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Mechanisms of Action of Metformin

Samira Abdulla Mahmood

Abstract

Metformin is the first-choice drug for treatment of type 2 diabetes notably those associated with obesity. It does not only reduce hyperglycemia, but also possesses pleiotropic effects opening the pave for numerous potential clinical applications. In this chapter we illustrate the various mechanisms of metformin action in reduction of hepatic glucose output, improvement of insulin action, restoration of fat metabolism and gut microbiome, reduction of inflammation, upregulation of antioxidant enzymes, and attenuation of tumor growth. Understanding of such mechanisms might propose further clinical applications for metformin.

Keywords: 5′ AMP-activated protein kinase (AMPK), metformin, gluconeogenesis, antioxidant, mammalian target of rapamycin (mTOR), complex 1

1. Introduction

The mechanisms underlying metformin actions appear to be complex and responsible for the pleiotropic effects of metformin. These mechanisms remain a topic of considerable debate. Actually, in the last decade we moved from a simple picture, that metformin acts via the liver 5′ AMP-activated protein kinase (AMPK), to a much more complex one, reflecting its various mechanisms in different cells and tissues.

Since the early studies have suggested that metformin acts by inhibition of complex 1 in mitochondrial electron transport chain [1] and subsequently activation of AMPK [2, 3], AMPK-independent targets have also been reported. These comprise dephosphorylation the ribosomal protein S6, suppression of mammalian target of rapamycin complex 1 (mTORC1) activation and signaling via Rag GTPase [4], attenuation of hepatic glucose 6 phosphate levels [5], suppression of redox transfer by mitochondrial glycerophosphate dehydrogenase (mGPD) [6], as well as modulation of inflammation/oxidative stress and oncogenic signaling pathways.

2. Primary molecular mechanism

Metformin, a hydrophilic drug with Pka 12.4, cannot readily be diffuse passively through the cell membrane due to its existence as cation (ionized) at physiological pH 7.4 [7]. As hydrophilic drug it needs a carrier mediated pathway to efficiently pass through the cell membrane. This is facilitated by the organic cation transporter

1(OCT 1) [8], a member of the soluble carrier family 22 (SLC22) of membrane proteins. OCT1 is mostly expressed in the liver for transferring of cations including metformin, but also facilitates the uptake of metformin from the gut lumen to the interstitium [9]. Cells express OCT1 are able to facilitate cellular uptake of metformin which is in consistence with its accumulation in particular targeted organelles. Also, other types of OCT proteins are present at apical or basolateral sites with different functions.

Within the mitochondria metformin accumulates in the matrix and inhibits complex1 electron transfer chain NADH ubiquitin oxidoreductase (NADH) [1, 10, 11], which promotes proton generation. This inhibition reduces NADH oxidation and ultimately prevents ATP production from ATP synthase. By this way, the ratios of AMP: ATP and ADP: ATP increase, **Figure 1**. Increment in these ratios, which accompanied with reduction in cellular energy activates the cellular energy sensor (house keeper enzyme) AMPK [11]. Another consequence of complex 1 inhibition is the higher levels of AMP, which in turn induces AMPK-independent effects. Moreover, metformin directly inhibits hepatic GPD2, the enzyme involves in substrate (glycerol) gluconeogenesis. Its inhibition by metformin leads to increase cytosolic redox and suppression of gluconeogenesis [12].

AMPK is a heterotrimeric protein complex that consists of α, β, and γ subunits. The α subunit represents the catalytic site that can be activated (phosphorylated) by liver kinase B1 (LKB1) [13] at Thr-172 [14] and also by calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) at Thr-172 [15]. The β and γ denote regulatory subunits. In mammals, the γ subunits contain nucleotide-binding sites for AMP or ATP [16]. In case of cellular energy stress with low ATP, AMP or ADP directly and mutually bind to the γ subunits causing conformational change leading to AMPK activation. Metformin induces activation of AMPK by LBK1 pathway and also by AMP/ADP induced conformational changes, too. It is worth to mention that higher levels of AMP protect AMPK from dephosphorylation by phosphatases [17]. AMPK plays a role in several cellular events, including glucose metabolism, lipid metabolism, redox regulation, anti-aging and anti-inflammation [18, 19].
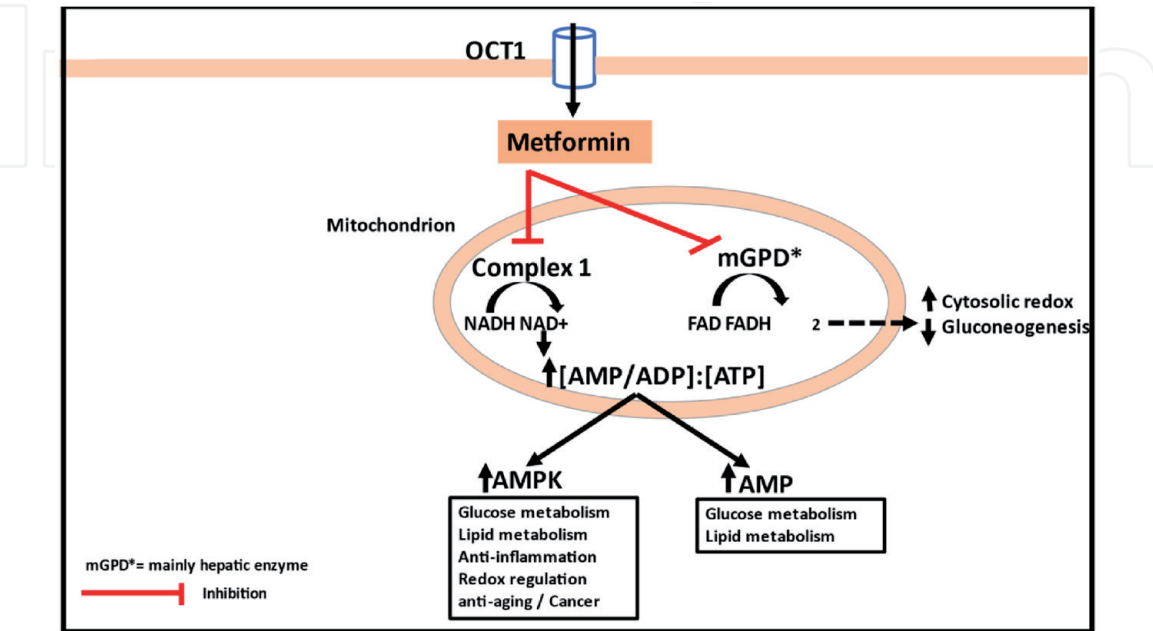

