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REVIEW ARTICLE

Is Metformin a Perfect Drug? Updates in Pharmacokinetics and Pharmacodynamics

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Abstract: Metformin, a synthetic biguanide, is currently one of the most frequently recommended medications for type 2 diabetes treatment around the world. This review presents the latest discoveries in the pharmacokinetics of metformin, especially the role of transporters (*e.g.* Organic Cation Transporters OCTs, Multidrug and Toxin Extrusion transporters MATE) in oral absorption, distribution, elimination and biochemical effects of metformin in humans. We also review the associations between genetic variations of metformin transporters, their pharmacokinetics and drug efficacy or drug responses.

In the second part of this paper, we highlight the current knowledge on novel metformin actions including favourable effects on lipid profile (*e.g.* decreasing plasma triglycerides (TG) and low density lipoprotein (LDL) cholesterol levels) and the cardiovascular system (*e.g.* decline in systolic and diastolic blood pressure, and vasoprotective effects). Furthermore, we provide an up-to-date overview of multidirectional activities of metformin, including the effects on coagulation and fibrinolysis, polycystic ovary syndrome, as well as the anti-ageing and anti-inflammatory properties. Over the past two decades, metformin's antineoplastic properties have been drawing increasing attention of scientists; herein, we outline the state-of-the-art discoveries concerning metformin use in the field of oncology. Finally, we review the newly synthesized derivatives and pro-drugs of metformin and other biguanides.

Keywords: Biguanides, metformin, pharmacokinetics, glycaemia, coagulation, pro-drugs.

INTRODUCTION

Metformin is a synthetic biguanide which was originally described in 1922. However, it is related to guanidines found in the French lilac (*Galega officinalis*) that has already been used in the Middle ages to decrease blood sugar and relieve the symptoms of diabetes mellitus [1,2]. Metformin is currently one of the most recommended medications for diabetes treatment around the world. The drug was accepted for the therapy of hyperglycaemia successively in England in 1958, Canada in 1972, and the US in 1995 [3-5]. It has been estimated that nowadays, approximately 120 million people use metformin worldwide [6,7]. The most common indication is the treatment of type 2 diabetes; however, it is also used for polycystic ovarian syndrome, metabolic syndrome, and diabetes prevention [8-10].

Metformin is slowly and incompletely absorbed from the intestine, and therefore, the pharmacologically active doses are relatively high (0.5-2.0 g per day). Unfortunately, treatment with high doses of metformin is associated with gastrointestinal adverse effects, such as nausea, vomiting, diarrhoea, abdominal pain and loss of appetite, which is frequently the reason for discontinuation of therapy. However, overall metformin is well tolerated and accepted as anti-diabetic agent, since the side effects occur most frequently during initiation of therapy and resolve spontaneously in most cases. Lactic acidosis is a rare (5 per 100000 population) but serious (high mortality in the absence of prompt treatment) metabolic complication related to metformin accumulation. Reported cases of lactic acidosis in patients taking metformin have occurred primarily in diabetic patients with significant renal failure [11].

Metformin has several glucose lowering mechanisms, which will be summarized in this review. It mainly inhibits hepatic gluconeogenesis and increases glucose consumption in muscles. It also increases insulin sensitivity and inhibits intestinal glucose absorption [3]. However, scientific society has also been stunned by the multidirectional activities of metformin, including lipid lowering, anti-ageing and anti-inflammatory properties, which will be outlined in this review. Over the past two decades, metformin has been drawing increasing attention of scientists due to its antineoplastic properties in relation to several oncologic diseases; therefore, we also review the ground-breaking evidences concerning metformin use in the field of oncology. However, we would like to start from summarizing the latest discoveries in the pharmacokinetics of metformin and finally provide a section of newly synthesized derivatives of metformin.

ABSORPTION AND DISTRIBUTION OF METFORMIN

Metformin is slowly absorbed after oral administration, predominantly from the small intestine. The bioavailability shows some intra-subject, as well as inter-subject variability. Bioavailability after oral administration has been estimated at approximately 50-60% with a plasma half-life of 1.5-4 h [12]. Considering its chemical structure, metformin is a biguanide (1,1-dimethylbiguanide hydrochloride). Due to its very polar guanidine structure, metformin is a highly hydrophilic base that exists as cationic spe-

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cies at physiological pH, with a minimal passive diffusion through the cell membranes [2,13].

Metformin is not bound to plasma proteins [13,14]. Metformin diffuses into erythrocytes, most likely as a function of time. The volume of distribution (Vd) has been established to range from 63 to 276 L after intravenous administration. The apparent volume of distribution after oral administration (Vd/F) of 2000 mg of metformin daily is approximately 600 L. Clinicians indicate that due to uncompleted absorption, the actual Vd ~~(Cross out volume of distribution)~~ during multiple dosage is about 300 L. The high value of Vd means that metformin is able to be taken up by various tissues [13].

Numerous studies have confirmed that after a single oral dose, metformin concentrations in the kidneys, adrenal glands, pancreas and liver exceed the serum concentrations up to seven times. Lower concentrations of metformin are observed in the lungs and muscles [13,15].

It is of great importance to mention that metformin passes through the placenta [16] and the concentrations in the foetus are only slightly lower than in the mother [13]. Another crucial fact is that the pharmacokinetics of metformin in pregnant women is altered because of the higher glomerular filtration rate (GFR). This leads to lower plasma concentrations of metformin during pregnancy in comparison to non-pregnant women [13,17].

It has been established in numerous studies that metformin is a substrate for several organic cation transporters (OCTs), which determines its oral absorption, distribution, elimination (hepatic uptake, renal excretion) and biochemical effects of metformin in man (Scheme **1**) [13]. Therefore, within this section, we will investigate the most important aspects of transporters' role in metformin pharmacokinetics. Scientists have focused their efforts on the role of genetic factors in predicting response variations to metformin [18]. In addition to pharmacokinetic individual variability, the response to metformin differs significantly, with approximately 30% of subjects receiving metformin classified as non-responders [19,20]. Numerous researches have been conducted in order to find out the associations between genetic variations of metformin transporters, their pharmacokinetics and drug efficacy or drug responses [18]; therefore, we have presented here the major findings within this area.

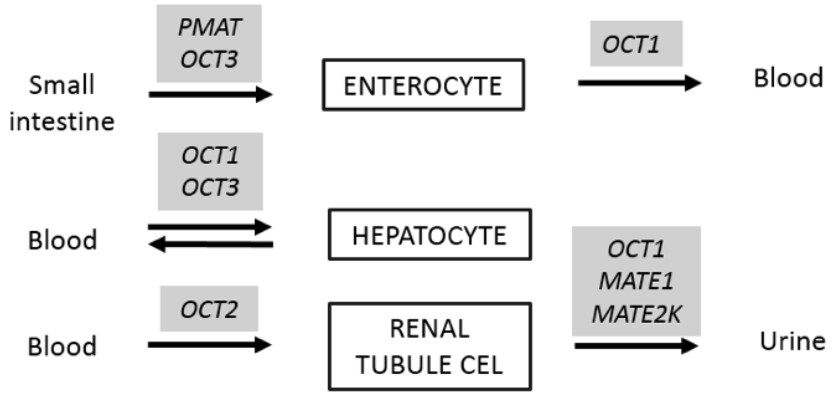
As reported by Zhou *et al*., [21] one of the transporters responsible for the uptake of metformin from the intestine appears to be plasma membrane monoamine transporter (PMAT) which is localized on the luminal side of enterocytes [20]. Generally, OCT transporters (three subtypes OCT1, OCT2, OCT3) play an important role in the tissue distribution of a wide variety of positively charged molecules, including drugs and endogenous substrates [22,23]. Kimura *et al.* in *in vitro* study compared the specificity of OCT1 and OCT2 for several guanidine compounds, including creatinine, guanidine, aminoguanidine, etc [22]. The results of the study showed that many guanidine compounds had relatively equal affinity to OCT1 and OCT2. The researchers found that among the studied compounds, aminoguanidine had greater affinity for OCT2 than OCT1 which may lead to the conclusion that OCT2 can act as a transporter for aminoguanidine in renal proximal tubules [22].

Graham *et al.* reported that OCT 1 and OCT 3 are also present in low amounts in the gastrointestinal tract [13]. OCT3 transporters, localized in the brush border of enterocytes, may act as a carrier of metformin into enterocytes. The author speculates also that OCT1 may take part in the transport of the drug into the interstitial fluid [13]. Based on the extensive review, Gong *et al*. stated that the role of OCT1 and OCT3 in the intestinal transport of metformin needs to be defined [18].

It should be underlined here that genetic variants of OCT1 and OCT3 might contribute to a decrease in the ability to transport metformin into model cells [24]. For instance, Shu *et al.* [24] reported that among the subjects being heterozygotes with one of the several variant OCT1 transporters, the plasma concentrations of metformin were only slightly higher in comparison with the normal (wild-type) OCT1, whereas in those being homozygotes carrying poorly functioning transporters, major changes such as higher area under the plasma concentration-time curve (AUC), and higher maximal plasma concentration (Cmax) could be seen.

The hepatic uptake of metformin is mediated primarily by OCT1 and to a lesser extent by OCT3 [18]. These transporters are localized on the basolateral side of hepatocytes [13,18]. In 2007, Shu *et al.* [25] published a study in which they reported that in OCT1 deficient mice, the hepatic metformin concentration in the liver was significantly lower than that in control mice, and most importantly, the glucose-lowering activity of metformin disappeared completely [25]. In turn, Nies *et al.* [26] reported that the hepatic expression of OCT1 and OCT3 in Caucasians was significantly affected by cholestasis and certain genetic variants. Due to the fact that OCT1 and OCT3 are involved in the hepatic uptake of metformin, these conditions may lead to the variability in drug response [26].

