

## **Revisiting the mechanisms of metformin action in the liver**

### ***Les mécanismes d'action de la metformine dans le foie révisités***

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## **Abstract**

Although considerable efforts have been made since the 1950s to better understand the action of metformin, the first line therapeutic for type 2 diabetes, its mechanisms of action has not been fully elucidated. The main antidiabetic effect of this drug is to decrease hepatic glucose production. A plausible molecular mechanism of action now emerges from recent breakthroughs that place metformin at the control of energy homeostasis. Metformin was shown to induce a mild and transient inhibition of the mitochondrial respiratory chain complex 1. The resulting decrease in hepatic energy state activates the AMP-activated protein kinase (AMPK), a cellular metabolic sensor, and provided a generally accepted mechanism for metformin action on hepatic gluconeogenic program. However, the role of AMPK activation in metformin action has recently been challenged by loss-of-function experiments. Recent evidence showed that metformin-induced inhibition of hepatic glucose outputs mediated by reducing cellular energy charge rather than direct inhibition of gluconeogenic gene expression. Furthermore, recent data support a novel mechanism of action for metformin involving antagonism of glucagon signaling pathways by inducing the accumulation of AMP, which inhibits adenylate cyclase and reduced levels of cAMP.

## **Keywords**

Metformin ; hepatic glucose production; energy charge; AMP-activated protein kinase; adenylate cyclase.

## **Résumé**

*Malgré l'utilisation depuis les années 50 de la metformine, l'antidiabétique le plus couramment utilisé dans le traitement du diabète de type 2, son mécanisme d'action n'a pas encore été totalement élucidé. Sa cible principale reste le foie où elle diminue la production hépatique de glucose. De nouvelles pistes ont commencé à émerger avec la description de son effet sur le métabolisme énergétique. La metformine est un inhibiteur modéré du complexe 1 de la chaîne respiratoire, ce qui provoque une diminution de la charge énergétique dans le foie et l'activation du senseur énergétique AMPK (protéine kinase activée par l'AMPK), permettant ainsi d'apporter une explication au mécanisme d'action de la metformine dans l'inhibition de la gluconéogenèse hépatique. Cependant, le rôle de l'AMPK a récemment été remis en question dans des expériences de perte de fonction. Il a été mis en évidence que la metformine inhibe le flux gluconéogénique en réduisant la disponibilité en ATP plutôt que par un effet direct sur l'expression des gènes de la gluconéogenèse. De plus, un nouveau mécanisme d'action de la metformine a été proposé impliquant un antagonisme des effets du glucagon par une accumulation de l'AMP intracellulaire, ce qui provoque l'inhibition de l'adénylate cyclase et une réduction des taux d'AMP cyclique.*

## **Mots clés**

*Metformine, production hépatique de glucose; charge énergétique ; protéine kinase activée par l'AMP; adénylate cyclase.*

## **Introduction**

In the last few decades, prevalence of type 2 diabetes (T2D) has reached to epidemic proportions in many countries worldwide(1) and promotes the risk for cardiovascular diseases and early mortality(2). Prevention and management of T2D has become a major public health challenge around the world. T2D is defined by elevated serum glucose levels (higher than 7 mM) (3) manifest by an impaired insulin-stimulated glucose uptake and an impaired reduction in glucose output from the liver (4). A mainstay of pharmacological therapy for individuals with T2D is the biguanide drug metformin, being prescribed to at least 120 million people worldwide. Despite being introduced clinically in the 1950s (although it was only available in the United States from 1995), the exact mechanism of action of metformin has persistently remained elusive. The present review provides new mechanisms of the action of metformin in the liver, which is an important site for its therapeutic effect.

