use, leaving metformin as the sole biguanide available for T2D. In the USA, however, clinical use of biguanides was suspended altogether, as metformin did not gain

approval until much later, in 1995 [18]. Also in the 1990s, improved epidemiology established that treatment with metformin was associated with improved outcomes in T2D, compared with other treatment approaches. Thus, compared with sulfonylureas, in prospective studies, T2D patients treated with metformin exhibited a reduced relative risk of all mortality, cardiovascular mortality [19,20] and incidence of cancer [2] (reviewed in [21]). Current professional guidelines indicate the priority of metformin over all other options in the treatment of T2D [22–26].

Pharmacokinetics: key regulatory processes in transport and clearance of the drug

Metformin, an organic cation, is actively transported via organic cation transporters primarily in the intestine, liver and kidneys. **Figure 1** shows how metformin is transported into the intestinal epithelial cells across the apical membrane from the gut lumen via the plasma membrane monoamine transporter (PMAT; encoded by *SLC29A4*) and organic cation transporter 3 (OCT3; encoded by *SLC22A3*), and transported across the basolateral membrane into the bloodstream via organic cation transporter 1 (OCT1; encoded by *SLC22A1*). Once in the portal circulation, metformin is delivered to the liver where it is transported into the hepatocytes primarily via OCT1 and, to a lesser extent (in mice), via OCT3. Mice that lack OCT1 show reduced efficacy of metformin [27] and, while it is difficult to be certain how dramatically the dose–response curve of metformin uptake was shifted in this study as it was carried out at a single physiologically relevant dose, this work did establish a key role of OCT1 in metformin uptake and underlined the pivotal role of the liver as the main site of action for metformin action. Metformin is not metabolized, and elimination is primarily via the kidneys. Again the organic cation transporters play a key role in this process, with transport into the renal tubular epithelial cell at the basolateral membrane via OCT2, and efflux into the urine via multidrug and toxin extrusion (MATE) 1 and 2 (encoded by *SLC47A1* and *SLC47A2*, respectively). The renal excretion of metformin means that metformin accumulates in renal impairment, increasing the risk of lactic acidosis. For this reason the dose of metformin should be limited to 1 g daily when the eGFR is below 45 ml/min, and metformin should not be used when the eGFR is <30 ml/min. There is