Recently, considerable variation in the hepatic expression of OCT1 has been reported, and some authors have highlight that it should be regarded as clinically important [13,26,27]. For instance, Sogame and colleagues [28] revealed that both metformin and phenformin are transported actively by OCT1, with the active transport components much greater than passive transport ones



Scheme 1. Transporters involved in the absorption, distribution and urinary excretion of metformin. MATE - multidrug and toxin extrusion transporter; OCT - organic cation transporter; PMAT - plasma membrane monoamine transporter.

which suggests that functional changes in OCT1 might affect the transport of the drugs. This, in turn, may be the cause of lack of metformin's activity in some subjects [28]. Gambineri *et al.* also confirmed that genetic variation in OCT1 may be associated with heterogeneity in the metabolic response to metformin in women with PCO [29].

A study conducted by Christensen *et al.* [30] demonstrated a huge inter-individual variability in thorough steady-state metformin concentration in diabetic patients. The authors established a mean trough steady-state plasma concentration to be 576 ng/mL and a nearly 80-fold range from 54 to 4133 ng/mL which, in fact, corresponds with inter-individual variability. The scientists concluded that the metformin pharmacokinetics is affected by the OCT1 activity, which in turn is associated with a reduction in the absolute decrease in Hb1Ac level [30].

In the case of inter-subject differences in the expression of OCT3 protein, there are not available scientific evidences yet, however, differences in the expression of OCT3 have been detected [13,26]. It should be mentioned that the above discoveries have some limitations. For example, we cannot exclude the effect of other administered drugs on the variation in OCT1 and OCT3. It has also been established that the levels of OCT1 and OCT3 were lower in livers in patients with cholestasis [13]. These changes in variation in hepatic OCT1 and OCT3 transporters might be the reason for the differences in the clinical response to metformin.

Apart from hepatic location, both OCT1 and OCT3 are also expressed in skeletal muscles [31]. It has been reported that the expression of mRNA of OCT3 is higher than that of OCT1 [13,31]; however, the activity of both of these transporters has not yet been evaluated. The clinical importance of OCT3 variants on the response to metformin also has not yet been determined [13].

The high levels of OCT1 and OCT3 have been established in the adrenal gland which are consistent with the substantial levels of metformin at this site [13,32]. However, the correlations between the metformin's mechanism of action and its distribution in the body need further examination [13].

METABOLISM AND EXCRETION OF METFORMIN

Several papers have reported that metformin is not metabolized, and no metabolites or conjugates of metformin have been identified. The fact that metformin does not undergo liver metabolism clearly differentiates the pharmacokinetics of metformin from those of other biguanides [33]. Approximately 30-50% of an oral dose is excreted in the urine as the unchanged drug within 24 hours, and 30% of the dose is eliminated unchanged with the faeces [2]. The elimination half-life ($t_{0,5}$) of metformin during long-lasting treatment in patients with physiological renal function is approximately five hours [13]. It has been calculated that the population mean renal clearance (CL_R) of metformin is approximately 510 ± 130 mL/min, and its apparent total clearance after oral administration (CL/F) 1140 ± 330 mL/min, in healthy subjects [13]. In patients with decreased renal function (lower creatinine clearance), the plasma and blood half-life of metformin is prolonged and the renal clearance is decreased together with the decrease in creatinine clearance [13]. According to the most recent FDA (Food and Drug Administration) recommendations, metformin administration in patients with chronic kidney disease should be based on estimated glomerular filtration rate (eGFR) rather than creatinine clearance. If the eGFR is below 30 mL/min per 1.73 m^2, which is defined as advanced renal disease, the drug is contraindicated [33].

The process of metformin excretion is also based on its transporter mechanism. As previously reported, the OCT2 transporter, localized in basolateral membrane in the renal tubules, is mainly engaged in the uptake of metformin from circulation into renal epithelial cells [18]. Approximately 90% of the variation in renal metformin clearance (CL_R) has been attributed to genetic characteristics [30]. According to Ogasawara *et al.* [34] the expression of OCT2 mRNA in the human kidney varies over 100-fold, which contributes to considerable inter-subject variation in the expression of OCT2 protein and, consequently, in metformin CL_R [34]. In the case of OCT2 polymorphisms, while several papers have analysed the impact of the minor allele in rs316019 on renal metformin clearance, no firm conclusions can be drawn as some report increased renal clearance [35], some decreased [36] and others no effect at all [37]. A retrospective data analysis by Yoon *et al.* [38] found that certain genetic polymorphisms of OCT2 (OCT2-808 G>T) exert a significant effect on metformin pharmacokinetics, contributing to increase in the serum concentration of metformin, suggesting that in certain circumstances, *e.g.* in renal dysfunction when elimination of metformin is reduced, the dose of metformin should be titrated. However, the authors claim that this recommendation needs more extensive research for confirmation [38].

In a study of drug transporters under acidosis, Gaowa *et al.* [39] found the CL_R of metformin and creatinine and expression of OCT2 to remain unchanged under acidotic conditions. In addition, the amount of OCT1 protein was reduced, and that of MATE1 protein was increased in these conditions [39].

Various transporters other than OCT2 can take part in the process of metformin excretion. One such example is OCT1, which is expressed in the apical membranes (luminal side) in the proximal and distal tubules [37].

Excretion of metformin from the renal tubule cell to the urine is facilitated by MATE1 and MATE2-K (multidrug and toxin extrusion) [40,41], which are expressed in the apical membrane of the renal proximal tubule cells [18]. MATE1 was described for the first time in 2005 as an efflux transporter, and shortly after, other isoforms such as MATE2 and MATE2-K were discovered [42]. In the kidney and liver, MATEs work together with OCTs, and are responsible for modulating the elimination of organic cations [18,43]. In the case of SNP (single nucleotide polymorphism) marked rs2289669 in MATE1 and rs622342 in OCT1, the number of minor alleles was associated with an additive pharmacodynamics of metformin's effect [30]. The authors reported interaction between the two polymorphisms for homozygous rs622342 patients, who had more efficient glucose-lowering effects of metformin with increasing numbers of rs2289669 [30,44]. Genetic variants of MATE1 or MATE2K have not yet been fully characterized, but are associated with differences in clinical responses to metformin [18]. However, Kusuhara *et al.* note that the administration of a pyrimethamine, a MATE inhibitor, contributed to a significant increase in metformin Cmax and AUC [45]. A study of the effects of novel promoter variants in MATE transporters in the pharmacokinetics and pharmacodynamics of metformin by Stocker *et al.* found that MATE1, a reduced expression promoter variant, is associated with increased response to metformin in healthy subjects and diabetic patients who were homozygous for the OCT1 reference allele [20]. In the case of MATE2, the enhanced expression promoter variant, the researchers found a reduced response to metformin in healthy volunteers. In addition, CL_R and secretory clearance of metformin was significantly greater in subjects with the promoter variant of MATE2 who were also MATE1 reference [20]. The authors concluded that promoter variants of both MATE 1 and 2 are important factors regarding metformin disposition and response in healthy subjects and patients with type two diabetes [20]. Elsewhere, it was reported that MATE1 dysfunction contributes to a marked elevation in the metformin concentration in the liver and causes lactic acidosis. This finding implies that the homozygous MATE1 variant could be one of the risk factors for metformin-induced lactic acidosis [46].

DRUG-DRUG INTERACTIONS WITH METFORMIN

Recent studies have reported that drug-drug interactions through the inhibition of metformin transporters (OCTs and MATEs) are clinically relevant, and contribute to variability in the

drug response [18]. For instance, proton-pump inhibitors decrease metformin uptake *in vitro* by inhibiting OCT1, OCT2, and OCT3 transporters [47]. Similarly, other oral anti-diabetic drugs, such as repaglinide and rosiglitazone, inhibit OCT1 transporter in *in vitro* models [48]. Another study notes that cimetidine, a substrate for cationic transporters, decreases the CL_R of metformin, thus increasing the systemic exposure to metformin, and that it also inhibits transporter OCT2 depending on the genetic variant of the transporter [49]. Indeed, cimetidine decreases metformin's CL_R by about 50% in patients with the reference OCT2. Among those with the heterozygous or homozygous variant, the CL_R of metformin is even lower (20% of the standard value) [50]. In turn, results of Misaka's research indicate that cimetidine and trimetoprim significantly reduce OCT1-, OCT2-, MATE1-, and MATE2-K-mediated metformin uptake [51]. A number of other studies have been conducted in order to identify any possible pharmacokinetic interactions between metformin and other anti-glycaemic drugs such as glyburide, vildagliptin, sitagliptin, and rosiglitazone. However, the effects of these drugs on metformin's CL/F, and vice versa, have not been of clinical significance [13]. One of the most recent studies indicated clinical implications in the disposition, efficacy and toxicity of metformin while simultaneous use of tyrosine kinase inhibitor (imatinib, nilotinib) [52].

Regarding MATE transporters and drug-drug interactions, changes in the renal excretion of substrate drugs such as metformin result in inadequate pharmacotherapy or occurrence of toxic effects [42]. It has been reported that interaction between metformin and cimetidine is also possible by means of MATE1 [53].

EFFECTS OF METFORMIN ON GLYCAEMIA

Type 2 diabetes mellitus is a progressive metabolic disease which comprises three characteristic pathophysiologic abnormalities: relative insulin deficiency, insulin resistance, and hepatic insulin resistance (resulting in increased gluconeogenesis and impaired glycogen synthesis) [54]. Biochemical abnormalities of type 2 diabetes include hyperinsulinemia, high levels of serum triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL), increased levels of low-density lipoprotein cholesterol (LDL), increased concentrations of plasminogen activator inhibitor-1 (PAI-1) and C-reactive protein (CRP) [12].