## **Clinical effects of metformin**

Metformin (1,1-dimethylbiguanide), a biguanide derivate, is currently the most widely prescribed drug to treat hyperglycemia in individuals with T2D and is recommended, in conjunction with lifestyle modification (diet, weight control and physical activity), as a first line oral therapy along in the recent guidelines of the American Diabetes Association and European Association of the Study of Diabetes(5, 6), if there is no contraindication. It is safe and effective as monotherapy and in combination with other hypoglycemic agents. It improves HbA1c by 1 to 5% depending on dose and duration of treatment. Metformin has some side effects, principally gastrointestinal disturbances associated with abdominal pain, nausea, vomiting and diarrhea, which occur in 20%–30% of patients. Very rarely, metformin causes lactic acidosis and the mortality risk is less than 0.015 cases per 1000 patients year. In addition to its efficacy in lowering blood glucose levels, metformin has the clinical advantages of inducing mild weight reduction in people with higher BMI with only a minimal risk of hypoglycemia(7, 8). Additional benefits of metformin therapy include improved insulin sensitivity and insulin secretion, although these effects could be secondary to reductions in serum glucose and insulin levels. The improvement in insulin sensitivity by metformin could be ascribed to its positive effects on insulin receptor expression and tyrosine kinase activity (9). Metformin may also exert its beneficial metabolic actions in part through the modulation of multiple components of the incretin axis(10). Maida *et al.* have recently reported that metformin acutely increases plasma levels of (GLP-1) and induces islet incretin receptor gene expression through a mechanism that is dependent on peroxisome proliferator-activated receptor (PPAR)- $\alpha$ (11).

The intensive glucose control with metformin appears to decrease the risk of diabetes-related endpoints and death in overweight diabetic patients, and is associated with less weight gain and fewer hypoglycemic attacks when compared to insulin and sulphonylureas. The reduction of cardiovascular mortality by metformin compared with any other oral diabetes agent or placebo was confirmed by recent meta-analysis including more than 30 clinical trials(12, 13). In addition to T2D and associated cardiovascular complications (14), metformin is considered a therapeutic option for other diseases associated with insulin resistance, such as polycystic ovary syndrome (PCOS) and gestational diabetes(15, 16). There is also evidence that metformin use is associated with a significant decrease in the relative risk of specific cancers suggesting anticancer properties. These observations initially came to attention by epidemiological

studies but are consistent with *in vitro* and *in vivo* studies showing antiproliferative action of metformin on various cancer cell lines and animal models(15).

### **History of metformin**

Metformin has an interesting history. In the 1920s, the biguanides(two linked guanidine rings) were developed from galegine (Figure 1), a derivative of guanidine found in *Galega officinalis* (also known as French lilac or goat's rue), a traditional botanic medicine used to treat diabetes across medieval Europe. Metformin and phenformin, the two main biguanides, became available for diabetes therapy in the 1950s. Metformin was clinically developed in 1957 by the French physician Jean Sterne, who gave it its first trade name, Glucophage ("glucose eater"). Phenformin was quite popular in the 1960s, but was withdrawn in the early 1970s due to the emergence of frequent lactic acidosis and increased cardiac mortality. Metformin, a less lipophilic biguanide, proved safer and has supplanted phenformin. After 20 years of use in Europe, metformin was approved for use in the USA in 1995. In 2002, metformin became available as a generic medication, making it one of the least expensive diabetes treatments.

### **Pharmacokinetics of metformin**

After oral administration, metformin is slowly absorbed from the proximal small intestine and absorption is apparently complete within 6 hours of ingestion(17). Metformin is rapidly distributed following absorption and does not bind to plasma proteins (18). The mean plasma elimination half-life after oral administration is between 4.0 and 8.7 hours. The clearance of metformin is dependent on renal elimination as metformin does not undergo relevant biotransformation in the liver or biliary excretion. Gastrointestinal absorption is the rate-limiting step of metformin disposition because the rate of metformin absorption is slower than its rate of plasma elimination. More metformin is absorbed after a lower dose than after a higher dose. Thus, an inverse relationship was observed between the amount of metformin ingested (from 0.25 to 2.0 g) and its bioavailability (from 86 to 42%), suggesting the involvement of an active and saturable absorption process. It has been shown that metformin only negligibly permeates the plasma membrane by passive diffusion (19) and solute carrier organic transporters such as organic cation transporters OCT1, 2 and 3 are, to date, the main transporters involved in metformin absorption in the intestine, liver and the kidneys. Using OCT1<sup>-/-</sup> mice, OCT1 was demonstrated to play an important role in both hepatic and intestinal metformin uptake(20). In addition, metformin has also been identified as a substrate for the multidrug and toxin extrusion (MATE) transporter contributing to the renal excretion of metformin and for the plasma membrane monoamine transporter (PMAT) participating in the intestinal absorption of metformin(21).