Figure 1.
Primary molecular mechanism of metformin action. For explanation see text.

3. Antihyperglycemic mechanisms of action

Metformin is currently the drug of choice in treating patients with type 2 diabetes mellitus (T2DM). Its mechanisms are still elusive. Nevertheless, it lowers blood glucose through multiple mechanisms. First, it inhibits intestinal absorption of glucose. Second, it suppresses glucose production by the liver. Third, it facilitates glucose uptake into tissues, thus reducing blood glucose levels enabling better health to pancreatic beta-cells. Finally, it improves insulin sensitivity and inflammation. The most accepted action of metformin in T2DM is inhibition of gluconeogenesis and reduction in hepatic glucose output (HGO).

3.1 Mechanisms to lower hepatic gluconeogenesis

Metformin is taken up into the hepatocyte via the OTC1 [20]. Due to the difference in hepatocyte pH and pka of metformin, the drug becomes ionized and positively charge and accumulates in the cells and, further, in the mitochondria to concentrations up to 1000-fold higher than in the extracellular medium [21]. The uptake of positively charge metformin into the mitochondria is derived by the membrane potentials across the plasma membrane and mitochondrial inner membrane (positive outside) [1], Within the mitochondria, metformin inhibits complex1, which reduces ATP production and increases AMP and ADP levels. One consequence of respiratory chain inhibition is increment in ADP:ATP ratios that modestly suppress gluconeogenesis as seen experimentally in cells carrying this process [22], and hinder the hepatocytes from synthetizing the high energy requiring gluconeogenesis [23], **Figure 2**. Other consequence is changes in NAD^+:NADH ratios involving in a negative impact on gluconeogenesis [10].

Criticized comments on this mechanism are based on the higher concentration (in millimole levels) on metformin required for rapid complex 1 inhibition, although experimentation in in vitro studies have shown that inhibition of comlpex1 in rat hepatoma (H4IIE) cells does occur at lower concentrations ((50–100 µmol/l)) after long periods due to a slow transport of metformin across mitochondrial membrane [1]. This observation has been confirmed experimentally [24].
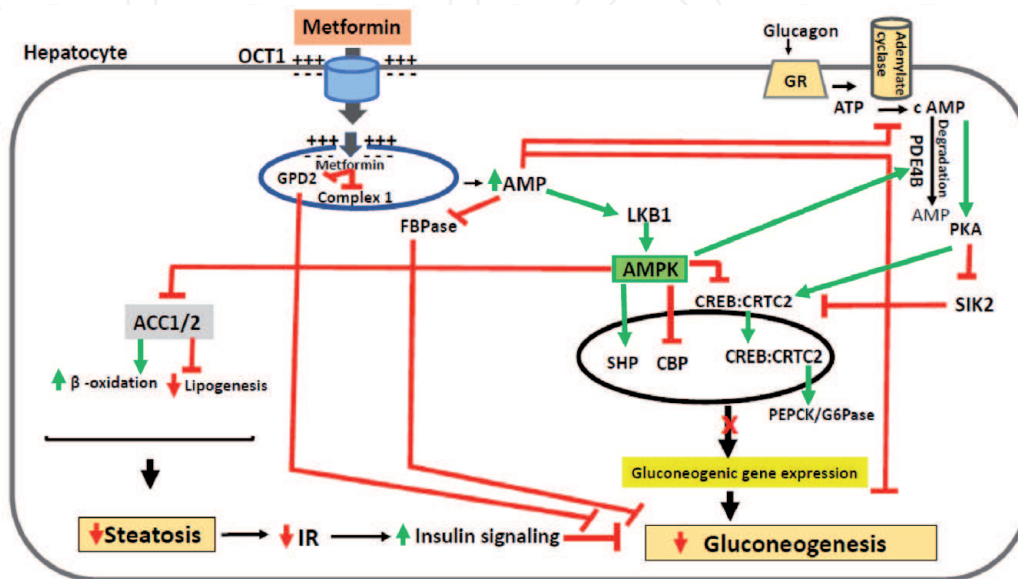

Figure 2.
Mechanism of lowering hepatic gluconeogenesis by metformin (see text).

3.2 Activation of hepatic AMPK

Metformin induced reduction in cellular energy and increment in AMP:ATP ratios are indicative for activation of the energy sensor AMPK by LKB1 (see primary mechanism). Stimulation of AMPK results in repression of anabolism (fatty acid and cholesterol synthesis, gluconeogenesis) and switching on catabolism (fatty acid uptake and oxidation, glucose uptake) [25] in order to restore cell energy hemostasis and prevent cells from damage [26]. The first observation of involvement of AMPK in metformin action was reported in vitro of rat hepatocytes and rat liver in vivo [27]. Moreover, AMPK can also be activated by glucose starvation, exercise and metformin activated lysosomal mechanisms [28].