Conventional therapeutic strategy usually begins with lifestyle interventions supported by the prescription of a single anti-diabetic drug. When insulin resistance progresses and the monotherapy option becomes insufficient to control the level of plasma glucose, patients are usually switched to a double-drug regimen. In the worst case scenario, further progression of insulin resistance and decline in endogenous insulin production require the administration of exogenous insulin [55]. Apart from insulin, several drug categories have been developed to treat type 2 diabetes mellitus. These include sulfonylureas, meglitinides, biguanides, inhibitors of α-glucosidase, thiazolidinediones, dipeptidyl peptidase 4 inhibitors (gliptins), glucagon-like peptide-1 (GLP-1) analogues and amylin analogues [56].

Most newly-diagnosed patients with type 2 diabetes are placed on metformin monotherapy which is then supplemented or often substituted by other oral anti-diabetic drugs [55,57,58]. The mitochondria are believed to be the primary molecular target of metformin [59]. The drug accumulates within the matrix of mitochondria, where it inhibits complex I of the mitochondrial electron transport chain, resulting in a reduction in NADH oxidation and ultimately a reduction in the synthesis of ATP [2,59]. These changes result in activating the 5'-adenosine monophosphate (AMP) kinase (AMPK) by means of a liver kinase B1 (LKB1) dependent mechanism [60]. As a result, metformin inhibits hepatic gluconeogenesis and increases glucose consumption in muscles [61]. It is noteworthy that Foretz *et al.* also note the presence of an LKB1- and AMPK- independent pathway for the inhibition of hepatic gluconeogenesis [62]. The main mechanisms involved in the glucose-lowering effect of metformin are presented in Scheme **2**.

AMPK activation has a number of other beneficial effects, mainly referring to the cardiovascular system, such as reduction of inflammatory cell adhesion to endothelium, decrease in lipid accumulation and proliferation of inflammatory cells, stimulation of gene expression responsible for cellular antioxidant defence and stimulation of enzymes responsible for nitric oxide formation [63]. These will be discussed in more detail below.

Administration of metformin leads to amelioration of hyperinsulinemia due to increased insulin sensitivity [64], *i.e.* indirect induction of insulin receptor expression, and a decrease in blood glucose level by inhibition of gastrointestinal absorption of glucose [56,65]. Metformin improves insulin resistance in several mechanisms such as increased insulin receptor tyrosine kinase activity, enhanced glycogen synthesis, and an increase in the recruitment and activity of GLUT4 glucose transporters [66].

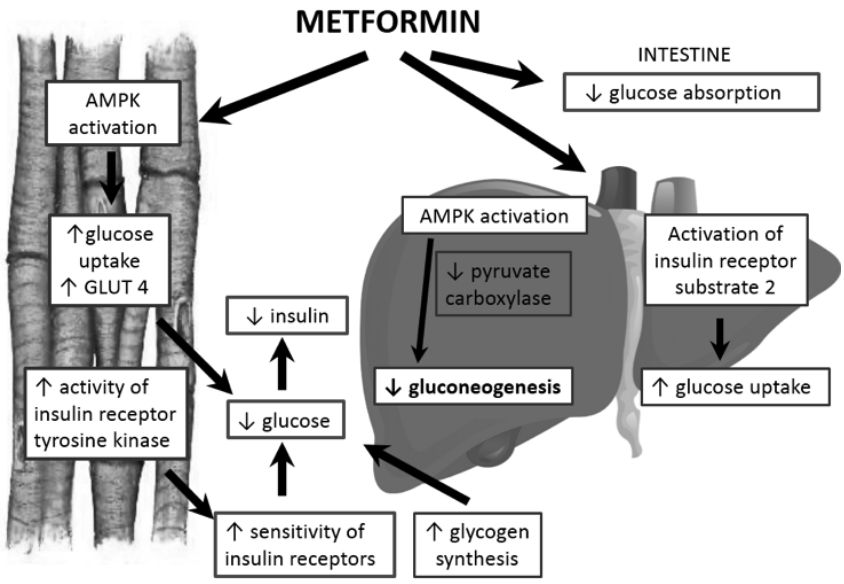
Metformin also increases the level of plasma glucagon-like peptide 1 (GLP-1) and the gene expression of the incretin receptor via peroxisome proliferator activated receptor-α (PPAR-α) [3,67,68], and in adipose tissue, promotes the re-esterification of free fatty acids and inhibition of lipolysis, contributing to reduced lipotoxicity [66].

A meta-analysis of 10 randomized placebo-controlled studies examining the efficacy of metformin in lowering blood sugar level by Johansen found metformin monotherapy contributed to a reduction of fasting blood glucose (FBG) concentrations by 2.0 mmol/L compared with placebo, and by 0.9 % compared with HbA_{1c} (glycated hemoglobin) values. No significant difference was found between metformin monotherapy and placebo with regard to their effect on body weight [69].

Numerous studies have demonstrated the beneficial effects of metformin in combination with other anti-diabetic drugs [54], such as insulin [70], sulfonylureas [71,72], thiazolidinediones [73,74], meglitinides [75], and α-glucosidase inhibitors [76]. For instance, coupling metformin with sulfonylurea provides superior glycaemic control than sulfonylurea monotherapy [77]. Another study, conducted among non-insulin-dependent obese diabetics, found that metformin not only improved glycaemic control, but was also effective in ameliorating many risk factors for coronary heart disease, resulting in a less atherogenetic blood profile [70].

A review the effects of thiazolidinediones and metformin on metabolic control in patients with type 2 diabetes by Seufert *et al.* [78] found that long-term monotherapy with thiazolidinediones appeared to be more effective than metformin itself. Moreover, thiazolidinediones were found to be more effective in promoting an increase in whole body insulin sensitivity and contributed to a greater reduction in concentrations of both plasma triglycerides and free fatty acids. Metformin was also more effective in promoting weight loss [78]**.** These results were confirmed by Phielix *et al.* [79]. Seufert and Urquhart [80] simultaneously conducted two clinical studies in which they evaluated the effectiveness of pioglitazone as add-on medication to metformin or sulfonylurea for reducing post-load serum glucose levels. They found that two-year treatment with pioglitazone as an add-on to either failing metformin or sulfonylurea therapy improved post-load glucose excursions without any effect on insulin secretion. On the other hand, glucose excursions were not improved by gliclazide or metformin add-on therapy, despite increases in post-load insulin levels [80].

Apart from its use in diabetes treatment, Slama notes that metformin might be effective in the prevention of diabetes: Therapy with metformin significantly reduced the incidence of diabetes in subjects with IGT (impaired glucose tolerance) and high-normal fasting plasma glucose [81]. Moreover, health economic analyses imply that metformin treatment is cost-effective in the US and Europe [81].



Scheme 2. Anti-diabetic role of metformin.

METFORMIN'S EFFECTS ON LIPIDS

Apart from its hypoglycemic effect, metformin has been shown to be beneficial in improving lipid metabolism [67]. Both animal [82,83] and clinical studies [84,85] demonstrated that metformin might improve prognosis of fatty liver disease by reversing hepatic steatosis, also in models of acute and chronic alcohol exposure [86]. Reduced hepatic lipid content after metformin treatment is associated with an increase in both fatty acid oxidation and inhibition of lipogenesis, probably mediated by AMPK activation [67,82,87] which leads to alterations in the hepatic lipid metabolism [88]. Metformin activates AMPK, which consecutively induces the phosphorylation and inactivation of acetyl-CoA carboxylase (ACC). ACC constitutes an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is a critical precursor for the biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation [89]. It has been found that in human hepatoma HepG2 cells, metformin enhances ACC phosphorylation and, as a result, induces the reduction of triglycerides levels. Intracellular triacylglycerol and cholesterol levels were also decreased. This phenomenon can be supported by increased oxidation of fatty acid and its decreased synthesis [67,90].

Several studies have demonstrated that AMPK suppresses expression of lipogenic genes such as fatty acid synthase, S14 and ACC by direct phosphorylation of transcription factors such as carbohydrate response element binding protein (ChREBP) and hepatocyte nuclear factor 4 (HNF4) [67,91,92]. It has been shown that HNF4-α might play a role in the mechanism of action of metformin on hepatic apolipoprotein B (ApoB) secretion by regulating the genes that control ApoB expression. The second mechanism involves the reduction of lysophosphatidylcholine (lysoPC) level in hepatocytes [93].

According to Li *et al*., metformin takes part in the regulation of lipogenesis gene expression by down-regulating sterol regulatory element-binding protein-1c (SREBP-1c) gene expression, and inhibits the proteolytic processing of SREBP-1c and its transcriptional activity [94].

Recently, it has been proposed that metformin affects the process of the biosynthesis of monounsaturated fatty acids from saturated fatty acids through AMPK-mediated thyroid hormone receptor 4 (TR4) phosphorylation and control of stearoyl-CoA desaturase 1 (SCD1) expression [67,95].

Another mechanism of metformin action is the promotion of carnitine palmitoyltransferase I (CPT-1) expression and reduction of fatty acid-binding protein 4 (FABP4) expression, involved in palmitic acid induced lipid accumulation in cells. Further molecular studies have shown that metformin decreases FABP4 expression by promoting Forkhead transcription factor (FOXO1) nuclear exclusion and subsequently restricting its activity [96].