### **Metformin and its molecular mechanism of action in the liver**

Early work on the mechanisms of the blood glucose lowering action of metformin indicated that inhibition of gluconeogenesis was the most likely mechanism (22). Metformin does restore glucose homeostasis in T2D patients mainly by decreasing hepatic glucose production, even if a moderate increase in glucose disposal rate has been also reported (23). Although the molecular target of metformin was elusive for

several years, the demonstration that metformin activates AMP-activated protein kinase (AMPK) has shed new light on the mechanism of action of the drug(24). AMPK is a phylogenetically conserved serine/threonine protein kinase viewed as a fuel gauge monitoring systemic and cellular energy charge and which plays a crucial role in protecting cellular function under energy-restricted conditions in the liver (25). AMPK is activated in response to metabolic stress characterized by a rise in AMP paired with a fall in ATP levels, reflecting a decrease in cellular energy state, as seen following hypoxia, glucose deprivation and inhibition of mitochondrial oxidative phosphorylation (26, 27). AMPK is a heterotrimeric protein consisting of a catalytic  $\alpha$ -subunit and two regulatory subunits  $\beta$  and  $\gamma$  and each subunit has at least two isoforms. AMPK activation requires phosphorylation on Thr172 within the activation loop of the catalytic  $\alpha$ -subunit by upstream kinases, identified as the tumor suppressor serine/threonine kinase 11 (STK11/LKB1) (28-30) and CaMKK $\beta$ (31, 32), which is further stimulated by the allosteric activator AMP (33). Activated AMPK switches cells from an anabolic to a catabolic state, shutting down the ATP-consuming synthetic pathways and restoring energy balance. This regulation involves phosphorylation by AMPK of key metabolic enzymes and transcription factors/co-activators modulating gene expression (25). As a result, lipid and protein synthesis as well as cell growth are inhibited whereas fatty acid oxidation and glucose uptake are stimulated.

Metformin did not activate AMPK or affect its phosphorylation by LKB1 in cell-free system, indicating that it acted indirectly(34). Rather, there is evidence that AMPK activation by metformin is secondary to its effect on the mitochondria, the primary cellular target of the drug. It was reported in the early 2000s that metformin induced a time and concentration dependent inhibition of the mitochondrial respiratory chain complex 1 in isolated rat hepatocytes (35, 36) and this original observation was also recently demonstrated in isolated human hepatocytes (37). These results strongly suggest that metformin activates AMPK by inhibiting ATP synthesis and thus increasing cellular ADP and AMP levels. This was confirmed by using cells stably expressing AMPK complexes containing AMP-insensitive variants that were completely resistant to the effect of metformin to activate AMPK(38). In addition, bypassing the mitochondrial respiratory chain complex 1 by using methyl succinate, a substrate of respiratory chain complex 2, antagonized the metformin-induced AMPK activation in a pancreatic  $\beta$ -cell line(39). In line with this, AMPK activation was abolished in bovine aortic endothelial cells depleted of mitochondria ( $\rho 0$  cells) (40).

The glucose lowering effect of metformin has been initially attributed to its ability to suppress hepatic gluconeogenesis through the LKB1/AMPK signaling pathway(41). Deletion of LKB1 in the liver abolished the blood glucose lowering effects of metformin and prevented activation of AMPK in the liver of high fat fed diabetic mice (41). The LKB1/AMPK signaling pathway regulates the phosphorylation at Ser171 and nuclear exclusion of the transcriptional coactivator CREB-regulated transcription coactivator 2 (CRTC2, also referred to as TORC2)(41), a pivotal regulator of hepatic glucose output in response to fasting by directing transcriptional activation of the gluconeogenic program (42). Non-phosphorylated CRTC2 translocates to the nucleus, where it associates with phosphorylated CREB to drive the expression of peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) and its subsequent gluconeogenic target genes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (Fig. 2). A possible alternative mechanism for metformin inhibitory action on CRTC2-mediated gluconeogenesis has been recently proposed (Fig. 2), involving deacetylation of CRTC2 by the NAD<sup>+</sup>-dependent protein deacetylase sirtuin 1 (SIRT1)(43), resulting in