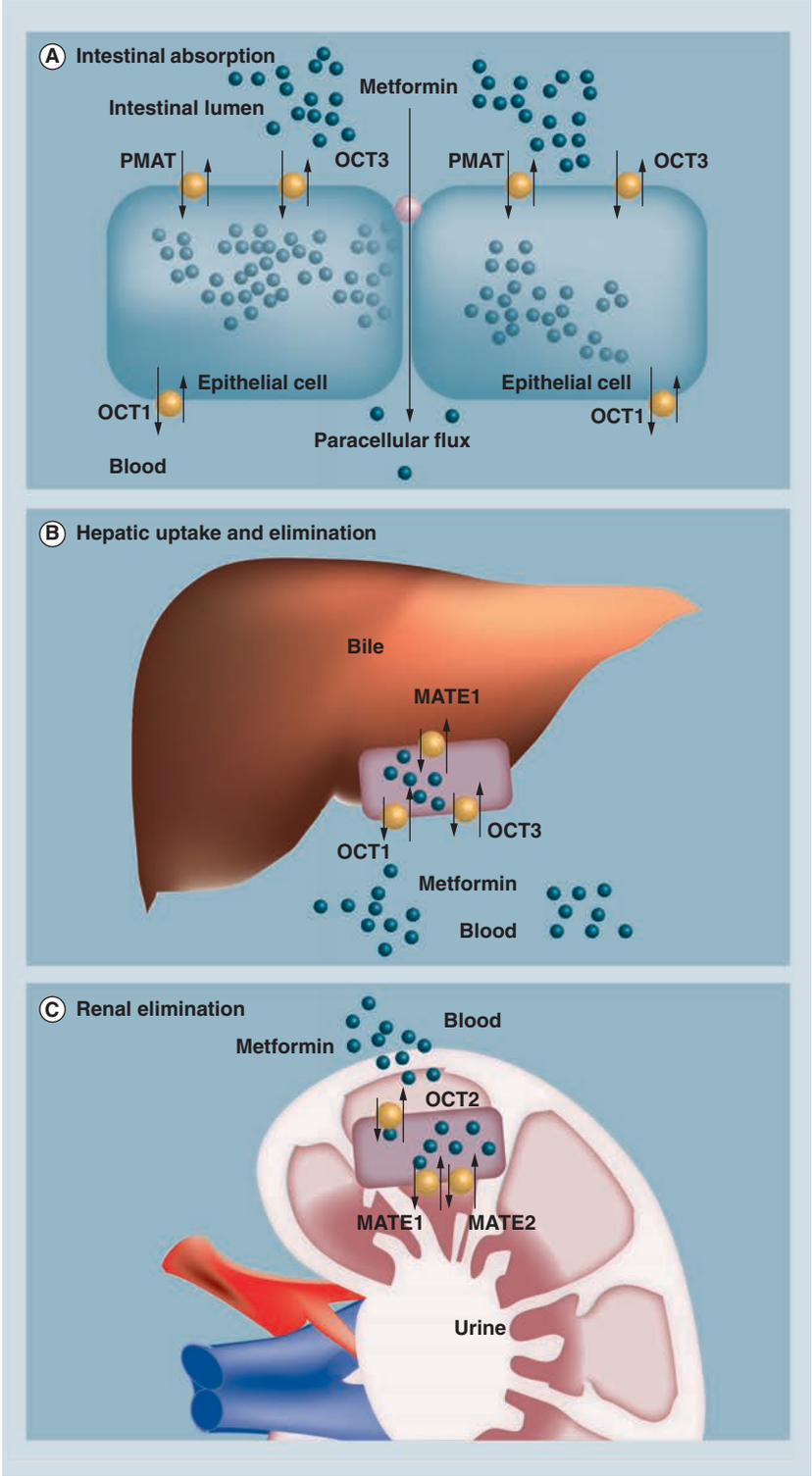

Figure 1. Transport of metformin by organic cation transporters. The distribution of organic cation transporters and their role in metformin absorption in the intestine **(A)**, uptake into the liver **(B)** and elimination via the kidney **(C)**.
MATE: Multidrug and toxin extrusion; OCT: Organic cation transporter; PMAT: Plasma membrane monoamine transporter.

considerable interindividual variation in the metformin concentrations achieved despite the same daily dosing, with one large study showing that trough steady state concentrations on 1 g twice-daily metformin dosing varied within a range of approximately 0.4 μM to 32 μM, with a mean concentration of 4.5 μM [28]. In response to oral administration, intestinal mucosal cells receive the highest concentrations of metformin [29,30], with the concentration in intestinal epithelial cells between 100- and 1000-fold that seen in the serum. This may underlie the gastrointestinal side effects (e.g., diarrhea and abdominal discomfort) frequently observed in metformin use [31].

Molecular action of metformin: what is known and not known about how metformin actually works

Early molecular studies on the diguanides (forerunners of biguanides) had established that hypoglycemia with these agents was accompanied by a striking reduction in oxygen consumption [32], implicating reduced respiration as one possible locus of action of other guanidine-based antidiabetic agents quite early on. Later work found that guanidine [33,34], diguanides [34] and phenformin [35,36] were all effective inhibitors of oxygen consumption in isolated mitochondria from a variety of tissues.

These common effects promoted the idea that biguanides inhibited gluconeogenesis indirectly, through inhibition of mitochondrial respiration [37]; however, others maintained that if mitochondrial effects contributed anything to biguanide drug action, it was perhaps more likely to be side effects such as lactic acidosis, rather than therapeutic effects [38]. There were several reasons for this scepticism. For example, antihyperglycemic effects did not always correlate with effects on respiration even amongst the guanidine-related drugs described above. Particularly where metformin was studied, it was sometimes difficult to obtain any measurable impact on cellular ATP levels in response to treatment, even at concentrations well above those achieved *in vivo*. However, in 2000, studies employing freeze-clamped livers, as well as hepatocytes and mitochondria isolated from rodents, suggested that metformin represses hepatic glucose output by inhibiting complex I of the mitochondrial electron transport chain **(Figure 2)** [29,39]. This oxygen-consuming process couples glucose breakdown and fatty acid oxidation to ATP production, providing the bulk of most cells' energy requirements. One key piece of evidence supporting complex I involvement in both these studies was that metformin inhibited the mitochondrial oxidation of glutamate and malate much more effectively than succinate [29,39]. Succinate is a complex II substrate that can bypass complex I inhibition; therefore, the differential effects of metformin on metabolism of these substrates suggested that it acts on complex I. These findings were similar to earlier studies on guanidine and related alkylguanidines [33,34]; however, understanding of the molecular details of mitochondrial respiration was insufficient to allow the data to be interpreted in this way until later. One caveat is that recent studies have identified cell-to-cell variations in the effects of metformin on mitochondrial respiration, but further work is required to determine the underlying reasons for these variations [40]. It is also worth noting that it has not yet been possible to validate by genetic experiments whether or not complex I is the only mitochondrial target of metformin.

In the studies with metformin, the correlation between the magnitude of inhibition in gluconeogenesis and that of the respiratory chain suggested that cellular energy depletion caused by metformin results in a shortfall of ATP required for energy-consuming hepatic gluconeogenesis [29]. In addition, this work also provided an explanation of lactic acidosis. As a consequence of respiratory chain inhibition, an elevation of glycolytic lactate production would be predicted to occur. Compared with other biguanides that have been used clinically in the past, incidence of lactic acidosis is much less common during metformin treatment, possibly because metformin uptake into the mitochondria is thought to require them to be actively respiring [29]; thus, this inhibition is thought to be self-limiting. This property may also explain why metformin is much less toxic than other well-known complex I inhibitors such as the neurotoxic pesticide rotenone [29]. Interestingly, thiazolidinediones, another clinically used T2D drug, are also mild inhibitors of respiratory chain complex I, which may at least partially contribute to their antihyperglycemic efficacy [41].