3.3 AMPK dependent mechanisms

Activated AMPK phosphorylates the cAMP specific 3′,5′-cyclic phosphodiesterase 4B (PDE4B) and activates cAMP degradation (↓cAMP) [21]. Consequently, it prevents the activation of cAMP-dependent protein kinase A (PKA), the enzyme that phosphorylates the transcription factor cAMP response element binding protein (CREB), and then activates CREB-CBP-CRTC2 (CREB:CRTC2) transcription complex involving in transcription of the genes encoding the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and Glucose 6-phosphatase (G6Pase) [29], **Figure 2**. On the other hand, AMPK induces phosphorylation of CREB binding protein (CBP) at serine 436 leading to dissociation of the CREB-CBP-CRTC2 transcription complex, thus repression of PEPCK and G6Pase [29]. In addition, AMPK or salt-inducible kinase 2 (SIK2) phosphorylates CREB-regulated transcriptional coactivator-2 (CRTC2), thus, inhibits its nuclear translocation and retains in cytoplasm [30]. Moreover, AMPK upregulates the orphan nuclear receptor small heterodimer partner (SHP), that functions as transcription repressor [31] through competition with CRTC2 binding in CREB–CBP complex, **Figure 2**.

Another mechanism mediated by AMPK is inhibition of fat biosynthesis and activation of fat beta-oxidation, resulting in long term enhancement of hepatic insulin sensitivity, which is clinically relevant. Metformin-induced hepatic AMPK phosphorylates the isomers of acetyl-CoA carboxylase (ACC1/ACC2) at serine residues responsible for fat beta-oxidation [32]. Phosphorylation of ACC1 and ACC2 inhibit the conversion of acetyl-CoA to malonyl-CoA resulting in reduction of liver lipogenesis and hepatosteatosis (fatty liver) and increment in fatty acids oxidation, which are factors contributing in improvement of insulin sensitivity/signaling and hyperglycemia. Likewise, activation of AMPK suppresses the expression of lipogenic genes by direct phosphorylation of transcription factors including carbohydrate response element binding protein (ChREBP), **Figure 2**, and by this means regresses the lipogenesis [33], **Figure 2**. Taken together, the role of AMPK involves in phosphorylation of key metabolic enzymes and transcription co-activators/factors modulating gene expression leading to inhibition of glucose, proteins and lipid synthesis and stimulation of glucose uptake and fatty acid oxidation.

3.4 AMPK independent mechanisms

Metformin induced a rise in AMP levels inhibits gluconeogenesis independent of AMPK. AMP allosterically inhibits fructose-1,6-bisphosphatase, a key enzyme of gluconeogenesis and AMP sensitive [34]. This action might be responsible for acute metformin action. In addition, AMP inhibits adenylate cyclase producing cAMP in response to glucagon released in starvation leading to lowering cAMP and reducing expression of gluconeogenesis enzymes [35], **Figure 2**.

Recent proposed mechanism of increased hepatic gluconeogenesis is related to impaired white adipose tissue lipolysis with resultant increase in hepatic uptake of non-esterified fatty acids (NEFA). Hepatic beta-oxidation of NEFA can produce acetyl-coenzyme A (acetyl-CoA), the allosteric activator of the enzyme pyruvate carboxylase that is implicated in the first step f gluconeogenesis by supplying oxaloacetate [36]. Insulin regulates lipolysis of white adipose tissue, thereby, indirectly regulates hepatic gluconeogenesis [37]. Insulin resistance with inflammation in white adipose tissue increases glycerol turnover. Thus, metformin improves insulin sensitivity and reduces resistance leading to suppression of gluconeogenesis.

In addition, white adipose tissue delivers glycerol to the liver. In the liver, glycerol is phosphorylated. Through mitochondrial glycerol-3-phosphate dehydrogenase (GPD2), glycerol is converted into dihydroxyacetone phosphate (DHAP), a component included in gluconeogenesis.

Metformin inhibits GPD2, leading to suppression of DHAP and subsequently gluconeogenesis in substrate (glycerol) specific manner [12]. In context of obesity and T2DM, inhibition of gluconeogenesis from increased supply of glycerol due to dysregulation of white adipose tissue may partially benefit uncontrolled type2 diabetic patients with dysregulated white adipose tissue lipolysis [38].

As discussed above, metformin suppresses gluconeogenesis through interactions with regulatory process of gluconeogenesis as shown in inhibition of transcription (downregulation of gluconeogenic genes expression), substrate (suppression of glycerol induced DHAP formation) and increase cytosolic redox **Figure 2**.

3.5 Mechanisms in skeletal muscle

Metformin affects skeletal muscle metabolism by direct and indirect mechanisms. Emphasis has been placed on the metformin's effect to increase insulin-stimulated peripheral glucose uptake and to reduce glucotoxicity, which indirectly improves muscle glucose uptake [12]. Metformin reduces gluconeogenesis and hepatic glucose output leads to reduce blood glucose levels in type 2 patients, which accompanied by improvement in insulin action. Improvement in insulin levels in circulation under metformin treatment attenuates the hyper-insulinemic pressure on insulin receptors (insensitive phosphorylated receptors) leading to upregulation and increase sensitivity of receptors to insulin [39]. Consequently, muscle glucose uptake is indirectly stimulated by metformin due to reduce insulin resistance in skeletal muscle and peripheral tissues.

