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New Insight into Metformin Mechanism of Action and Clinical Application

Yun Yan, Karen L. Kover and Wayne V. Moore

Abstract

Metformin is the first-line medication for Type 2 diabetes (T2D) treatment, and it is the only US FDA approved oral antidiabetic medication for pediatric patients with T2D 10 years and older. Metformin is also used to treat polycystic ovary syndrome (PCOS), another condition with underlying insulin resistance. The clinical applications of metformin are continuing to expand into other fields including cancer, aging, cardiovascular diseases, and neurodegenerative diseases. Metformin modulates multiple biological pathways. Its novel properties and effects continue to evolve; however, its molecular mechanism of action remains incompletely understood. In this chapter, we focus on the recent translational research and clinical data on the molecular action of metformin and the evidence linking the effects of metformin on insulin resistance, prediabetes, diabetes, aging, cancer, PCOS, cardiovascular diseases, and neurodegenerative diseases.

Keywords: metformin, insulin, insulin resistance, diabetes, aging, PCOS, cancer, cardiovascular, neurodegenerative

1. Introduction

Synthesis of metformin was reported in 1922 and its effect of lowering glucose was reported soon after. Metformin was first reported to be used for the treatment of diabetes by French physician Jean Steme in 1957. The effect of metformin on improvement of morbidity and mortality in type 2 diabetes (T2D) was confirmed in the United Kingdom Prospective Diabetes Study (UKPDS), a large clinical trial performed in 1980–1990s [1]. It was approved for T2D treatment in adults by US FDA in 1994 and for pediatric patients 10 years and older in 2000. Metformin is prescribed world-wide as the first-line oral drug for adults and children with T2D. Its physiological effects related to T2D include increase in insulin sensitivity, reduction of gluconeogenesis in the liver, enhanced glucose uptake by muscle, and reduced intestinal glucose absorption. Several molecular mechanisms of action have been proposed but more remain to be discovered. In this chapter, we will review molecular mechanisms of action of metformin and its prospect for clinical application.

2. Mechanisms of action

The potential mechanisms of metformin action involve several pathways. The AMPK-pathway plays an important role in metformin actions [2, 3]. Metformin inhibits the mitochondrial respiratory chain (complex I), which increases the AMP to ATP ratio, leading to the phosphorylation of AMP-activated protein kinase (AMPK) at Thr-172. We have demonstrated that metformin treatment increases protein level of phosphorylated AMPK in high-glucose-treated endothelial cells [4]. The phosphorylated AMPK subsequently phosphorylates multiple downstream effectors to regulate cellular metabolism and energy homeostasis [5]. These downstream effectors include thioredoxin interacting protein (TXNIP) and TBC1D1, a RAB-GTPase activating protein and a member of the tre-2/BUB2/cdc1 domain family. Phosphorylated TXNIP and TBC1D1 increase the plasma membrane localization of glucose transporter 1 (GLUT1) and GLUT4, respectively [6, 7], and regulate glycogen synthases (GYS1 and GYS2) to prevent the storage of glycogen [8]. Some actions of metformin have been found to be AMPK-independent [9].

In diabetic mice, metformin has an effect on gut microbiota by inducing a profound shift in the gut microbial community profile, resulting in an increase in the *Akkermansia* spp. population [10] and cAMP-induced agmatine production [11], which may decrease absorption of glucose from the gastrointestinal tract and increase lipid metabolism respectively. In addition, metformin decreases insulin-induced suppression of fatty acid oxidation and lowers lipid content of hepatic cells [12].

3. Insulin resistance

Insulin resistance (IR) is a condition in which the cellular response to insulin is decreased resulting in elevated insulin levels (hyperinsulinism). When the beta cells are not able to overcome the resistance by producing more insulin, hyperglycemia develops. Insulin resistance is more prevalent in certain racial populations suggesting a genetic basis for the resistance. The major “environmental” risk factors for insulin resistance are obesity and sedentary lifestyle. Exercise and weight loss are established approaches to improve insulin sensitivity and decrease insulin resistance [13]. Insulin resistance may also be the basis for polycystic ovary syndrome (PCOS) in women. Some studies have suggested that metabolic syndrome (insulin resistance, type 2 diabetes, obesity, hyperlipidemia, and hypertension) and PCOS (insulin resistance, hyperandrogenism, amenorrhea, non-obese) are the ends of a spectrum of insulin resistance. The loss of microvascular insulin response and reduction of muscle glucose uptake are early events in the pathogenesis of insulin resistance [14, 15].

Metformin can increase insulin receptor tyrosine kinase activity, enhance glycogen synthesis, and increase the recruitment and activity of GLUT4 glucose transporters. In high-fat-diet-fed insulin resistant rats, metformin improved the insulin sensitivity of vascular and skeletal muscle and restored glucose uptake in insulin resistant skeletal muscle [16]. In adipose tissue, metformin promoted the re-esterification of free fatty acids and inhibited lipolysis, which indirectly improved insulin sensitivity through reduced lipotoxicity [17].