A review of several clinical studies examining the effects of metformin on total cholesterol (TC), TG, LDL-cholesterol, and HDL-cholesterol levels suggests that metformin has a favourable impact on lipid profile, including decreasing plasma TG and LDL cholesterol levels [97]. Mourão-Júnior [98] conducted a cohort study on type 2 diabetic patients with metabolic syndrome treated with insulin and metformin to evaluate their impact on glycaemic control, blood pressure, and lipid profile. The body mass index (BMI), waist circumference, lipid profile, HbA_{1C} level, fasting blood glucose level, daily dose of NPH insulin (Neutral Protamine Hagedorn), systolic and diastolic blood pressure were measured before the start of metformin therapy and six months later. Following the addition of metformin, glycaemic control significantly improved, while total cholesterol, BMI and waist circumference were significantly reduced. However, the authors did not notice any effects on HDL cholesterol or blood pressure [98]. The results of a meta-analysis including 41 randomized-controlled trials demonstrated that metformin treatment contributes to decreased plasma total cholesterol and LDL-cholesterol. In contrast to these findings, however, it was found to have no effect on HDL-cholesterol or TG in patients with type 2 diabetes [97].

On the other hand, there are also other studies which do not confirm these positive effects, therefore no consensus about its beneficial effects on these parameters can be reached. For instance, a study by Tessier *at al*. comparing gliclazide and metformin in patients with type 2 diabetes mellitus with regard to the efficacy and lipid peroxidation profile found both drugs increased serum vitamin E and decreased the level of lipid peroxidation markers in LDL-cholesterol and HDL-cholesterol. Despite this, neither drug was associated with any changes in the standard lipid profile [99].

Other studies examine combination of anti-diabetic therapy. In one carried out in rats, rosiglitazone and metformin were found to exhibit a hypolipidaemic effect when administered alone or in combination. In comparison, nateglinide, when used alone, contributed to a significant increase in cholesterol and total lipid levels. This unfavourable effect was hidden when nateglinide was administered together with metformin [97].

This review of the most recent literature suggests that metformin has a favourable effect on the lipid profile, including decreasing plasma TG and LDL-cholesterol levels. However, the findings regarding its impact on HDL-cholesterol remain unclear. Scheme **3** summarizes up the main mechanisms of metformin action on lipid profile.

METFORMIN'S ACTION ON THE CARDIOVASCULAR SYSTEM

The results of the UKPDS trial showed that metformin significantly reduced diabetes-related death by 42%, and also all-cause mortality by 36% [72]. Similar results have been obtained in other clinical or epidemiological studies [100,101,102]. It has been proposed that these results might be not only due to better glycaemic control but also because of weight loss induction. Metformin treatment represents a relevant element of an integrated lifestyle modification-pharmacotherapy to prevent both type 2 diabetes and cardiovascular disease [103]. Therefore, metformin is regarded as a valuable drug on account of its anti-diabetic properties and beneficial effects on mortality in this population [67,104].

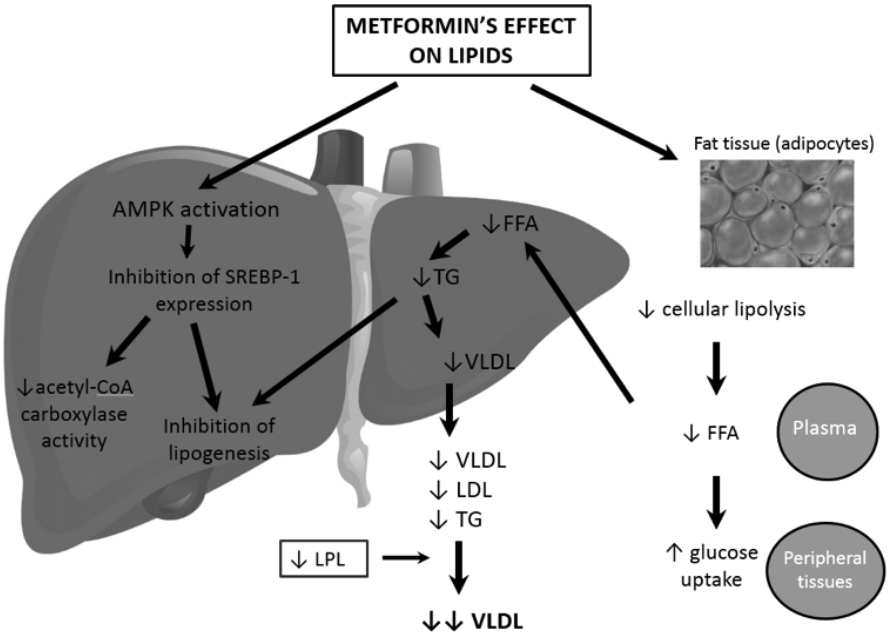
Potentially beneficial effects of metformin, including a reduction in the risk of cardiovascular disease, have been demonstrated not only in subjects with type 2 diabetes, but also with insulin resistance, obesity, polycystic ovary syndrome, or HIV with fat redistribution [105].

A study of non-obese, non-diabetic, hypertensive patients treated with metformin revealed a significant decline in systolic and diastolic blood pressure. The favourable changes remained stable for two months after discontinuation of metformin administration [106]. However, it has to be stressed that this study has three major limitations: comparison of the results to the initial point of the study, lack of control group and a small number of subjects. A review of other clinical trials did not identify any clinically relevant antihypertensive effect of metformin in humans [107,108]. Any discrepancies in the effect of metformin on blood pressure observed between these studies might result from differences in the study design and small number of patients. It must be borne in mind that as these studies are uncontrolled, they cannot establish a clear cause and effect relationship between administration of metformin and effects on blood pressure. Schafers (2003) concluded that metformin does not exert any clinically significant antihypertensive effect, and that the vasoprotective effects of metformin appear to be independent of changes in blood pressure [109].

The mechanisms of metformin action on cardiovascular system is not fully understood, but it has been proved that the drug promotes myocardial preconditioning, reduces cardiomyocyte apoptosis during ischemia, enhances the adaptation of cardiomyocytes metabolism during ischemia, and protects against the development of heart failure [67].

Animal studies have demonstrated that metformin treatment improves cardiac function and reduces the infarct size after a myocardial infarction [110]. Another study examined myocardial tolerance to ischemia in rats with neonatal streptozotocin type 2 diabetes mellitus after metformin treatment. The findings indicate no difference between controls and metformin-treated animals with regard to infarct size or postischemic recovery of left ventricular function. In additions, the infarct size in the type 2 diabetes mellitus animals was significantly lower than that in the controls, indicative of the metabolic preconditioning in type 2 diabetes mellitus, a protective mechanism reducing the risk of heart failure [111].

A rat model study proved that metformin inhibits cardiac hypertrophy in a mechanism based on the reduction of angiotensin II-induced protein synthesis and enhanced phosphorylation of AMPK and eNOS (nitric oxide synthase 3), leading to higher NO production [112]. In addition, metformin contributes to beneficial alterations in cardiac metabolism during myocardial ischemic condition. This mechanism is based on the modification of the cardiac lipid/glucose oxidation ratio [67,113]. Another cardio-protective property of metformin is the ability to reduce the production of pro-



Scheme 3. Effects of metformin on lipid profile.

apoptotic proteins, with a simultaneous increase in anti-apoptotic proteins and decrease in the percentage of apoptotic cardiomyocytes [114]. Furthermore, Gundewar and associates published a study in which they proved that metformin significantly improves left ventricular function and survival in a murine model of heart failure [115].

The molecular mechanism behind the favourable effect of metformin on cardiomyocytes is mainly based on improved myocardial cell mitochondrial respiration and ATP synthesis, which depends on the activation of AMPK and its downstream mediators, eNOS and PGC-1α (peroxisome proliferator-activated receptor-gamma coactivator 1 alpha)[116,117].

Recent studies have thrown new light on the metformin administration in patients with a history of heart failure. The results of several trials have exhibited that the use of metformin in monotherapy or in combination with sulfonylurea reduces the mortality and the morbidity in diabetic patients with heart failure in comparison to sulfonylurea monotherapy [118,119]. Metformin also decreases the mortality in a group of patients with atherothrombosis [120].

Several animal studies suggest that metformin may lower the prevalence of plaque formation in atherosclerosis [121,122]. In most studies, the beneficial effect of metformin on arterial lesion formation was not related to the level of plasma lipids, suggesting that the drug has a vascular effect, mainly based on the prevention of endothelial lesions and damage [103]. The main mechanisms for the atheroprotective effect of metformin involve inhibition of leukocyte-endothelial interaction, foam cell formation, smooth muscle cell proliferation and platelet aggregation [123]. Ghatak *et al.* investigated the effects of metformin on atherothrombotic risk factors in experimental hyperlipidemic rats [124]. They found that treatment with metformin at a dose of 400 mg/kg improved overall lipid profile and provided a significant reduction in oxidative stress. Metformin reduced also endothelial cell damage in ferrous chloride-induced thrombosis in carotid arteries [124].

Metformin was also found to prevent micro- and macrovascular complications of diabetes mellitus by improving vascular endothelial functions [125] in an AMPK-dependent manner. By activation of AMPK, metformin reduces hyperglycaemia-induced mitochondrial reactive oxygen species (ROS) production (induction of Mn-SOD) and promotion of mitochondrial biogenesis (activation of the PGC-1α pathway) in HUVEC cells [126]. Animal studies have also confirmed that metformin elicits a cardioprotective effect in both non-diabetic and diabetic hearts. The diabetic hearts treated with metformin showed more organized and elongated mitochondria and demonstrated a significant increase in phosphorylated AMPK and in PGC-1α expression in comparison with diabetic non-treated hearts [127].