In skeletal muscle, activation of AMPK by metformin increases proliferator-activated receptor γ coactivator-1α (PGC-1α), which in turn stimulates glucose transporter 4 (GLUT4) gene transcription [40]. This mechanism induces GLUT4 and others mitochondrial genes required for catabolism. In addition, activated AMPK stimulates the translocation of GLUT4 to the plasma membrane and acutely increase skeletal glucose uptake.

Moreover, AMPK induced phosphorylation of acetyl-CoA-carboxylase-2 (ACC-2) results in reduce malonyl-COA, which is the inhibitor of carnitine *O*-palmitoyltransferase, leading to increase transport of fatty acids into mitochondria. Thus, fatty acid-oxidation is acutely increased. It worth to mention that these mechanisms are regulated by PGC-1 α, which initiates many genes involved in AMPK functions in skeletal muscle [40], **Figure 3**.

3.6 Mechanism in fat tissue

Obesity has been found to be the most crucial factor for insulin resistance (IR). In addition, insulin sensitivity decreases with age. Therefore, glucose entry into
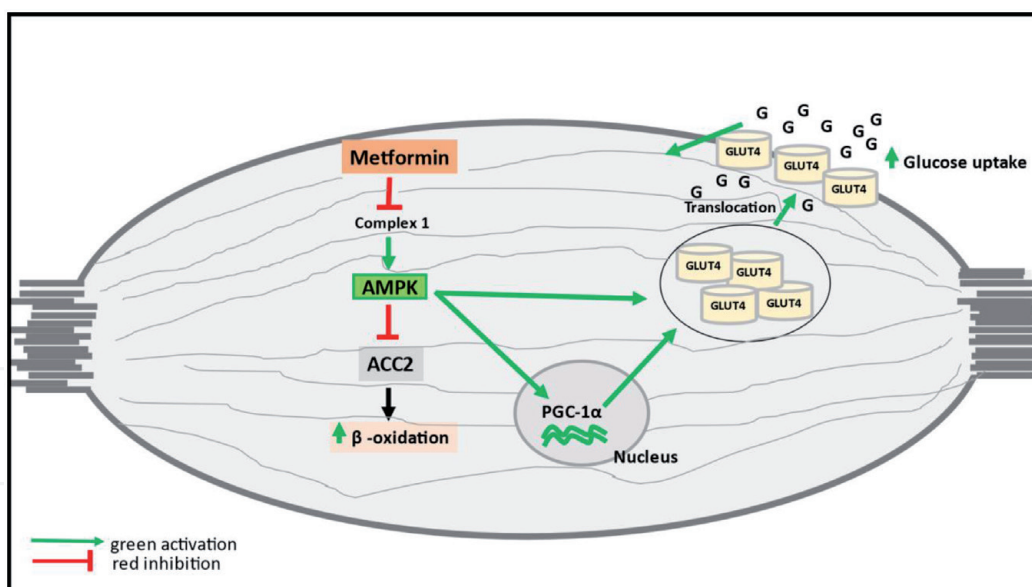
Figure 3.
Mechanism of increase muscle responsiveness by metformin (see text).

tissues, including muscle and fat, decreases, since adipocytes have fewer insulin receptors. Metformin improves lipid profile in patients with T2DM. Insulin resistance (IR) means reduces tissue responsiveness to insulin with resultant elevation in insulin levels (hyperinsulinemia). Beta-cells produce more insulin, but ultimately fail to overcome IR with resultant loss of beta-cell function and development of hyperglycemia. Risk factors for IR are obesity and inactivity.

The signaling pathway connected with IR is phosphatidylinositol-3-kinase/protein kinase B protein (PI3K/PK), which also known as Akt. Akt is also important for translocation of GLUT4 onto the cell membrane surface of muscle and fat cells for glucose entry [41]. Akt inactivation or defect can lead to impairment of membrane transposition of GLUT4, which results in IR accompanied with hyperinsulinemia, hyperglycemia and cardiac impairment [42]. Metformin activates and restores the PI3K/Akt/GLUT4 signaling in rats with type 2 diabetes [43], thereby suppresses IR.

Metformin enhances disposal of blood glucose into skeletal muscle and fat, thus insulin resistance associated with diabetes is overcome. This can be translated into increase storage of glycogen in skeletal muscle, enhancement of fatty acid oxidation and reduced lipogenesis in adipose tissue, which in general reduce the body fat content.

3.7 Mechanisms in the intestine

Beside the liver, the intestine is also considered as an important target for metformin actions. Metformin lowers blood glucose not only through the action in the circulation, targeting the liver and other organs, but also through one in the intestine. The proposed actions are increase in intestinal glucose entry mainly in enterocytes with anerobic utilization, resulting in reduced net glucose uptake into blood with enhanced lactate production [44, 45], increase in glucagon like peptide-1 (GLP1) levels, increase bile acid pool within the intestine and modulation of microbiome [46].

Activated AMPK phosphorylates the glucose transporter 2 (GLUT2), which then translocated to the apical membrane of intestinal cells, mainly enterocytes, where it promotes glucose uptake into enterocytes [6]. Metformin increases uptake and utilization (anerobic) of glucose, where subsequently an increase in plasma lactate is resulted. In fact, the intestine and the liver are implicated in metformin-related

lactate production. The effect of metformin in intestinal glucose utilization has been shown in positron emission tomography–computed tomography (PET-CT) imaging of patients treated with metformin. This imaging technique uses positron-emitting **18**F-fluorodeoxyglucose (^{18}F-FDG), that its intestinal (mainly in the colon) uptake increases in metformin treated patients confirming increase glucose uptake and metabolism in the gut [46].