In 2001, Zhou *et al.* proposed a novel molecular insight that an enzyme called AMPK might be the key mediator of metformin's effects in lowering blood glucose and lipid levels [42]. AMPK is an important regulator of cellular energy homeostasis that coordinates metabolic pathways in order to balance nutrient/substrate supply with energy demand. AMPK is activated by metabolic stresses

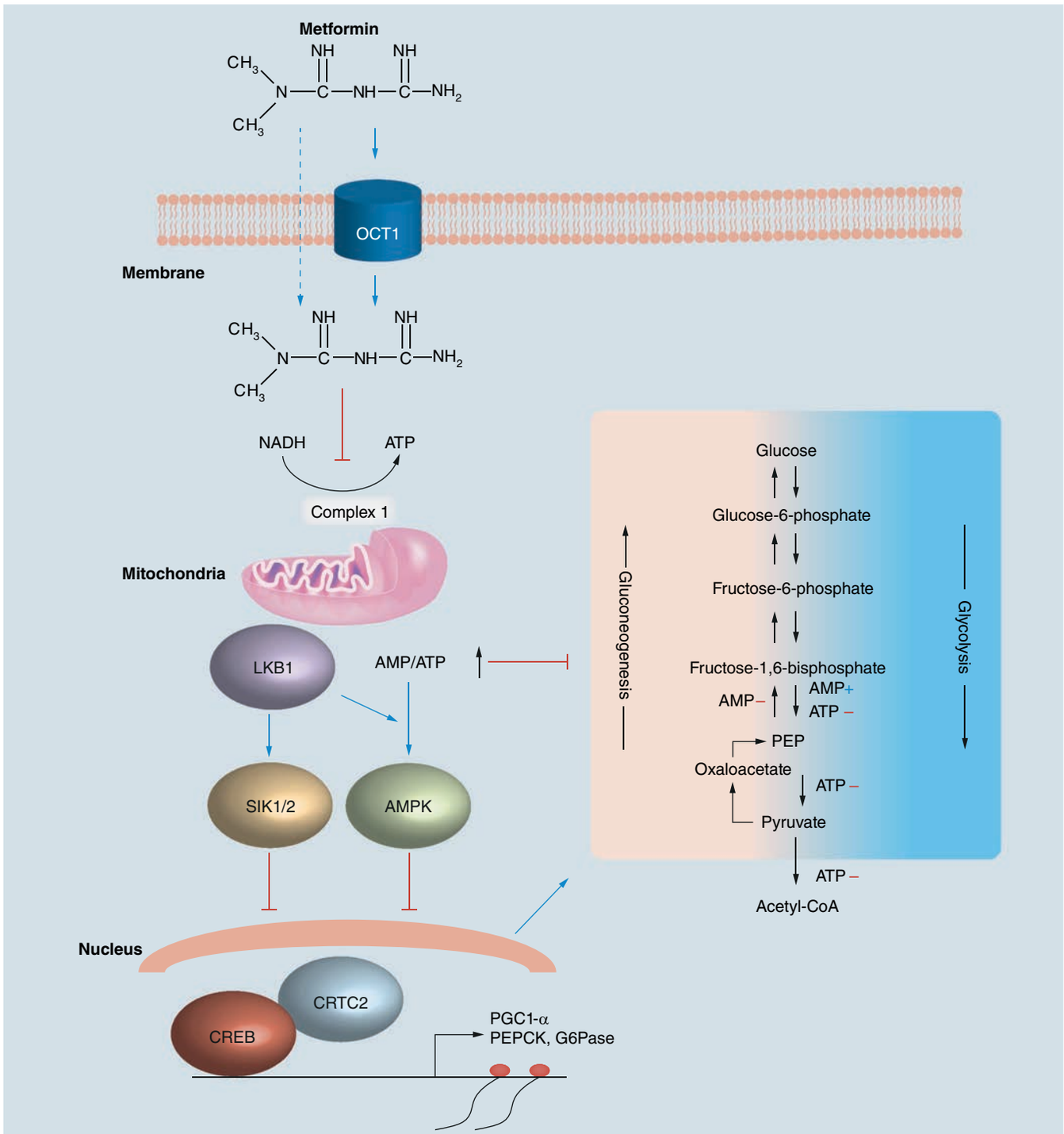

Figure 2. Currently proposed molecular pathways that control hepatic gluconeogenesis by metformin. Metformin is thought to be transported into hepatocytes mainly via OCT1, resulting in an inhibition of the mitochondrial respiratory chain (complex I) by an unknown mechanism(s). This causes an increase in the AMP:ATP ratio, which leads to the activation of AMPK via LKB1. LKB1/AMPK-dependent pathways have been proposed to inhibit gluconeogeic gene expression, and hence glucose output. By contrast, a recent genetic study by Foretz *et al.* has demonstrated that the LKB1/AMPK pathway is dispensable and AMPK-independent mechanisms such as AMP- and ATP-mediated metabolic events directly effect glycolysis and gluconeogenesis [64].
+: Activation; –: Inhibition; OCT1: Organic cation transporter 1; PEP: Phosphoenolpyruvate.
Adapted with permission from [65].