In 2006, a research has been published regarding endothelial vascular reactivity in first line relatives of type 2 diabetic patients with metabolic syndrome and normal glucose tolerance. Contrary to placebo group, which did not show any significant changes in endothelium-dependent and independent vasodilators, patients treated with metformin presented an improvement in the level of endothelium-dependent FBF (forearm blood flow) of up to 111%, while independent factors showed no considerable changes. No differences were reported in patients who were concurrently on antihypertensive drugs [128]. A study by Machado *et al.* [108] whose objective was to evaluate the effects of metformin on vascular reactivity, haemostatic factors and glucose and lipid profiles in patients with type 2 diabetes found that metformin application was associated with protection against macrovascular diabetes complications, increased systolic carotid artery diameter and total systolic blood flow. Such effects may have some yet undiscovered potential in the treatment of complications connected with cerebral blood flow, which are very likely in patients suffering from type 2 diabetes [108].

Recently, metformin has been found to have effects on the soluble intercellular cell-adhesion molecules (ICAMs) and the soluble vascular cell-adhesion molecules (VCAMs) [129]. It has been found that by lowering the plasma level of ICAM-1 and VCAM-1 metformin might decrease cardiovascular events. Importantly, metformin decreases ICAM-1 and VCAM-1 independently of its anti-diabetic properties [130]. Apart from ICAM-1 and VCAM-1, metformin decreases also PAI-1 (plasminogen activator inhibitor-1), vascular endothelial growth factor (VEGF) levels, soluble E-selectin, and vWF (von Willebrand factor) [130].

On the basis of the presented data, we may conclude that metformin exerts a beneficial influence on the cardiovascular system through complex activities on endothelial functions, ROS production and cardiomyocyte functionality.

EFFECTS OF METFORMIN ON COAGULATION AND FIBRINOLYSIS

Hypercoagulability, a tendency toward thrombosis or abnormal blood clotting which predisposes the patient to developing atherosclerosis, has a great tendency to occur in diabetic patients. Impaired fibrinolysis is often reported to be the greatest contributor to cardiovascular complications in diabetes mellitus [131]. It leads to occlusion of blood vessels that often results in lethal consequences, if no immediate action is taken to clear or dilate it [131].

Studies of elderly type 2 diabetes treated with metformin showed a reduction of platelet factor 4 and beta-thromboglobulin. As these proteins are proved to be markers of platelet activation, such an outcome strongly suggests that metformin has platelet-stabilizing potential, as does the fact that metformin has an antioxidant effect on platelets. However, another study did not show any clear effect of metformin on platelets. It is difficult to determine whether either metformin influences physiological activity of platelets directly or its effect on biochemical path alteration is not so clear [132]. Colwell notes that metformin prevents platelet aggregation [133]. Another study conducted in patients with insulin-dependent diabetes mellitus showed that metformin decreased maximum aggregation of platelets induced by adenosine diphosphate (ADP) *in vitro*. The authors concluded that this effect was not dependent on other metabolic factors such as blood glucose, cholesterol, triglyceride and fibrinogen levels [134]. Nevertheless, Nagi and Yudkin demonstrated that metformin does not exert any influence on platelet function [135]. Also Pentikainen *et al.* in a study on 24 nondiabetic patients with hyperlipidaemia demonstrated that metformin had no effect on platelet counts and platelet aggregation induced *in vitro* by ADP, adrenaline, or collagen [136].

The Diabetes Prevention Program Research Group investigated that diabetic patients are generally characterized by increased level of fibrinogen in comparison with multi-ethnic populations. What is important to mention, in this study, fibrinogen did not correlate significantly with changes in glucose, HOMA-IR (insulin resistance index) or demographic variables. The overall level of fibrinogen reduction in patients who underwent metformin therapy was modest, yet significant (0.3%), whereas changing to an intensive lifestyle resulted in reductions of up to 2.0%. Increased level of fibrinogen has been proved in prospective studies to predispose the subject to the development of type 2 diabetes [137]. Other studies also have suggested either a small fall or no change in fibrinogen levels to be associated with metformin use [132].

Gathak *et al.* [124] report that pre-treating rats with diverse doses of biguanide leads to prolongation of APTT (activated partial thromboplastin time) while the greatest prolongation was observed with 300 mg/kg, and a dose-dependent reduction with 400 mg/kg and 500 mg/kg respectively. The most effective dose for this parameter was assessed at 300 mg/kg. In comparison with rats without induced hyperlipidaemia, none of the groups were reported to present any significant difference in the fibrinogen level [124]. In another study metformin-fenofibrate combination treatment was

compared to fenofibrate monotherapy in the case of the global effect on haemostasis. The authors found that metformin tended to increase INR, prolong the partial thromboplastin time (PT) and reduce the level of von Willebrand factor (vWF) and factor VII activity [138].

Nevertheless, there are various alternative ways in which metformin affects clot structure, for example by reduction of crosslinking mediated by FXIII which as a consequence, influences the process of fibrinolysis. Despite the predominant use of metformin in diabetic patients, the ability to penetrate clots has also been demonstrated in non-diabetic obese patients who were subjected to metformin therapy [139,140]. Metformin reduces on the activity of FXIII and the levels of FXIII A and B subunits in plasma after 12 weeks of treatment compared to placebo. In addition, the authors report that metformin altered fibrin structure/function by interfering with the processes involved in fibrin polymerization and lateral aggregation [140]. The effects on clot stabilization (fibrin crosslinking) and activity of FXIII are explained by the effects of metformin on the formation of advanced glycation end products [141]. There is also one study indicating that metformin use is associated with a reduction in coagulation FVII activity levels [142].

Many studies have reported metformin to have fibrinolytic potential which contributes to hindering coagulation processes. It is commonly known that imbalances in clot formation and lysis have a significant influence on developing cardiovascular diseases. Numerous studies on this subject have shown that the main factor with positive impact on listed processes is the reduction in the concentration of PAI-1. Plasmin, the enzyme formed as a result of interaction of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), has been shown to mediate degradation of fibrin clots. The action of these activators is determined by specific inhibitors (PAIs), of which PAI-1 is considered to have the most potent regulative effect on tPA and uPA in humans [143]. Increased PAI-1 level is one of the major risk factors of obesity and metabolic syndrome, as well as various diseases, especially atherosclerosis. Several studies in type 2 diabetic subjects have reported that the use of metformin is associated with an increase in fibrinolysis in both Caucasian [144,145] and Asian subjects [135,146]. Therefore, diabetic patients treated with metformin are less likely to develop these ailments. Maintaining metformin doses of around 2550 mg daily leads to a reduction of PAI-1 levels from a mean of 200.7 ng/mL at baseline to 173.7 ng/mL within 12 weeks [147].

Interesting fact is that PAI-1 level correlates with of plasma insulin level, which implies a relationship between PAI-1, insulin resistance and hyperinsulinemia. Metformin, which is used to decrease insulin level and diminish insulin resistance, additionally controls reduction of PAI-1 [148]. Further support for the effect of metformin on PAI-1 levels comes from a randomized, double blind trial in 27 patients with non-insulin dependent diabetes mellitus. Treatment with metformin contributed to a significant decrease in PAI-1 concentrations when compared with baseline values or placebo treatment [135].

The ability of metformin to reduce PAI-1 levels not only has practical implications regarding fibrin: lower PAI-1 levels were found to be present in alcohol-induced liver injury in a mouse model. Acute alcohol ingestion generates hepatic PAI-1 mRNA, peaking 2 and 12 hours after ethanol consumption. Interestingly, metformin had no effect on PAI-1 expression in alcohol-free mice [88]. The results of this study could potentially refer to humans with alcohol use disorders and therapy of complications caused by excessive expression of the gene encoding PAI-1, with use of biguanides.

In another study, Machado *et al.* evaluated the effects of metformin on haemostatic factors. They found that the improvements in fibrinolysis after metformin treatment was due to the increase in plasminogen levels. Interestingly, the authors indicated that metformin leads to an unexpected decrease in another marker of fibrinolysis — t-PA activity [108]. In contrast to these results, an increase [132] or no effect [135,146] in t-PA activity was observed during metformin therapy.

To summarize, the results of several experimental and clinical studies highlight the multidirectional effect of metformin on haemostasis, including platelet and plasma haemostasis with both coagulation and fibrinolysis system.

METFORMIN'S EFFECTS ON POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome (PCOS) is one of the most common endocrinological disorders, affecting up to 4-12% of women and has a highly unpleasant impact on female sexuality. It is usually manifested by hyperandrogenism, anovulation, and infertility, with incidents of irregular menstrual cycles, acne and hirsutism. One of the most common reported complaints of women with polycystic ovary syndrome is anovulatory infertility. Such women are also at high risk of developing cardiovascular diseases [149].

Metformin was introduced as a hypoglycaemic agent for medicinal purposes in order to determine the extent to which hyperinsulinemia influences the pathogenesis of the PCOS. Initial results of the studies aroused a tremendous amount of interest as it seemed to be a breakthrough. However, subsequent randomized clinical trials and numerous meta-analyses have diminished earlier enthusiasm. Currently, it is obvious that more work is required to establish the role of metformin with particular regard to the mode of prevention and treatment of various gestational or long-term medical conditions [150].

There are certain effects that have been reported in relation to metformin in PCOS patients, for example restoring ovulation, weight loss, lowering levels of circulating androgen, diminishing the frequency of abortion and reducing the risk of developing gestational diabetes mellitus (GDM). Further studies have shown that the addition of mentioned biguanide to the regime of ovarian stimulation in *in vitro* fertilization (IVF) enhances the outcome of pregnancy [150].