Metformin inhibits mitochondrial glycerophosphate dehydrogenase, so the conversion of cytosolic pyruvates to lactate is reduced [6], thus, intracellular lactate levels are built up and then released into the plasma. This has been proved in rat studies, where the hepatic portal vein has been shown as the area with the higher peak of plasma lactate concentrations, implicating the intestine as the main site of metformin-associated anerobic glucose utilization and lactate production (estimated by 10% increase in intestinal lactate concentration) [47].

Another intestinal action of metformin directs to GLP-1, which is secreted from L cells distributed throughout the gut but concentrated in the ileum. As reported in mice studies, metformin increases the expression of the precursor proteins (pre-proglucagon and proglucagon) of GLP-1, thus potentially increasing GLP-1 production and secretion [48]. In addition, metformin affects the enzyme degrading GLP-1, dipeptidyl peptidase-4 (DPP4) by mechanisms that are not well clarified [49]. Moreover, stimulation of GLP-1 secretion can occur indirectly, via the bile acid pool alteration by metformin [46]. Metformin activated AMPK directly phosphorylates and represses bile acid sensor, the farnesoid X receptor (FXR), on ileal cells, which results in reduced FXR transcription activity and subsequently reduced sensing and ileal absorption of bile acids [50]. By its turn, the higher level of bile acid pool stimulates bile acid receptors TGR5 on L cells, inducing secretion of GLP-1 [51]. Furthermore, the consequences of reduced bile acid absorption are lower levels of cholesterol in patients taken chronic metformin [52] and diarrhea associated with metformin intolerance due to osmotic effect mediated by increased luminal bile slats levels [53].

The gut microbiome composition has been shown to contribute to the development of obesity and type 2 diabetes, which implicated in a reduction in bacteria producing short chain fatty acids (SCFAs) such as butyrate-producing bacteria and an increase in opportunistic pathogens as shown in type 2 diabetics [54]. SCFAs are considered as important signaling metabolites that impact hepatic gluconeogenesis and fatty acid metabolism [55]. Metformin modulates gut microbiota and increases SCFAs metabolizing bacteria, which lead to suppression of hepatic gluconeogenesis, reduction in FFA release from adipocytes and appetite suppression via incretin [56].

Metformin alters the microbiome composition in mice and humans, where the bacterium Akkermansia muciniphila is increased, accompanied with associated increase in mucin-producing goblet cells as demonstrated in mice model. Akkermansia muciniphila can increase endocannabinoids, which improve the thickness of gut mucous barrier and reduce inflammation [57], and so improve glucose tolerance. On the other hand, an increase in such bacteria by metformin triggers production of short chain fatty acids butyrate and propionate, which results in reduction of hepatic gluconeogenesis, appetite and weight [46]. Taken together, alteration of microbiome composition by metformin can improve metabolic disorders which needed further investigations.

4. Mechanisms of antiinflammatory/antioxidant

Beyond the glucose lowering actions, metformin can directly and indirectly modulate inflammation. Several experimental and clinical studies demonstrated

the anti-inflammatory actions of metformin in endothelial cells (EC) and smooth muscle cells (SMC), monocytes, macrophages and other cell types, where it suppresses the main components of inflammation and restores cell functions [58, 59]. Since inflammation is linked to a number of clinical disorders, thus, metformin can possibly interfere with and ameliorate metabolic disorders, cardiovascular diseases, atherosclerosis, obesity cancer and aging. Although the crucial mechanisms are not well elucidated, accepted anti-inflammatory mechanisms of metformin, which are common and implicated in the before mentioned disorders are presented below.

Activation of AMPK by metformin inhibits nuclear factor kappa light-chain-enhancer of activated B-cells (NF-κB) transcription [60]. NF-κB is a transcription regulator implicated in various inflammatory pathways. Metformin induced NF-κB inhibition suppresses inflammatory pathways, proinflammatory cytokines and reactive oxygen species (ROS) production [61]. Likewise, activation of AMPK-phosphatase and the tensin homolog (PTEN) pathway by metformin suppresses phosphoinositide 3-kinase (PI3K)-Akt pathway that activates NF-κB in human vascular SMC. In this way, NF-κB is also inhibited, **Figure 4**. In addition, metformin suppresses Poly [ADP ribose] polymerase 1 (PARP-1), which functions as a coactivator of NF-κB transcription to stimulate pro-inflammatory pathways. Nitric oxide (NO), a mediator of in nerve, immune and CVS is decreased in oxidative stress induced by hyperglycemia. Metformin increases NO via activation of AMPK, which antagonizes inflammation and ROS production [62]. As well, metformin inhibits the differentiation of monocytes to inflammatory macrophages [63] through activation of AMPK, which reduces the phosphorylation of signal transducer and activator of transcription 3(STAT3), **Figure 4**.