Insulin resistance is a risk factor for the development of T2D [18] and occurs earlier than hyperglycemia. Blood-based biomarker that identify insulin resistance earlier than current glycemia-based approaches, including fasting glucose and HbA1C [19] might identify individual's at risk for developing diabetes, and provide a novel tool to monitor metformin treatment in the high risk population. Several blood-based biomarkers of insulin resistance have been identified [19]. Branched-chain amino acids [20] and asymmetric dimethylarginine (ADMA) [21] show an

association with insulin resistance. Metformin decreases the level of circulating branched-chain amino acids and reduces insulin resistance in a high-fat diet mouse model [22]. Metformin treatment lowers plasma ADMA which is associated with improved glycemic control in patients with T2D [23].

Recent studies indicate that phosphatidylinositol-3-kinase/protein kinase B protein (PI3K/PKB, also known as Akt) signaling pathway is associated with insulin resistance, and plays a critical role in insulin stimulation of glucose transport into cells [24–30]. The key molecules involved in this pathway are PI3K, Akt, 3-phosphoinositide-dependent protein kinase 1 (PDK1), and phosphoinositide 3.4.5 trisphosphate (PIP3).

Akt has three isoforms Akt1, Akt2 and Akt3 (also referred to as protein kinase B (PKB) α , $-\beta$ and $-\gamma$, respectively). Their domain structures are similar, including a pleckstrin homology (PH) kinase domain at the amino-terminal and a hydrophobic motif (HM) domain at the carboxyl-terminal [31]. Three isoforms share many substrates, but each isoform also has specific substrate. Akt2 is specific for the insulin signaling pathway and plays a critical role in glucose homeostasis. Akt2 deficient mice have insulin resistance, hyperglycemia, and loss of pancreatic β cells while Akt1 deficient mice do not exhibit diabetes phenotypes [32, 33].

PIP3 binds to PDK1 and Akt protein and recruits Akt protein to the plasma membrane. PDK1 phosphorylates Akt at Thr308/309 of Akt1/Akt2, respectively of the kinase domain leading to partial Akt activation. PI3K might directly phosphorylate Akt1 at Thr308 [34]. Full Akt activation is associated with a second PI3K phosphorylation of Akt at Ser473/474 of Akt1/Akt2, respectively in the carboxyl-terminal hydrophobic motif [34]. Subsequently, the phosphorylated Akt2 recruits insulin-regulated GLUT1 and GLUT4 glucose transporters from the cytoplasm onto the cell membrane surface and thereby increases glucose uptake [35].

GLUT1 is an insulin independent transporter whereas GLUT4 is an insulin dependent transporter. Insulin increases GLUT4 in the cell membrane and promotes the glucose transport into muscle and fatty cells (**Figure 1**). Any defect in Akt pathway along with the downstream molecules could result in insulin resistance [29]. Clinical data indicate that acute myocardial insulin resistance that occurs after cardiac surgery with cardiopulmonary bypass is attributed to Akt inactivation.

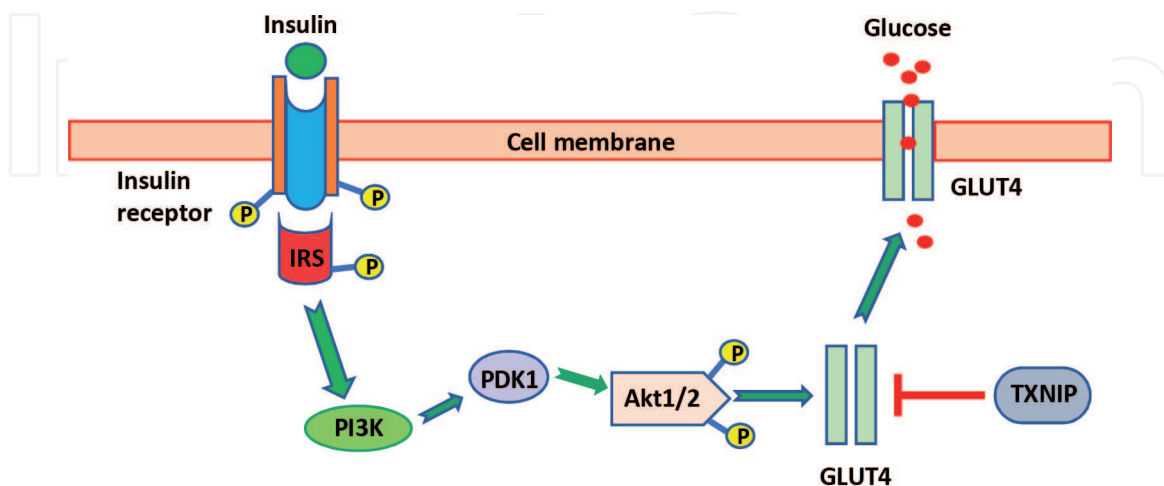


Figure 1. Insulin binds to insulin receptor and induces its dimerization and auto phosphorylation of tyrosine residues in two transmembrane β subunits, which further lead to the phosphorylation of tyrosine residues on the IRS protein. These molecules can further activate PI3K, resulting in activation of PDK1/2. AKT is recruited and gets phosphorylated by PDK1/2. Once activated, AKT promotes GLUT4 translocation to plasma membrane and facilitates glucose into cell. TXNIP inhibits glucose transporter by promoting GLUT4 endocytosis.