that increase cellular ADP:ATP and AMP:ATP ratios either by reducing catabolic generation of ATP (e.g., glucose deprivation, hypoxia, ischemia and treatment with metabolic poisons), or by accelerating ATP consumption (e.g., muscle contraction) [43]. Several excellent and extensive review articles on AMPK can be found elsewhere [44–46]. Zhou *et al.* reasoned that AMPK is an attractive and logical molecular target/mediator of metformin [42], as: physiological consequences of AMPK activation (e.g., glucose uptake in muscle, inhibition of lipid synthesis and promotion of lipid oxidation in the liver [45]) mimic the therapeutic effects of metformin; and metformin was known to cause energy stress/depletion (i.e., decreased cellular ATP levels) in hepatocytes [29,39] that were also known to trigger AMPK activation. They elegantly demonstrated that metformin stimulates AMPK in a dose- and time-dependent manner, significantly elevates fatty acid oxidation and suppresses the expression of lipogenic enzymes in rat primary hepatocytes [42]. Moreover, a prolonged incubation of isolated skeletal muscle *ex vivo* with a high concentration of metformin (2 mM, 3 h) resulted in an increase in AMPK activity, which was associated with a modest but significant increase in glucose uptake that was also observed to be additive with the effect of insulin treatment [42]. Although these interesting findings by Zhou *et al.* were rather observational, they were substantiated further by the use of a novel and selective pharmacological AMPK inhibitor termed 'compound C'. When primary hepatocytes were pretreated with this compound, metformin-induced inhibition of glucose production was reversed, and suppression of acetyl CoA carboxylase (ACC), a target of AMPK that functions to play a key role in lipid metabolism, was attenuated [42]. It should be noted that although compound C has been a useful tool to understand the downstream effects of AMPK activation in intact cells, it has recently been shown that it is not a specific inhibitor for AMPK and produces numerous off-target effects [47,48]. It has been demonstrated that metformin does not directly modulate AMPK activity (as it does not activate purified AMPK in cell-free assays); rather, as described above, it indirectly stimulates AMPK through an increase in cellular AMP:ATP and ADP:ATP ratios via the inhibition of the mitochondrial respiratory chain.

Some early studies suggested that one of the key effects of metformin (in addition to its effects on liver) was to potentiate the effect of insulin [49] and/or stimulate glucose uptake (independently of the insulin pathway) in muscle [42,50,51], although other studies failed to observe a similar effect [52]. A growing body of current literature from both human and animal studies suggests that the antihyperglycemic action of metformin is primarily to reduce hepatic glucose output, by suppression of gluconeogenesis [53,54]. Although the drug may accumulate over longer periods in muscle and other tissues to produce some effects [55], the concentration of metformin (1–2 mM, 3–16 h) that was needed to stimulate glucose transport and activate AMPK in isolated skeletal muscle [42] or cultured muscle cells [50] was 1–2 orders of magnitude higher than those (10–20 μM) estimated in human plasma after therapeutic doses [56,57]. However, as described earlier, the majority of the effects of the drug are believed to occur via inhibition of hepatic gluconeogenesis, and being supplied directly from the gut by portal vein, the liver would be exposed to a much higher concentration of orally administered metformin than peripheral tissues (e.g., muscle, adipose) [58]. In addition, this preferential action of metformin in hepatocytes might be accelerated further via OCT1 and other transporters as described earlier.

The role of liver AMPK during metformin treatment was further highlighted by findings from a mouse model in which an upstream kinase regulator of AMPK, called LKB1, was specifically ablated in hepatocytes (from adult animals) [59]. The mice showed a marked increase in fasting blood glucose levels and impaired glucose tolerance. In liver LKB1-deficient mice, metformin treatment failed to activate hepatic AMPK and also failed to elicit a glucose-lowering effect when these mice were rendered hyperglycemic using high-fat diet. To investigate the molecular mechanism underlying the effects of LKB1 deletion on glucose homeostasis, Shaw *et al.* examined the expression of several genes that are involved in hepatic gluconeogenesis and also genes involved in lipogenesis that were significantly elevated in mice lacking hepatic LKB1 [59]. The LKB1–AMPK pathway is thought to regulate a transcription coactivator called CREB-regulated transcription coactivator 2 (CRTC2), which was identified as a key regulator of hepatic glucose production in response to fasting by directing transcriptional activation of the gluconeogenic program (reviewed in [60]). Briefly, in the fed state, CRTC2 is sequestered in the cytoplasm; however, in response to fast (or stimuli

mimicking fasting such as glucagon treatment), CRTC2 is dephosphorylated and transported to the nucleus, where it enhances the transcriptional activation of the gluconeogenic genes. This includes induction of CREB-dependent transcription of PPAR-γ coactivator-1α (PGC-1α), master regulator of cellular energy control [61], and its subsequent gluconeogenic target genes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6Pase), key enzymes in gluconeogenesis. A specific covalent modification of CRTC2 (phosphorylation on Ser171 residue) catalyzed by AMPK is critical for determining the activity, cellular localization and degradation of CRTC2. However, it has also been reported that a family of protein kinases that have sequence homology to AMPK (therefore called 'AMPK-related kinases' [62]) can regulate/phosphorylate CRTC2 via the same (Ser171) and/or other sites (including Ser275) on CRTC2, which suggests that multiple signaling pathways converge to control CRTC2 activity [61]. A more recent study has reported that members of class IIa histone deacetylase (HDAC4, 5 and 7), modulators of transcription including gluconeogenic genes, are regulated by AMPK, which might contribute to the glucose-lowering effect of metformin [63].