Inhibition of NF-κB transcription triggers consequences in different tissues. In macrophages, inhibition of NF-κB activation by metformin can result in reduction of NO, PGE2, and proinflammatory cytokines, such as IL-1β (interleukin-1 β), TNF-α (tumor necrosis factor - α) [64], IL-6 and IL8 (responsible for calling monocytes and adhesion of endothelial cells) [65]. In human adipocyte, metformin induced inhibition of NF-κB pathway leads to suppression of proinflammatory cytokine-induced 11β-HSD1 (11 β -hydroxysteroid dehydrogenase type 1) expression [66]. 11β-HSD1 is elevated in human adipose tissue in obesity and metabolic syndrome, generates active glucocorticoids and is associated with chronic inflammation. Moreover,
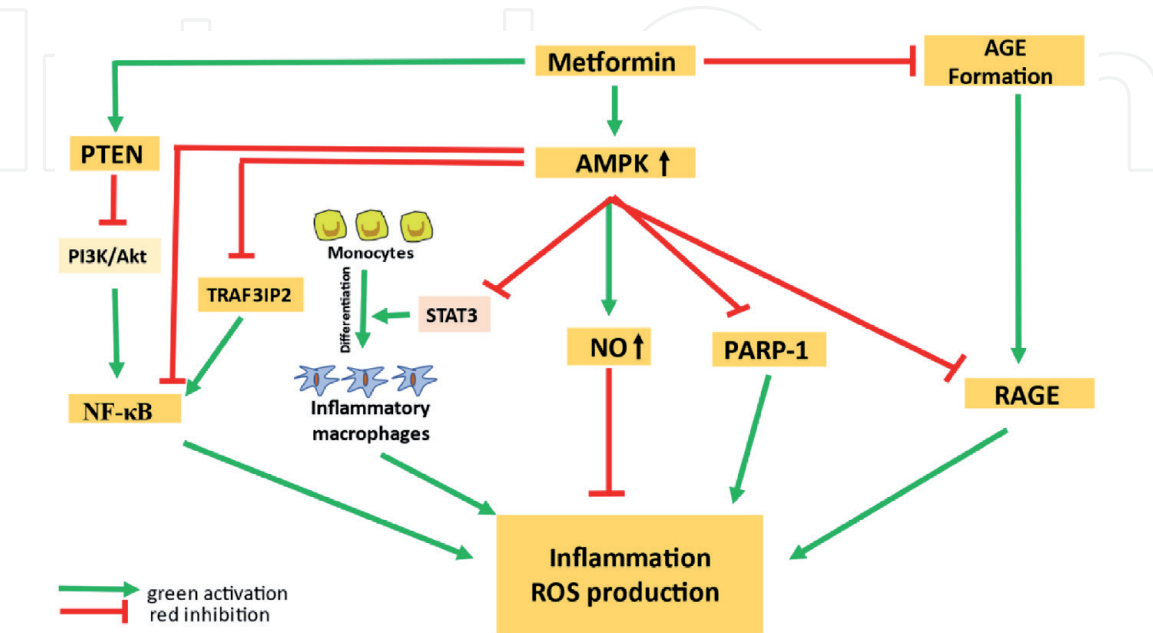

Figure 4.
Potential mechanisms of metformin to attenuate inflammation and production of reactive oxygen species.

inhibition of NF-κB suppresses the expression of CXCL8, a cytokine responsible for changing the microenvironment around the tumor by attracting leukocytes and endothelial progenitors contributed in angiogenesis [67]. Metformin applies its anti-inflammatory action for antifibrotic effect on heart muscle cells through activation of AMPK and inhibition of the pro-inflammatory mediators of the TRAF3 interacting protein (TRAF3IP2) molecule, which induced by aldosterone and enhances production of NF-κB [68]. Furthermore, anti-inflammatory mechanisms associated with atherosclerosis, allergic asthma, hepatic steatosis and vascular injury have been ascribed to metformin but required further elucidations.

In regard of macrophages activity, activated AMPK by metformin reduces phosphorylation of STAT3 (signal transducer and activator of transcription 3), thereby, inhibits the differentiation of monocytes into inflammatory macrophages (M1) [69], while promotes polarization into anti-inflammatory macrophages (M2). These mechanisms place metformin as potential anti-inflammatory targeting macrophages differentiations and polarization with benefits in vascular injury, atherosclerosis, certain cancer and insulin resistance [63].

Further mechanism associated with anti-inflammatory actions of metformin is the inhibition of advance glycation end-products (AGEs) [70], which are one of the crucial inflammatory factor in diabetes, promoting inflammation, ROS production and atherosclerosis [71, 72]. In fact, during hyperglycemia accumulation of glucose in cells facilitates the binding of each two closest glucose molecules with each other to form dicarbonyl compounds, which are percussors of AGEs. AGEs bind to their receptors (RAGE) in different target cells including macrophages, where they promote expression of IL1, IL6, TNF α and RAGE, and activate NF-κB pathway [68], leading to inflammation, apoptosis and fibrotic reactions, as observed in tubular cells. Metformin not only binds chemically to these precursors and renders them inactive, thereby reduces the formation of AGEs, but also suppresses RAGE via activation of AMPK [73]. Altogether, metformin suppresses RAGE/NF-κB pathway, leading to regression of RAGE effects on macrophages and change of their surface markers from inflammatory (M1) to anti-inflammatory (M2) phenotype. **Figure 4** illustrates the potential anti-inflammatory mechanisms of metformin.

Beside the direct effect on proinflammatory pathways, metformin can indirectly reduce inflammation through metabolic consequences. Reduction in hyperglycemia and subsequently the weight as well as the atherogenic LDL cholesterol levels can have favorable effect on chronic inflammation, atherosclerosis and cardiovascular disorders.

As mentioned before, metformin inhibits mitochondrial complex 1 electron transfer complex chain and reduces the production of ROS, which normally formed by synthesis of ATP from ATP synthase. Metformin can reduce ROS through activation of AMPK which inhibits TFG-β, a potent inflammatory factor stimulating the production of ROS and induce endogenous antioxidants such as glutathione reductase (GSH), superoxide dismutase (SOD) and catalase (CAT) [74]. Independent of AMPK activation, metformin can activate antioxidant SOD and clean the damaging effects of ROS in tissues. In addition, it can direct trap hydroxyl peroxide and activate antioxidant enzymes such as catalase, which decomposes H_2O_2. Reduction of ROS reduces IL1β [68]. Therefore, metformin has been shown to play a role in controlling and changing oxidative/inflammatory pathways in clinical and laboratory conditions through various mechanisms.