Metformin, as the first drug which causes sensitization to insulin (ISD), played a pioneering role in PCOS treatment to determine the mechanism of insulin resistance in the syndrome pathogenesis. Velazquez and colleagues reported a noteworthy progress in menstrual regularity and decline in circulating androgen levels, as well as a notable loss of body weight which confounded their findings [151]. Lord and colleagues [152] concluded consequently that metformin was an effective drug to enhance ovulation in PCOS women and that it was reasonable to use it as a first-line therapy. Nevertheless, they strongly accentuated that it should be applied simultaneously with a change in lifestyle. They involved 7 studies engaging a total of 156 PCOS patients who underwent therapy with metformin, of whom 72 (46%) responded with ovulation; 154 either received placebo or no therapy, and of these, 37 (24%) demonstrated recurrence of ovulation [152]. Lord and associates [149] claim that metformin is sufficient for inducing ovulation in patients with PCOS. However, it has to be taken into account that meta-analysis is valid only if the same general population is represented in all the analysed studies [149]. Furthermore, studies carried out by Ghandi and colleagues [153] showed that metformin appears to have a considerable influence on ovulation rates, which is 30%. Moreover, in comparison with the baseline, treatment with metformin appeared to show a significant reduction in body mass, waist circumference, serum luteinizing hormone (LH) and triglyceride level. Treatment with this biguanide also resulted in a reduction in total testosterone and cholesterol levels but the differences were negligible [153].

Another aspect of polycystic ovary syndrome is that affected women have a tendency to show much higher risk of abortion compared with healthy women. The risk has been assessed at 30-50%

[154]. However, the exact mechanism of this process is not yet clearly established and the extent to which insulin resistance may play a role still remains unclear. Velazquez and colleagues [148] report that by its significant impact on decreasing fasting insulin and reaction to glucose level in hyperinsulinemic PCOS patients, metformin diminishes the effects of hyperinsulinemia-driven hyperandrogenism and can reverse endocrinopathy to an extent by which women to return to regular menstrual cycles, reversal of infertility, and spontaneous pregnancy (Scheme **4**) [148].

Metformin is considered to enhance perifollicular vascularization and ovarian artery impedance, which hypothetically can potentially return ovarian follicular development to physiological levels [150,155]. What is more, observational studies have implied that metformin therapy reduced the probability of miscarriage among women who suffer from polycystic ovary syndrome [156]. On the other hand, a meta-analysis by Palomba and colleagues' claimed that metformin had no advantageous influence on the miscarriage rate [157].

To summarize, metformin has certain effects on patients with PCOS, such as restoration of ovulation, weight loss, lowering levels of circulating androgen and diminishing the frequency of abortion, However, the other mechanisms of metformin action need further examination.

ANTI-INFLAMMATORY PROPERTIES OF METFORMIN

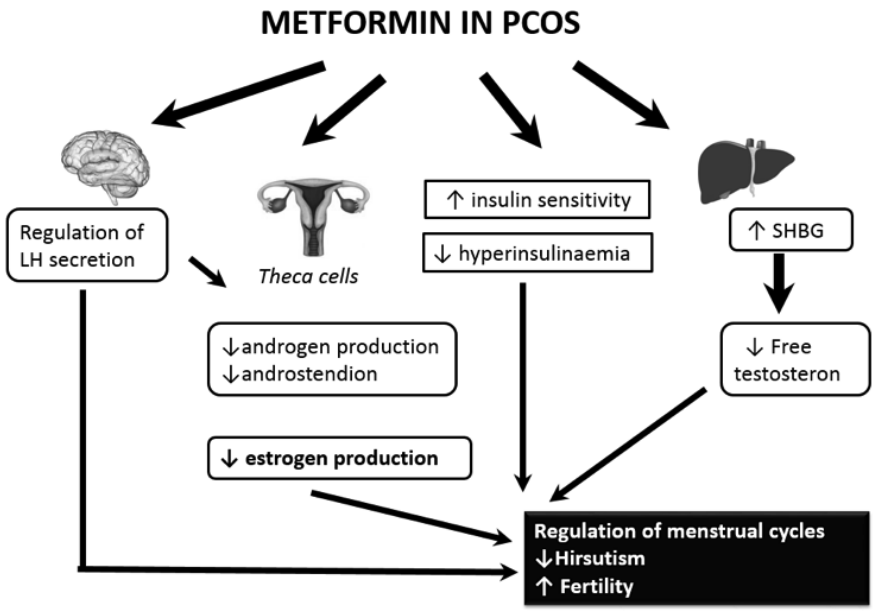
Yuan *et al.* [158] also report that metformin in lipopolysaccharide (LPS)-induced hepatic injury in D-galactosamine (D-Gal)-sensitized mice significantly reduced TNF-α (tumour necrosis factor) level and markers of hepatic efficacy: alanine aminotransferase (ALT), aspartate aminotransferase (AST) serum levels [158]. These changes were accompanied by improved histological alterations in liver sections, decreased myeloperoxidase (MPO) activity, reduced malondialdehyde (MDA) content in liver homogenates and increased survival rate of experimental animals. Metformin could also provide therapeutic benefits in endotoxin-induced hepatic injury, suggesting its pharmacological potential in inflammation-base disorders [158]. Elsewhere, metformin was found to have an influence on dimethylarginine (ADMA) metabolism in inflammation caused by lipopolysaccharide (LPS)/D-galactosamine (D-GalN) treatment [159].

Studies performed on human monocytes pre-stimulated with LPS and oxidized LDL showed that metformin addition resulted in reduced production of TNF and TF (tissue factor) [160]. Elsewhere, reduced production of inflammatory cytokines such as TNF-α, MCP-1 (monocyte chemoattractant protein-1), IL-1β (interleukin-1 β), MIP-1α (Macrophage Inflammatory Proteins-1α), IL-6, leptin, and IL-18 was observed in endotoxin-induced uveitis in rats after treatment with metformin [161]. Another laboratory study showed that macrophages stimulated with LPS and treated with metformin exhibited decreased production of TNF-α, IL-6, and IFN-γ (interferon-γ) in a dose-dependent manner [162]. Metformin was found to decrease IL-1β induced activation and nuclear translocation of nuclear factor κB (NF-κB) in smooth muscle cells [125]. What is more, metformin decreases IL-1β induced activation of proinflammatory phosphokinases Akt (protein kinase B), p38 (mitogen-activated protein kinase) and Erk (extracellular-signal-regulated kinases), however, it does not affect Class I Phosphoinositide 3-kinases (P-I-3 kinase) activity [125].

It has been proved that metformin inhibits mammalian target of rapamycine (mTOR) signalling which leads to the inhibition of mitogen-induced proliferation of B and T cells and reduction of IL-1 and TNF-α levels, therefore it has been implied that metformin might be used in rheumatoid arthritis treatment [163,164].

Metformin has also been shown to decrease the pro-inflammatory cytokine macrophage migration inhibitor factor (MIF) in the plasma and monocytes from obese patients when compared to untreated patients [165,166]. As reported by Shi *et al*., metformin decreases serum levels of CRP in patients with type 2 diabetes mellitus [167]. An *in vivo* study by Chakraborty *et al.* [168] in which different stress and inflammatory parameters were evaluated in diabetic patients found that ROS generation and advanced oxidation protein products were reduced by metformin treatment compared to placebo. The findings indicate that metformin administration also enhanced total thiol and nitric oxide level [168].

There also have been studies indicating the anti-inflammatory properties of AMPK which [169] suggest that metformin may also play an important role in targeting the inflammation present in the microenvironment of cancer tissues. Xavier *et al.* suggest that inhibition of angiogenesis by metformin might also contribute to the reduction of tumour growth [170].



Scheme 4. The role of metformin in PCOS.

Recent studies have also reported that AMPK might have an impact on pain in animal models of neuropathy and acute nociception [171,172]. Russe *et al.* [173] investigated the impact of AMPK in inflammatory nociception and found that metformin activation of AMPK results in analgesic effects similar to those observed with ibuprofen. The authors conclude that the mechanisms of this activity are based on regulation of the AMPKα2 subunit of the kinase in sensory neurons and immune cells [173]. Anti-inflammatory properties of AMPK have also been demonstrated in a study which revealed that AMPK activation is associated with decreasing IL-6 and IL-8 concentrations in adipose tissue of humans and in the skeletal muscles and muscle cells of rats [174].

Bearing in mind the anti-inflammatory properties of metformin and its several possible mechanisms of action, we may anticipate greater scientific interest in this field and novel clinical indications for metformin use.

METFORMIN AND AGEING

It is extremely important to emphasize that through induction of AMPK, metformin might present anti-ageing properties. It has been established that the AMPK signalling pathway is involved in the regulation of lifespan as its responsiveness deteriorates with ageing [3,175]. Apart from its effect on AMPK, metformin also exerts an influence on cellular metabolism and other age-related transcription factor pathways [3]. It also has been stated that the IGF-1 signalling pathway is involved in the mechanism of ageing. An animal study by Anisimov *et al.* found that the life-prolonging effects of calorie restriction may be due to falling IGF-1 levels [176]: metformin treatment slightly decreased food consumption, and slowed the rise in blood glucose and triglyceride levels. Most importantly, metformin prolonged the mean life span by 8% and the maximum life span by one month in comparison with the control mice group [176].

Scientific databases contain several articles on the role of metformin in the process of ageing. For instance, Na *et al.* [177] revealed that metformin has the potential to inhibit age- and oxidative stress-induced centrosome amplification (a hallmark of cancer) in Drosophila intestinal stem cells. The researchers confirm that metformin exerts this effect through down-regulation of AKT/target of rapamycin (TOR) activity [177]. A study conducted on nematodes has shown that metformin lengthens life span, improves locomotion and delays the occurrence of lipofuscin pigment, which is an indicator of cell ageing [175]. The next study performed on male rats with Huntington's disease has proved the effect of metformin on life prolongation [178].