To dissect if the effects of metformin on hepatic glucoeneogenesis are mainly mediated through AMPK or other LKB1-regulated kinases (i.e., AMPK-related kinases), Foretz *et al.* have recently generated and analyzed two mouse models either genetically lacking both AMPK catalytic subunits (α1 and α2) or LKB1 in hepatocytes [64]. They clearly demonstrated that AMPK and LKB1 are dispensable for metformin-induced reductions in hepatic glucose production (at least in mice). An acute administration of metformin to liver AMPK-deficient mice produced a normal glucose-lowering effect (comparable with control wild-type mice). In addition, treatment of primary hepatocytes lacking AMPK or LKB1 with metformin displayed a robust inhibition of glucose production [64]. It was somewhat surprising that metformin-induced inhibition of glucose output was normal in LKB1-deficient hepatocytes, as it seemed difficult to reconcile with the work of Shaw *et al.* described above [59]. The doses and delivery routes of drug administration were different. It should also be pointed out that Shaw *et al.* did not measure an acute effect of metformin treatment on hepatic glucose production, but rather they reported that the effect of daily injection of metformin to reverse high-fat diet-induced hyperglycemia was abolished in liver LKB-deficient mice. Therefore, it can be argued that in Shaw's study, metformin did not act directly on the regulation of glucose production, but perhaps indirectly exerted effects by improving liver steatosis imposed by a high-fat diet (thereby reducing lipotoxicity and insulin resistance known to enhance hepatic glucose production). In the study of Foretz *et al.*, metformin caused a reduction in cellular ATP and a concomitant increase in AMP, which the authors suggest is responsible for the metformin-induced reductions in hepatic glucose production [64]. Given that gluconeogenesis is an energy-demanding process (requiring six ATP equivalents per molecule of glucose synthesized), in order to maintain normal cellular energy homeostasis, hepatocytes would be obliged to balance this energy demand with production, likely via the oxidation of substrates such as glucose and fatty acids [65]. Since metformin is understood to be a mild 'mitochondrial poison' that causes a modest energy depletion, it is likely that energy charge decreases and glucose production is inhibited. Moreover, Foretz *et al.* [64] used a recently developed direct and widely used AMPK activator, A-769662 (that directly activates AMPK independently of AMP-binding site [66,67]) and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR, an AMP mimetic), and showed that the former had no effect on glucose production, but the latter robustly inhibited glucose production in liver AMPK-deficient mice, illustrating that AMP *per se*, but not AMPK, might play an important role in suppressing glucose output in the liver. Previous studies defined the exquisite control energy charge can exert on gluconeogenic flux through allosteric regulation of key enzymes in this pathway. For example, AMP inhibits the gluconeogenic enzyme fructose-1,6-bisphosphatase [68]. Finally, although mouse genetic models are powerful and elegant tools, we should keep in mind that a loss of key metabolic genes would possibly cause compensatory adaptations, leading to the creation of an alternative pathway(s) for survival, and results from mouse studies are not always applicable to human physiology.

New molecular insights: target of metformin

One important outstanding issue has been the identity of metformin's direct target. There is little evidence of direct binding of metformin to any

of the signaling or mitochondrial proteins already described. Recent research indicates that the ability of metformin to directly bind metal ions, which has been substantiated by x-ray crystallography [69] and other spectroscopic approaches [70–72], contributes to the biological action of the drug **(Figure 3)** [73]. Biguanide/metal interactions were first described in the 19th century [74] and also were investigated by Slotta and Tschesche in a paper published back-to-back with their seminal work on metformin's antihyperglycemic properties [9,75]. After Slotta, several decades of investigations have established that biguanides' extensive π-electron delocalization enables metformin to form a rigid metal-binding scaffold that generates unusual 'pseudoaromatic' planar ring structures, particularly with copper [70–72,76], with square planar geometry replacing more conventional tetragonal geometry [69,70]. Using analogs of metformin's structure, it has recently been shown that the drug's π-electron delocalization also enables biguanides to regulate AMPK, glucose production, gluconeogenic gene expression, mitochondrial respiration and mitochondrial copper binding [73]. By contrast, regulation of ribosomal S6 protein phosphorylation, a key event that regulates protein synthesis and cell growth, was prevented only by direct modification of the metal-liganding groups of the biguanide structure [73], supporting recent data that AMPK and S6 protein phosphorylation are regulated independently by biguanides [77,78]. The dependence of many of the hepatic effects of metformin on π-electron delocalization is consistent with earlier evidence that analogs of biguanides, where this delocalization is interrupted, do not exhibit antihyperglycemic properties [79]. In addition, striking similarities between this metal-coordinating core of metformin and the thiazolidinedione moiety of pioglitazone might underlie the similar effects of these two drug classes on mitochondrial respiration [29,39,41], AMPK and signaling to S6 protein [73,80,81]. Finally, supporting studies found that preincubation of cells with a copper-sequestering drug blocked metformin signaling responses [73].