5. Antineoplastic actions of metformin

The role of metformin in treatment of cancer has been reported in various recent sophisticated publications. Clinical observational studies in liver, colon and

pancreatic cancer have demonstrated that metformin prevents and decreases the risk of cancer development [75]. In addition, improvement overall survival outcomes have been reported in patients with colorectal and breast cancer [76], where metformin treated breast cancer patients showed a lower HER-2 positive rate and mortality rate than the control group [77] Besides that, metformin enhances the effects of anti-cancer drugs as shown in vitro and in vivo studies using vincristine, cisplatin, and doxorubicin [78, 79]. Altogether, the results point to involvement of metformin in chemotherapy as adjuvant or a potential anti-cancer candidate which require further experimentations.

Cancer growth and proliferation can be regressed direct and indirect by metformin. Metformin induced reduction in cancer growth has been shown to be indirect through systemic effects related to reduced blood glucose levels, improved insulin resistance and declined pro-inflammatory cytokines. This indirect action might explain the effect of metformin in several types of cancer linked to hyperinsulinemia as a risk factor. Also, metformin directly modulates several oncogenic signaling pathways described in the following text.

As mentioned in different sections of this chapter, the primary mechanism of metformin is to inhibit the oxidative phosphorylation by blockade of complex1 in mitochondria in target cells. Mitochondrial energy reduction and metabolic stress increase the endogenous levels of reactive oxygen species (ROS) which can mediate the death of cancer cells depending on oxidative phosphorylation for gaining energy [24]. Likewise, energy stress seems to hinder cancer cells from synthesis of energy requiring proteins, lipids and structural elements necessary for cancer growth and proliferation. This action can be considered as the first step of metformin induced tumor regression, and growth retardation. Furthermore, deprivation of cancer cells from ATP activates the tumor suppressor gene LKB1 which then phosphorylates AMPK [80] **Figure 5**. AMPK regulates several signaling pathways, primarily via inhibition of mammalian target of rapamycin (mTOR) signaling to suppress tumorigenesis as follows.

Metformin is taken up into cancer cells expressing OCT1, accumulates in mitochondria, blocks complex 1 and activates AMPK. AMPK phosphorylates p53 on ser15 (the tumor suppressor) which is required to start AMPK-dependent cell growth arrest and apoptosis [81], **Figure 5**. On the other hand, activated AMPK
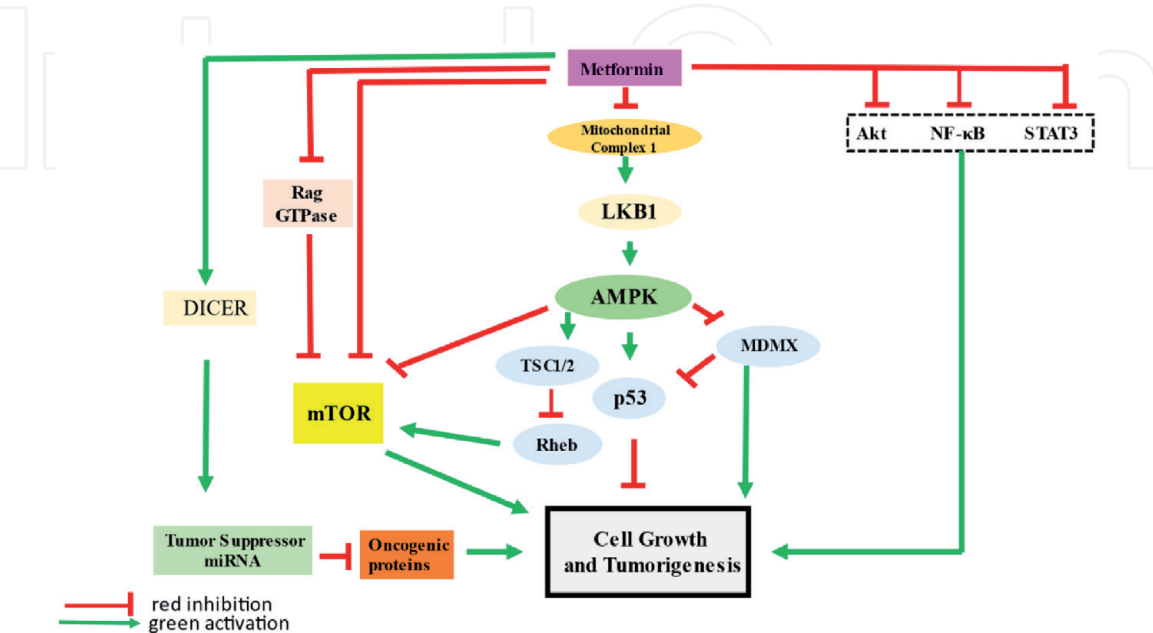

Figure 5.
Mechanisms of metformin suppressing tumorigenesis.

phosphorylates MDMX on ser 367 leading to MDMX inactivation and p53 activation [82]. MDMX and the human MDM2 are partner proteins monitor p53 in a negative feedback fashion and restrain its function to maintain the normal development and function of different tissues [83]. Phosphorylation one of them results in inhibition of ubiquitylation (a molecular change) of p53 leading to stabilization and activation of p53.

Beyond the effect on p53, metformin inhibits mTOR. mTOR is a catalytic subunit, composes of two protein complexes, mTORC1 and mTORC2, that regulate cell growth [84]. Inhibition of mTOR attenuates cell proliferation [85]. Metformin inhibits the activation of mTOR via AMPK-dependent and -independent mechanisms. By AMPK-independent way, metformin phosphorylates directly the regulatory associated protein (raptor) that inactivates mTOR. Likewise, metformin inhibits mTOR signaling by inactivating Rag GTPase [4]. On the other hand, AMPK directly phosphorylates the tumor suppressor tuberous sclerosis complex 2 (TSC2) leading to activation of complex 1 and 2, TSC1/2. TSC1/2 inhibits Rheb, which in turn inactivates mTOR [86] and suppresses cell proliferation.