In a most recent randomized, single-blind, placebo-controlled trial, it has occurred that, compared with baseline, metformin significantly improved metabolic parameters and insulin sensitivity, increased Sirtuin-1 (SIRT1) gene/protein expression, which is associated with metabolism and longevity, elevated mTOR gene expression, and modified the plasma N-glycan profile. Also, metformin exerted favourable effects on these factors in comparison with placebo [179]. The results of this first study on the anti-ageing properties of metformin conducted in humans show that metformin modulates effectors of pathways that regulate longevity in animal models [179].

Recent studies have examined the potential application of metformin for the treatment of Alzheimer's disease [180], amnestic mild cognitive impairment [181] and Parkinson's disease [182]. Considering the presented results, future interest in the use of metformin in the treatment of neurodegenerative diseases will undoubtedly grow.

ANTI-CANCER PROPERTIES OF METFORMIN

A number of *in vitro*, animal and clinical studies have demonstrated that metformin has an overall favourable effect on cancer; however, the precise molecular mechanism of this anti-cancer activity is still not fully understood.

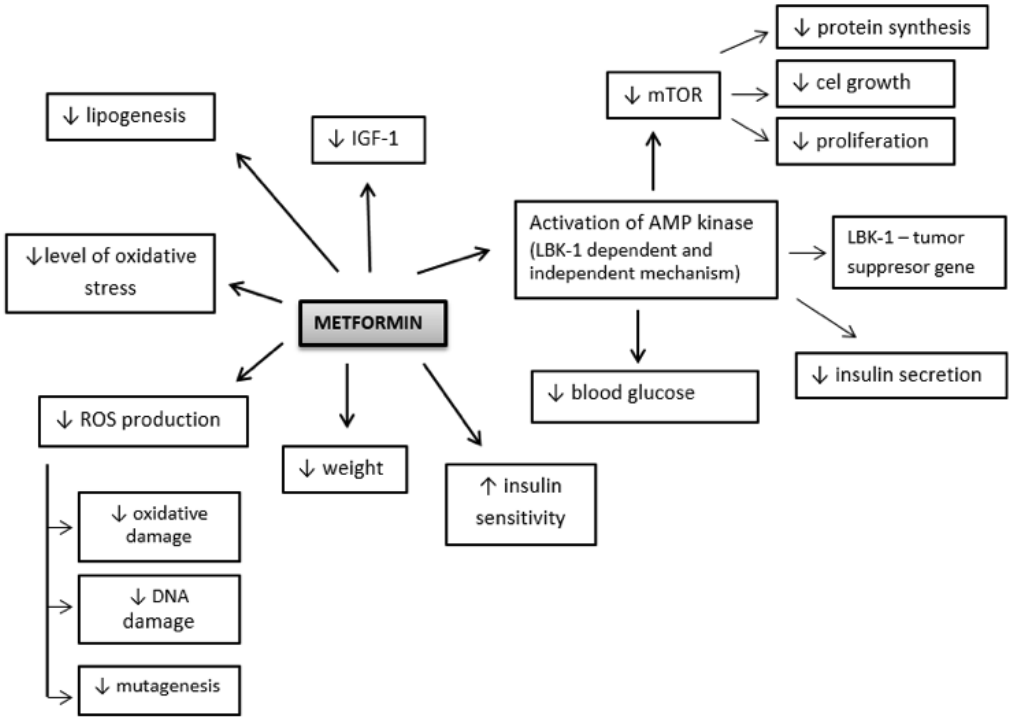
As reported above the main mechanism of metformin action is LKB1-based AMPK activation [183]. LBK1 is a tumour suppressor which constitutes one of the most commonly mutated genes in lung and pancreatic cancers and melanomas [184,185]. It has been stated that the absence or decreased expression of LKB1 in human breast carcinomas corresponds to higher mortality and poor prognosis [186]. In addition, metformin through AMPK activation leads to the inhibition of lipogenesis in malignant lesions [187,188], which as a consequence, might result in inhibition of the activity and expression of certain oncoproteins [189,190].

Another mechanism of metformin action is inhibition of the mammalian target of rapamycin (mTOR) through decreasing the levels of IGF-1 or inhibiting AKT by the AMPK-independent pathway [191,192]. Furthermore, by activation of the LKB1-mediated AMPK pathway metformin has been shown to inhibit mTOR and protein synthesis [193]. It is important to stress the importance of the mTOR signalling pathway, as it is a crucial factor in the regulation of cellular energy homeostasis [194,195], cell growth and tumorigenesis [196,197]. It has been reported that activation of mTOR correlates with cancer progression, adverse prognosis and resistance to chemotherapy [196,197].

Apart from the listed mechanisms, metformin's anti-cancer effect may result from the fact that it decreases the production of ROS independently of AMPK activation [198]. Metformin is also regarded as an 'antimetabolite drug' since several studies have reported that the drug alters folate metabolism in several types of cancer cell lines. Clinical studies confirm this fact, as an increased homocysteine and decreased folate and B_{12} vitamin levels have been reported in patients with type 2 diabetes mellitus treated with metformin [199,200]. In addition, it is important to point out that metformin's anti-cancer properties are also associated with its ability to reduce risk factors such as obesity, hyperinsulinemia and insulin resistance [7,201,202].

Another mechanism by which metformin exerts anti-cancer effects, independent of AMPK, is by decreasing the expression of the oncoprotein HER2 in human breast cancer cells *via* the direct inhibition of p70S6K1 activity [203]. Ben Sahra *et al.* showed also that metformin exerts a anti-neoplastic effect through the induction of cell-cycle arrest *via* decreasing cyclin D1 protein expression [204] and increasing REDD1 expression in a p53-dependent manner [67,205]. Metformin has also been shown to promote the death of cancer cells by promoting apoptotic pathways via caspase-dependent and caspase-independent mechanisms [206,207]. Another study reported that metformin enhances apoptosis of prostate cancer cells in a p53-dependent manner in the presence of 2-deoxyglucose [208]. It has also been stated that metformin decreases the production of tumour necrosis factor alpha (TNFα) in human monocytes, probably in AMPK-independent manner. As TNFα is involved in maintaining the process of chronic inflammation which sustains the basis of cancer progression, metformin may thus contribute to prevention of tumour development. Scheme **5** presents selected mechanisms behind the anti-cancer action of metformin [160].

A number of *in vitro* and preclinical studies have demonstrated that metformin has beneficial effects on various cancer cell lines [56]. For example, metformin may improve the efficacy of various chemotherapeutics and aids in overcoming chemotherapy resistance [74,78]. The results of several studies have revealed that metformin enhances the sensitivity of endometrial cancer cells to cisplatin and paclitaxel [209,210], increases the antiproliferative effects of cisplatin [211] and overcomes the resistance of trastuzumab in animal model of breast cancer [212]. A series of scientific works also outlines anti-proliferative properties of metformin. Indeed, metformin



Scheme 5. Schematic diagram of selected proposed mechanisms of anti-cancer action of metformin.

has been shown to inhibit the proliferation of human lung cancer [213], ovarian [214] and hepatocellular carcinoma cell lines [215].

A number of studies have evaluated the effects of metformin on gastrointestinal cancer [216-218]. In 2011 Zhang *et al.* presented the results of a meta-analysis on the relationship between metformin treatment in patients with type 2 diabetes and colorectal cancer [219]. Metformin treatment was associated with a significantly lower risk of colorectal neoplasm among diabetic patients [219]. Similarly, a 2011 retrospective cohort study including 800,000 patients from the Taiwanese National Health Insurance by Lee *at al.* [217] found interaction between metformin treatment and overall beneficial effects such as decline in incidence of total cancer, colorectal cancer, liver cancer and pancreatic cancer [217]. In contrast, a study of Bodmer [220] found that treatment with metformin was not associated with a decreased risk of developing colorectal cancer among diabetic patients [220]. The results of a recent meta-analysis of currently-available observational studies suggest that metformin administration appears to be associated with a reduced risk of pancreatic cancer in patients with type 2 diabetes mellitus [221]. However, it has been suggested that the results of a meta-analysis should be interpreted with caution mainly due to the fact that most of the studies were observational retrospective studies using historical medical or insurance data [56]. Similar findings concerning the effectiveness of metformin on gynaecologic cancers, including ovarian, breast and endometrial cancers are presented in Table **1**.

Despite being a well-tested anti-diabetic medication, metformin certainly has much more to offer, especially in the field of oncology. A number of clinical studies have indicated its promising anticancer properties; however, further studies are needed to fully establish the advantages of metformin in this regard.

PROMISING POTENTIAL OF METFORMIN PRO-DRUGS/DERIVATIVES

As noted above, huge inter- and intra-individual differences exist in the pharmacokinetics and clinical response to metformin, and the absorption of the drug from gastrointestinal tract is slow and variable. Therefore, there is a need to develop novel approaches in order to improve the bioavailability of metformin. Various formulation strategies, for example extended-release formulations, has been proposed for this end [230].

To illustrate this, Corti *et al.* prepared complexes between metformin hydrochloride and triacetyl-β-cyclodextrin and evaluated their suitability for the development of a sustained-release dosage form of metformin [231]. The authors found that the actual effectiveness of applied cyclodextrin as a carrier for obtaining a slow-dissolving form of metformin depends on the preparation technique of these complexes. While only one minute was needed to dissolve 100% of the pure drug, three, seven, 40, 120 and 420 minutes were needed for physically mixed, sealed-heated, kneaded, co-ground and spray-dried products, respectively [231].