At the molecular level, without excluding the possibility of important metal-independent cellular responses to metformin's rigid structure, future work will explore the ability of metformin to regulate metalloenzymes or perhaps even to act as a copper 'exchanger' using geometry switching to excise metals from high-affinity binding pockets in proteins. It has been known for many years that T2D results in altered copper metabolism [82] and, in addition, recent studies using genetic knockout of copper transporters suggest that the mitochondria are an important reservoir of cellular copper [83], dysfunction of which can result in cardiomyopathy [84]. Moreover, there is evidence that drug-based copper-sequestration improves left ventricular hypertrophy in people with diabetes, independent of effects on glycemia [85,86]; therefore, it will be interesting to investigate the role of copper in the cardioprotective properties of metformin.

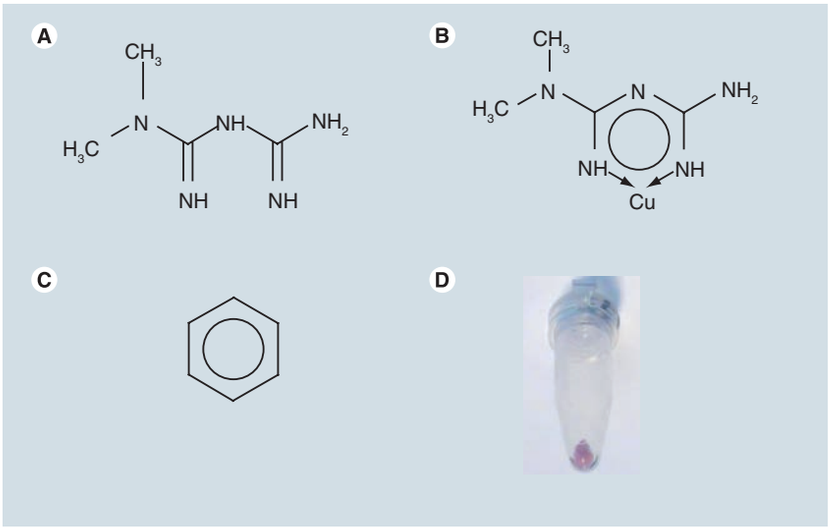

Figure 3. Metformin's antihyperglycemic properties depend on its ability to form 'pseudoaromatic' complexes with metal ions. In contrast to the paucity of evidence of direct interaction of metformin **(A)** with specific cellular proteins, X-ray crystallography and other spectroscopic analyses have provided compelling evidence of interaction of the drug with metals, particularly copper ions, and the structure of this interaction has been determined [69–72,76] **(B)**. The metformin/copper structure has been termed 'pseudoaromatic' by analogy with the aromatic structure of benzene **(C)**, with each possessing a ring of electron delocalization. Key aspects of this unusual structure are: the copper is induced to adopt square planar geometry; and the bonds that metformin shares with the metal are strengthened by considerable double-bond character (in other words they are stronger than usual metal/ligand bonds). Unlike most complexes with nitrogen-donor ligands, which are usually blue or green, these unusual features of the 2:1 metformin/copper interaction result in a pink-reddish colour **(D)**. As discussed in the text, investigation of responses to analogs of the drug suggest that the ability of metformin to form pseudoaromatic structures is required not only for the antihyperglycemic properties of the drug [73,79], but also key cellular responses, including AMPK activation and inhibition of mitochondrial respiration [73].

Pharmacogenetics of metformin: how an individual's genotype may be expected to alter the efficacy of action or the severity of side effects of this drug

There is considerable variation in the glycemic response to metformin. **Figure 4** shows the

distribution of absolute glycated hemoglobin (HbA_{1c})reduction in those starting on metformin monotherapy with a baseline HbA_{1c} between 7 and 8%. To date, the only clinical parameter associated with response (other than the baseline HbA_{1c}) is the creatinine clearance (greater response with decreased renal clearance); contrary to popular belief, BMI is not associated with response, with a similar response in obese and nonobese [87]. We are not aware of any studies that have investigated the association between insulin resistance and glycemic response to metformin, but by lowering hepatic glucose output in an insulin-independent mechanism there is no particular reason why metformin should work better in insulin-resistant patients. Given the lack of clinical association with response it may be that at least some of the variance can be explained by genetic factors. These genetic factors may be divided into variants that alter metformin distribution (pharmacokinetic pharmacogenetics) and those that alter metformin action (pharmacodynamic pharmacokinetics).

Given the recent findings for the role of the organic cation transporters on the distribution of metformin, there have been a number of recent papers investigating the effects of genetic variation in these genes on metformin pharmacokinetics and response; however, despite the clear role for these transporters in metformin transport *in vitro* or in mice, the impact of genetic variants on the treatment response in patients with diabetes remains inconsistent.