Besides AMPK and mTOR, metformin has been shown to affect other oncogenic signaling pathways. Metformin suppresses Akt (protein kinase B) expression which is associated with increased phosphatase and tensin (PTEN, a tumor suppressor gene) [87]. This is considered as main mechanism via which endometrial carcinoma is inhibited by metformin. Additionally, metformin inhibits activation of nuclear factor kappa light-chain-enhancer of activated B-cells (NF-κB) and phosphorylation of STAT3 in cancer stem cells [88]. The NF-κB and STAT3 transcription factors are involved in mediating an epigenic switch from non-transformed to cancer cells as shown in breast cancer model. This action suggests that metformin inhibits the anti-inflammatory pathway required for transformation and cancer stem cells formation [88].

Further mechanism of anticancer effect of metformin is modulation of microRNAs (miRNAs) expression (mainly tumor suppressor miRNA) through activation and upregulation of the RNAase III endonuclease (DICER). DICER is one of the key enzymes of microRNAs biosynthesis [89]. DICER has a role in formation of miRNAs and in assembly of their machinery to target mRNAs for degradation [90]. Downregulation of DICER is oncogenic and predict poor survival in lung, breast and ovarian cancer [91, 92]. In addition, impairment of metformin effect in vitro was shown in DICER-deficient tumor cells. As shown in **Figure 5**, metformin induced upregulation of DICER leads to expression of many suppressor miRNAs that target mRNA of coding genes for degradation, thus effectively reducing gene products such as oncogenic proteins [93].

6. Mechanisms of action in PCOS

One of the pleiotropic effects of metformin is to reduce insulin resistance (IR) and secondary hyperinsulinemia in diabetes mellitus and several clinical conditions associated with hyperinsulinemia. Hyperinsulinemia is linked with the pathogenesis of polycystic ovary syndrome (PCOS), a condition of primary ovulatory disfunction associated with metabolic disturbances. PCOS is the endocrine disorder characterized by hyperandrogenism, anovulation and infertility. Obesity further exaggerates IR in obese PCOS women. Importantly, IR in PCOs women is tissue selective, which means persistence sensitivity to insulin actions on steroidogenesis in ovary and adrenal gland, in face of resistance in skeletal muscle, adipose tissue and liver to metabolic actions of insulin. Paradoxically, in PCOS women, some tissues manifest IR, while steroid-producing tissues remain insulin sensitive [94].

Mechanisms of insulin action contributing to hyperandrogenism in PCOS are various. Insulin can enhance the amplitude of luteinizing hormone (LT) pulses to increase androgen production in theca cells [95] (similarly insulin increases thecal androgen response to LH through direct binding to insulin like growth factor – 1(IGF-1) receptors in theca cells). Also, insulin may stimulate the activity of ovarian cytochrome CYP17 (P450c17) and 17β-hydroxysteroid dehydrogenase (17βHSD) to promote androgen steroidogenesis [96]. In addition, insulin can decease the hepatic synthesis of steroid hormone binding globin (SHBG), which allows more free androgen and estrogen to be available. Finally, insulin inhibits the hepatic production of IGF binding protein-1 (IGFBP-1), which increases IGF-1 in circulation and allows greater local action [97].

Furthermore, increase androgen levels may be linked to decrease adiponectin secretion by adipocyte in PCOS women, thereby further increasing insulin resistance and subsequently insulin levels [98]. In addition, insulin may affect female subcutaneous adipose tissue and generate androgen from adipocytes by increasing the activity of aldo-keto reductase IC3 (AKRIC3) [99].

Metformin can ameliorate all the above-mentioned actions of insulin in PCOS. Treatment with metformin is useful in reduction of both hyperinsulinemia and circulating androgens and also restores ovarian function with the benefits of increase ovulation, reduce serum androgen levels and improve menstrual cyclicity.

Metformin acts directly on ovarian theca cells and suppresses androgen production by inhibition the enzymatic activity of P450c17 and 17βHSD [100] or indirectly via reduction of hyperinsulinemia and IR by multiple mechanisms. It has been shown that the metabolic actions of metformin on cells include increase in tissue responsiveness to insulin action, insulin receptor numbers in skeletal muscle and adipose tissue, tyrosine kinase activity and glucose uptake. Also, metformin decreases intestinal glucose absorption, plasma glucagon levels, gluconeogenesis and glycogenolysis in the liver. Most of these actions have been mediated through activation of AMPK cascade, which result in indirect reduction of hyperinsulinemia and IR the main pathogenic component in PCOS [101].

By means of anti-inflammatory/antioxidant mechanisms contributing to PCOS, metformin inhibits NF-kB, whose activation triggers IR and inflammation in PCOS [102]. Moreover, metformin increases the activity of the antioxidant enzymes such as catalase and CuZn superoxide dismutase, thereby, it scavenges the reactive oxygen species such as hydrogen peroxide (H_2O_2), superoxide (O2.) and hydroxyl (OH.) radicals, where metformin also directly reacts with the latter one [103]. More other related mechanisms of metformin in PCOS are still unclear and elusive.

7. Conclusion

Based on its multiple mechanisms of action and interference with signaling pathways, metformin represents as a promising potential drug for treating various medical conditions. Furthermore, the beneficial effects arising from these mechanisms can be demonstrated and clarified by substantial basic experiments and clinical trials.

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