Huttunen and Rautio team [230] propose the use of pro-drugs as a way of improving the oral absorption of metformin. A pro-drug is a pharmacologically-inactive derivative of drug which is converted into an active compound under biological conditions. Generally, pro-drugs are applied in order to improve the unfavourable physicochemical, pharmaceutical or biopharmaceutical properties of a parent drug [230].

The scientists synthesized several novel bio-reversible sulfenyl guanidine (N-S) pro-drugs (Table **2**, **I-II**) of metformin with improved oral absorption were produced [230], and which were stable in aqueous buffer solutions (> 80 h) at pH 4.0 and 7.4. *In vitro* bioconversion studies of pro-drugs showed that pro-drug **II** released metformin molecule extremely quickly with half-lives ranging from four seconds to 40 minutes, whereas the prodrug **I** released hardly any metformin for 24 hours. *In vivo* studies of pro-drugs **I** and **II** found that both pro-drugs were easily transformed into the active drug after intravenous administration. This enhanced oral absorption promoted the bioavailability of metformin from 43% to 65% in rats [230]. Pharmacokinetic studies revealed that pro-drug **I** showed a sustained-release profile and longer plasma half-life for metformin after oral administration [232].

Table 1. Selected clinical studies evaluating the effects of metformin on gynaecologic cancers.

Cancer	Study type/design	Total participants	Measured outcome	Results/conclusions/Remarks	Refs.
Breast	Retrospective cohort study	68,019	Risk of breast cancer in post-menopausal diabetic women	Lower breast cancer incidence among patients using metformin, slightly higher risk in women receiving other diabetic medications in comparison with non-diabetic women	[220]
Breast	Prospective study	39	Effects of metformin on Ki67 scores in tumour tissue	Metformin significantly decreased the mean percentage of cells staining for nuclear antigen Ki67 compared with controls	[221]
Breast	Meta-analysis	418,54	Risk of breast cancer with metformin treatment in postmenopausal diabetic women	Protective role of metformin on the incidence of breast cancer comparing to non-users	[222]
Breast	Retrospective study	1,983	Breast cancer-specific mortality of metformin users vs. nonusers in patients with human epidermal growth factor receptor positive breast cancer	Metformin usage in diabetic patients was associated with decreased breast cancer-specific mortality	[223]
Endometrial	Cohort study	1,241	Prevention of endometrial cancer	No association between metformin use and risk of endometrial cancer was found	[224]
Endometrial	Cohort study	1,495	Survival among patients with endometrial cancer	Non-metformin users had 1.8 times worse recurrence-free survival and 2.3 times worse overall survival after adjusting for age, stage, histology and treatment	[225]
Ovary	Case control study	10,781	Prevention of ovary cancer	Long-term use of metformin decreased ovarian cancer risk	[226]
Ovary	Case control study	215	Survival of patients with ovary cancer	Metformin use was associated with improved overall survival.	[227]

Huttunen *et al*. presented a synthesis of other (**III-VI,** Table **2**) consecutive metformin pro-drugs [233] which were characterized by increased lipophilicity. *In vitro* studies across a Caco-2 cell monolayer indicated that the novel pro-drugs expressed good permeability properties, with the octylthio (**V**) pro-drug being the most efficient [233]. The results of preliminary *in vivo* studies of pro-drug **V** were encouraging, because this pro-drug was found to be absorbed mainly intact after oral administration [233].

Huttunen's team synthesized a series of sulphonamide pro-drugs (**VII-IX,** Table **2**) [234]. Subsequently, they evaluated the bioconversion of these prodrugs by glutathione-S-transferase (GST) in *in vitro* models [234]. The results of the study revealed that pro-drug I**X** was bio-activated by GST and released metformin in a quantitative manner, whereas the two others were enzymatically stable, which suggests that pro-drug I**X** has the potential to increase the oral absorption of metformin [234].

Metformin has also been used as a substrate for further synthesis of novel derivatives with promising properties. For instance, Koh *et al.* [235] synthesized a novel metformin derivative (**X**) (HL010183) and a series of metformin salts which exerted more potent inhibitory effects on the proliferation and invasiveness of Hs578T breast carcinoma cells than metformin: **XI**-metformin gamma-aminobutyric acid (GABA) salt, **XII**-metformin pregabalin salt and **XIII**-metformin gabapentin salt (Table **2**) [235].

CONCLUSION

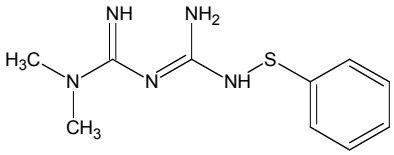
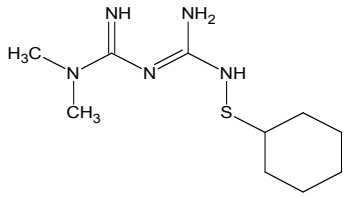
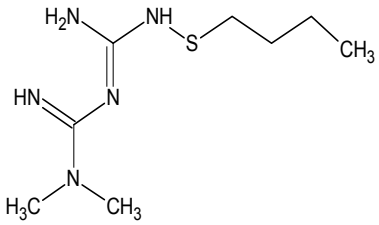
Metformin is an effective oral anti-diabetic drug with an established role in the treatment of type 2 diabetes. Recent studies have confirmed that metformin has a key role in several glucose-lowering mechanisms, including inhibiting hepatic gluconeogenesis and intestinal glucose absorption, increasing insulin sensitivity, and modulating of incretin axis (increase in glucagon-like peptide 1).

Despite possessing several advantageous pharmacological properties, the administration of metformin is associated with several problems including slow and incomplete absorption due to its physicochemical and pharmacokinetic properties, as well as its intra-subject and inter-subject variability in response to the drug. It has been established that metformin is a substrate for OCTs and MATE transporters, which determines its oral absorption, distribution, hepatic uptake, elimination (renal excretion) and biochemical effects in humans [13]. It has been found that promoter variants of both types of transporters might affect the pharmacokinetics and pharmacodynamics of metformin. However, it is necessary to be aware that until recently, its pharmacokinetic properties and the involvement of transporters were poorly understood.

Apart from anti-diabetic properties, there is also growing body of evidence indicating that metformin exerts a favourable effect on body weight, lipids, and cardiovascular risk associated with type 2 diabetes. Recent publications indicate that the potential spectrum of metformin beneficial effects has expanded to the treatment of polycystic ovarian syndrome, diabetic nephropathy and metabolic syndrome. Some scientists also highlight the anti-inflammatory and anti-ageing properties of metformin.

Currently available evidence suggests that metformin may play an important role not only in the treatment of certain types of can-

Table 2. Pro-drugs of metformin.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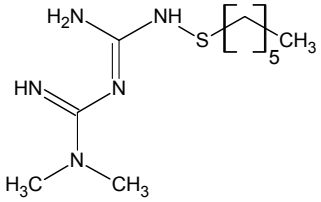	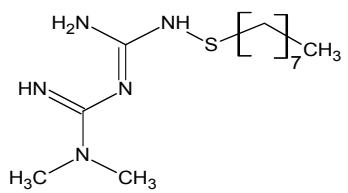	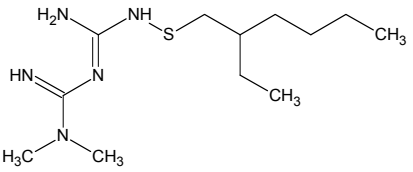
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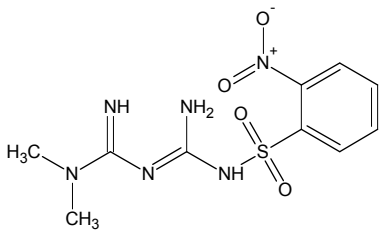	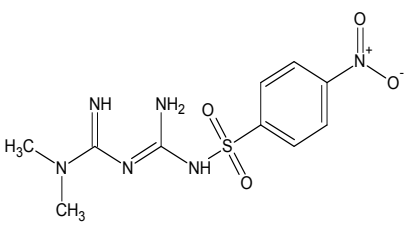	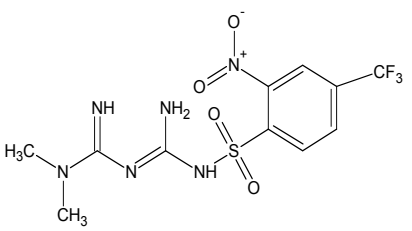
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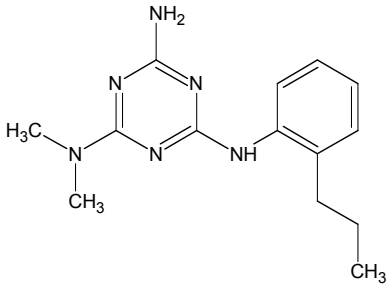	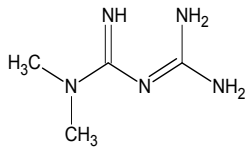 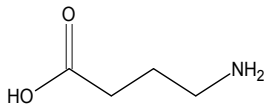	
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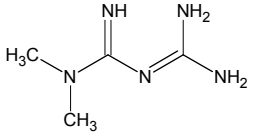 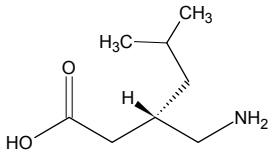	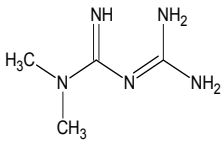 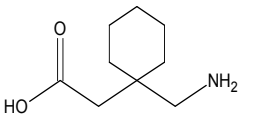	
XII	**XIII**	

cers but also in cancer prevention. However, further well designed, placebo controlled and randomised clinical studies for the evaluation of the effect of metformin on cancer tissue are needed to confirm its anti-neoplastic properties.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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