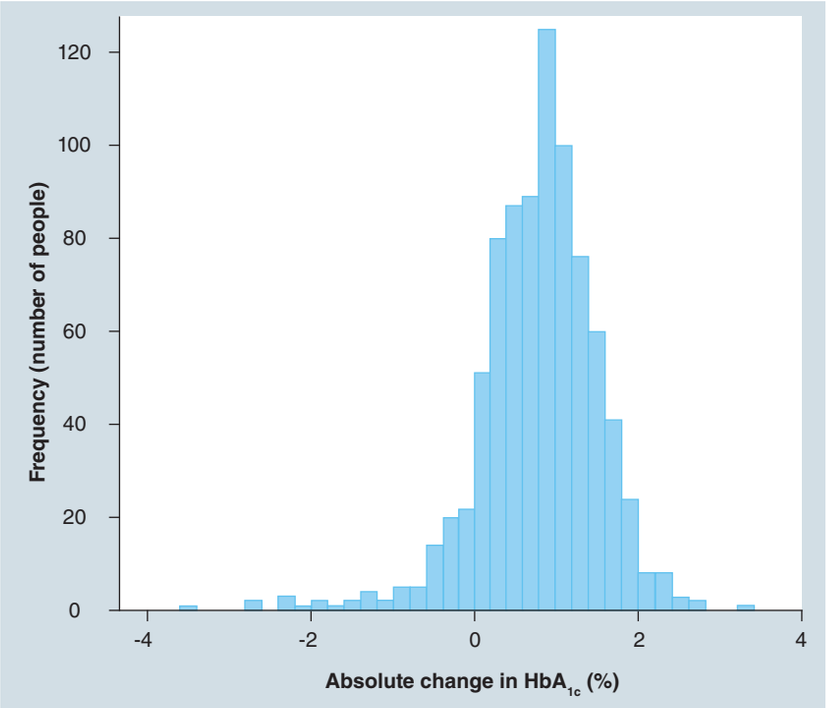

Figure 4. Histogram representing the absolute glycated hemoglobin change seen on starting metformin monotherapy for patients from Tayside, Scotland, with a baseline glycated hemoglobin between 7 and 8% (53–64 mmol/mol).
HbA_{1c} is a measure of glycemia reflecting glucose levels from the previous 2–3 months. The X-axis shows the HbA_{1c} change calculated by subtracting the minimum HbA_{1c} achieved in the first 18 months of treatment from the HbA_{1c} immediately prior to starting metformin, and the Y-axis represents the number of people who achieve that HbA_{1c} change.
HbA_{1c}: Glycated hemoglobin.

■ *SLC22A1*

A study investigating variants in this gene encoding OCT1 identified functional variants in human *SLC22A1* including R61C (rs12208357), G401S (rs34130495), 420del and G465R (rs34059508), and showed that individuals carrying these variants had a higher concentration of metformin after dosing (area under the curve and C_{max}) [88] and greater efficacy of metformin at reducing glucose excursion after an oral glucose tolerance test in 12 nondiabetic controls [27]. However, in contrast to this a study, in the GoDARTS study a population of 1500 patients with T2D treated with metformin showed no effect of the two most common loss of function polymorphisms (R61C and 420del) on initial HbA_{1c} reduction or time to failure of metformin monotherapy [89]. Other studies report variable outcomes with respect to variants in OCT1 in patients with diabetes. In a Danish prospective trial of 159 patients, there was a significant association of the number of reduced function alleles carried and the pharmacokinetics and pharmacodynamics of metformin response, primarily at 6 months [28]. This effect was reduced by 12 months, and was largely driven by the G401S variant, which was not tested in the GoDARTS study. In a study of 102 patients with diabetes from the Rotterdam study where 11 tagging single nucleotide polymorphisms (SNPs) were genotyped across OCT1, one SNP (rs622342) was associated with glycemic response although with only marginal significance after Bonferroni correction [90]. This same SNP was not found to be associated with the efficacy of metformin in delaying diabetes onset in the Diabetes Prevention Program [91], although another missense polymorphism, L160F, was reported to be associated in this study. In summary, OCT1 variants probably impact on metformin response, but this is less

dramatic than anticipated from mouse models. Other transporters may contribute more than is generally realized to metformin transport in these species. It may be significant, for example that OCT3, which is expressed in the liver, has Michaelis–Menten constant (K_m) and velocity maximum values similar to OCT1 [92]. A high interindividual variability of expression of both of these two transporters has been reported, and therefore OCT3 could be an important alternative to OCT1 in some cases [92]. In addition, OCT2 (described below) has a much lower K_m (and higher velocity maximum) for metformin than OCT1, whose K_m for metformin is in the millimolar range [93], but at least in healthy humans, OCT2 expression seems to be restricted to the kidney [94], as discussed below.

■ *SLC22A2*

This gene encoding the renal transporter OCT2 has been less studied than OCT1. Variants in human OCT2 have been associated with differences in pharmacokinetics [95–97]. In Han–Chinese, the A270S polymorphism is associated with increased plasma concentrations of metformin and increased plasma lactate concentrations, although this data arises largely from only two rare homozygotes [98]; this same SNP does not show association with metformin concentration or HbA_{1c} reduction in the Danish prospective trial described above [28]. Finally Jablonski *et al.* studied 44 tag SNPs for OCT2 and found no association with efficacy of metformin in the Diabetes Prevention Program, including the A270S variant [91].