the loss of protection from COP1-mediated ubiquitination and subsequent degradation (44). This likely occurs in parallel with other mechanisms entailing the disassembly of the CREB-CBP (CREB binding protein)-CRTC2 complex from gluconeogenic gene promoters (Fig. 2). The regulation of gluconeogenic gene expression by metformin appears to be dependent on the phosphorylation of CBP at Ser436 through AMPK-induced atypical PKC $\iota/\lambda$  activation and dissociation of the CREB co-activator complex (45). In addition, the metformin-induced inhibition of gluconeogenic genes in the liver is also mediated through AMPK-mediated upregulation of the orphan nuclear receptor small heterodimer partner (SHP), which operates as a transcriptional repressor (46). SHP inhibits CREB-dependent hepatic gluconeogenic gene expression via direct interaction with CREB and competition with CRTC2 binding in the CREB-CBP complex (47).

Understanding the mechanism of action of metformin is further complicated by recent studies establishing that both LKB1 and AMPK activities are dispensable for metformin-induced inhibition of glucose output or gluconeogenesis (48). It was reported that a reduction in hepatic energy status caused by metformin, but not a transcriptional repression of gluconeogenic genes, constitutes the critical factor responsible for the metformin-induced reduction in hepatic glucose production (48). Interestingly, forced expression of key gluconeogenic genes through PGC-1 $\alpha$  overexpression did not reduce metformin-induced reduction of glucose output, but was again associated with a significant depletion of energy stores (48). Since the rate of hepatic glucose production is closely linked to hepatic energy metabolism (6 ATP equivalents are required per molecule of glucose synthesized), disruption of the main energy supply in hepatocytes through inhibition of the respiratory chain complex 1 would have a profound effect on the flux through gluconeogenesis (Fig. 3). This energetic expense of glucose synthesis is exemplified in recent studies examining the decreased hepatic energy state associated with prolonged fasting or glucagon treatment in mice (49). In addition, as AMP tends to rise whenever ATP falls, this could also provide an alternative explanation for the acute inhibition of gluconeogenesis by metformin *via* allosteric regulation of key enzymes in this pathway (Fig. 3). For example, AMP synergizes with the regulatory sugar fructose 2,6-bisphosphate to stimulate phosphofructokinase and inhibit fructose-1,6-bisphosphatase (15). Furthermore, it was recently demonstrated that the resulting increase in intracellular AMP concentration elicited by metformin- or phenformin-induced energetic stress represent a novel mechanism by which these drugs antagonize the action of glucagon, thus reducing fasting glucose levels (50). AMP inhibited glucagon-stimulated adenylate cyclase, thereby reduced levels of cAMP and protein kinase A (PKA) activity and blocked glucagon-dependent glucose output from hepatocytes (Fig. 3). In hepatocytes lacking AMPK, phenformin blocked glucagon-dependent cAMP accumulation in a manner indistinguishable from that in control cells, indicating that the effects of biguanides on cAMP metabolism are independent of AMPK. Importantly, treatment of diabetic mice fed a high fat diet with metformin led to an elevation in hepatic AMP, which correlated well with a concomitant reduction in hepatic cAMP content and in the phosphorylation of key PKA target proteins in the liver (50). Thus, these data support a mechanism of action of metformin involving antagonism of glucagon.

## **Metformin and pharmacogenetics**

Considerable differences in the clinical efficiency of metformin has been recognized from patient to patient. It appears that the bioavailability of metformin is not complete, and that large interindividual differences in bioavailability after oral administration in the range of 20%–70% have been described. Given the importance of OCT1 in metformin uptake into the liver and thus metformin activity, several pharmacogenetic studies have focused on polymorphisms in the gene encoding OCT1, SLC22A1, as modifiers of the glycemic response. Recently, Shu *et al.* showed that *SLC22A1* variants reducing OCT1 function reduced the effect of metformin in lowering blood glucose levels in healthy volunteers compared to subjects with wild type alleles (20). Genetic polymorphism of *SLC22A1* and *SLC22A2*, the gene encoding OCT2, has also been described as modifiers of metformin renal clearance (51, 52). In addition, variations in genes other than those encoding OCTs are likely to modulate the pharmacokinetics or response to metformin therapy. Recently, a genome-wide association study showed association between glycemic response to metformin and the ataxia telangiectasia mutated gene (ATM), a gene involved in DNA repair and cell cycle control (53). This study concluded that ATM has a direct role in the pharmacologic action of metformin by regulating the phosphorylation of AMPK, the energy sensor widely considered to be involved in metformin metabolic effects. However, this conclusion has been challenged, suggesting that it is critical to perform additional studies to establish whether ATM or another gene in the ATM locus is the causative gene by which the response to metformin is modulated (54, 55).

## Conclusions

Metformin is currently the drug of first choice for the treatment of T2D. It exerts its antidiabetic effects by reducing hepatic glucose production through inhibition of gluconeogenesis, which is upregulated in T2D. Reduction of hepatic glucose production by metformin is mediated by inhibition of mitochondrial respiratory chain resulting in a decrease in cellular ATP and a concomitant increase in AMP. As the anabolic process of gluconeogenesis is an energetically costly program, glucose output is reduced accordingly upon treatment with metformin and concomitant reduction in hepatic energy state. However, although the energy sensor AMPK is activated in response to energy stress, it has been clearly showed that AMPK is dispensable for the inhibitory effect of metformin on hepatic gluconeogenesis. The metformin-induced inhibition of glucose production occurs through regulation of the gluconeogenic flux rather than direct inhibition of gluconeogenic gene expression. Moreover, metformin also produces its effect by inhibiting glucagon signaling pathways through the elevation in intracellular AMP. Thus, it appears that the decrease in hepatic energy charge following inhibition of respiratory chain complex 1 by metformin is the central explanation for the reduction of hepatic gluconeogenesis.

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### Disclosure of interest

The authors have no conflict of interest to declare.

### Figure Legends

**Figure 1 : Origin and chemical structure of guanidine, galegine and metformin.** The synthesis of the biguanide compound metformin (1,1-dimethylguanidine) originates from the plant *Galega officinalis*, a traditional botanic medicine used to treat diabetes across medieval Europe. Studies in the late 1800s indicated that *G. officinalis* was rich in guanidine but attention turned in the 1920s to galegine, a less toxic extract with antidiabetic properties. In 1929, the glucose-lowering biguanide metformin was synthesized.

**Figure 2 : Potential molecular mechanisms for the action of metformin on the gluconeogenic program.** The specific inhibition of respiratory chain complex 1 by metformin leads to the decrease in ATP:AMP ratio and activation of AMPK. The LKB1/AMPK signaling pathway regulates the phosphorylation, nuclear exclusion, degradation, recruitment in the CREB-CBP complex and binding competition with SHP at gluconeogenic gene promoters of the transcriptional coactivator CRTC2, a pivotal regulator of the gluconeogenic program.

**Figure 3 :Metformin-induced inhibition of glucose production by reduction in hepatic energy charge.**After hepatic uptake of metformin through OCT1, the mitochondria become the primary target of metformin exerting a mild and specific inhibition of the respiratory chain complex 1. The resultant mild decrease in cellular energy state leads to decrease in cellular ATP and a concomitant increase in AMP. Multiple regulatory points exist for direct AMP- and ATP-mediated effects on gluconeogenesis. ATP drop blocks the energetically costly process of gluconeogenesis and AMP accumulation inhibits gluconeogenesis through allosteric regulation of fructose-1,6-bisphosphatase and the reduction of glucagon-stimulated adenylate cyclase activity.