■ *SLC47A1*

This gene encodes the renal efflux transporter MATE-1. One small study suggested that a SNP rs2289669 was associated with metformin response [99], and this was replicated, again with borderline significance in the Diabetes Prevention Program study (rs8065082, $r^2 = 0.8$) [91]. However, once again this is not a consistent finding as there was no effect on plasma steady state levels or on glycemic response to metformin for rs2289669 in the Danish prospective study [28].

In considering the pharmacodynamic pharmacogenetics of metformin, it is surprising that only very few candidate studies have been reported. This may reflect the uncertainty around the molecular mechanisms for how metformin impacts on glycemia. A few very small studies have reported no association with metformin with variants in the AMP-activated protein kinase genes (*PRKAA1, PRKAA2, PRKAB1, PRKAB2, PRKAG1* and *PRKAG2*) and the upstream kinase LKB1 (encoded by *STK11*). The most comprehensive study to date was in the Diabetes Prevention Program where all these genes were tagged [91]. Variants in five genes were associated with response, but only with nominal significance that did not withstand adjustment for multiple testing. There is clearly a need for more comprehensive pathway-driven candidate gene studies for metformin. However, recently, a genome-wide approach has been employed, which makes no prior assumption about candidacy. The GoDARTS and UKPDS metformin pharmacogenetics study group carried out a genome-wide association study on approximately 1100 patients treated with metformin [100]. In this study, one locus on chromosome 11 was associated with metformin response with a p-value of 1.9×10^{-7}. This locus was subsequently replicated in two independent cohorts, including UKPDS, with a combined overall p-value of 2.9×10^{-9}. This genetic association has been subsequently replicated in additional European cohorts [101], making this the most robust metformin pharmacogenetic variant to date. However, of note, this locus only explains 2.5% of the variance in metformin response. The locus on chromosome 11, tagged by rs11212617, consists of a large linkage disequilibrium block encompassing seven genes. The causal gene and variant remains to be determined, but there is considerable supporting literature to point to the *ATM* gene as the likely candidate at this locus. The recent proposal that ATM is involved in metformin action through directly or indirectly controlling AMPK has been questioned due to off-target effects of the ATM inhibitor KU-55933 on OCT-1-dependent uptake of metformin [102–104]. Although it is worth noting that the original study found that KU-55933 inhibition of AMPK activity was sustained even at concentrations of metformin around ten-times above the K_m for OCT1 uptake [100], experiments using alternative approaches such as use of mouse knockout/knockin models are necessary to resolve the role of ATM in metformin action. Whilst this has yet to be confirmed, if established, the use of a genome-wide approach will provide novel insight into the molecular mechanisms of metformin and the role of ATM in mediating glucose response to metformin.

Conclusion & future perspective

■ Pharmacogenetics

The pharmacogenetics of metformin has progressed considerably in the last few years, providing insight into the distribution of metformin and its action. However, as yet, none of the organic cation transporter variants are consistently associated with response, and the one robust variant at the *ATM* locus only has a very small impact on response. Therefore, there has yet to be translation of these results to clinical practice. Hopefully, with increasing collaboration internationally, and the study of rare variants in large cohorts with extreme response/nonresponse, we will see the clinical application of pharmacogenomics within the next decade.

■ Molecular action

The most widely accepted model of metformin's antihyperglycemic action is that it occurs principally through suppression of hepatic gluconeogenesis. AMPK, a key metabolic sensor and regulatory protein, certainly responds to metformin and has been proposed as an important mediator; however, it is still questionable to what extent it plays a role in inhibiting gluconeogenesis (and also lipid accumulation in liver and stimulating glucose uptake in muscle). The most recent work, mainly from mouse genetic studies, has indicated that the effects of metformin on gluconeogenesis may depend more directly on the rate of mitochondrial respiration [64], as was originally suggested [29], or on as yet unidentified/undefined AMPK-independent cell responses to reduced ATP availability. In addition, some recent studies suggest that other cellular components, including the protein kinase ATM and copper ions, may also play an important role in mediating the antidiabetic action of this drug. Further work on these novel aspects of metformin action may, in addition, provide better insight into the cardioprotective and anticancer properties of this agent, which remain very poorly understood.

Although the main physiological mechanism whereby metformin lowers plasma glucose is by lowering hepatic glucose production in the fasting state [105], it has long been recognized that metformin exerts some of its antihyperglycemic effect by decreasing gut glucose absorption and increasing gut glucose utilization [106]. More recently, metformin has been shown to increase GLP-1 concentrations and GLP-1R expression in mouse islets in a PPARα-dependent, AMPK-independent mechanism [107], suggesting a role for the incretin axis in mediating some of the effects of metformin. Finally, it is important also to keep in mind that however important suppression of hepatic glucose output may be in controlling diabetes, the improved outcomes of metformin therapy over other treatments are not explicable on the basis of glycemic control alone [1], and potential glucose-independent protective effects of metformin are very poorly understood at present.

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