Inactivated Akt impairs the membrane transposition of GLUT4, which results in insulin resistance accompanied with hyperinsulinemia, hyperglycemia and cardiac dysfunction [36]. It has been reported that metformin attenuates insulin resistance by restoring PI3K/Akt/GLUT4 signaling in the hepatocytes of T2D rats [37]. Metformin combined with phloretin, a dihydrochalcone found in fruits, promoted glucose consumption and suppressed gluconeogenesis in skeletal muscle via PI3K/Akt/GLUT4 signaling pathway in T2D rat models [38].

TXNIP is being considered as a novel mediator of insulin resistance [39, 40]. TXNIP induced by high-glucose concentration is a key intracellular regulator of glucose and lipid metabolism [6]. We have demonstrated that metformin improves endothelial cell function via down-regulation of high-glucose-induced TXNIP transcription [4].

Over expression of TXNIP induces apoptosis of pancreatic β cells and endothelial cells, decreases muscle and adipose insulin sensitivity, promotes GLUT4 endocytosis and reduces glucose uptake in myocytes and adipocytes [4, 41–43]. Reduction of TXNIP expression by RNA interference gene-silencing significantly improves insulin induced glucose uptake in cultured human skeletal muscle cells [41]. TXNIP knockout mice had improved insulin sensitivity and increased glucose uptake in both adipose and skeletal muscle [39]. In PCOS, metformin improved insulin resistance in a PCOS rat model via an AMPK alpha-SIRT1 pathway [44].

4. Prediabetes

New criteria defining prediabetes includes the presence of one or more of the following, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and HbA1C of 5.7–6.4% [45]. The progression from prediabetes to diabetes is related to insulin resistance and β -cell dysfunction. Prediabetes is a serious health condition which increases the risk of developing T2D, heart disease and stroke. In the US, approximately 84 million American adults (more than 1 out of 3) have prediabetes but 90% patients with prediabetes are not aware of their condition [46]. Metformin improves insulin sensitivity and provides an attractive pharmacological intervention for prediabetes [47, 48]. Results from several clinical trials in the prediabetes population, including children, adolescents and adults, have indicated that metformin can delay or halt the progression from prediabetes to diabetes [49–51]. Metformin is generally well tolerated and has no significant safety issues with long-term use for diabetes prevention [48]. In the long-term “Diabetes Prevention Program Outcomes Study (DPPOS)”, either lifestyle intervention or metformin significantly reduced diabetes development over 15 years. Lifestyle intervention has been shown similar or greater effectiveness than metformin in clinical trials [52] and remains the cornerstone of care for patients with prediabetes. However, lifestyle interventions are difficult for patients to maintain and often fail to control weight over the long term. Metformin therapy was shown to be just as effective as lifestyle intervention in individual with prediabetes <60 years of age, BMI ≥ 35 kg/m² and in women with a history of gestational diabetes mellitus [51, 53]. A study showed that metformin was underused in patients with prediabetes and only 3.7% of adult patients with prediabetes were prescribed metformin [54]. Currently metformin is not approved by FDA for prediabetes. Overweight patients with comorbidities may be at increased risk of diabetes. New guidelines recommended that metformin therapy for T2D prevention should be considered in those with prediabetes, especially those with BMI ≥ 35 kg/m², those aged <60 years, and women with prior

gestational diabetes mellitus [55]. The combinations of metformin with lifestyle or other treatments have shown more beneficial effects in diabetes prevention [48, 49].

5. Diabetes

Metformin is approved for use in patients with T2D. It is still under debated whether metformin can be an adjunct therapy for T1D though many overweight T1D patients have been prescribed metformin due to its beneficial effects on improving insulin resistance.

5.1 Adult T2D

Metformin is considered first-line therapy to treat T2D due to its blood glucose-lowering effects, safety and relatively low cost. Metformin lowers blood glucose level by decreasing glucose production in liver, reducing intestinal glucose absorption, increasing insulin sensitivity and promoting muscle glucose uptake in muscle. Metformin treatment can be combined with lifestyle modification and other anti-diabetic drugs, such as dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists or sodium-glucose cotransporter-2 (SGLT2) [56, 57]. Combined therapy is individualized depending on effectiveness, safety, tolerability, and the characteristics of each patient [58].

Metformin is safe and tolerable with the exception of the risk of lactic acidosis in patients with risk factors for lactic acidosis [59], including impairment of renal, cardiac, and hepatic function [60–62]. Another concern is metformin-induced vitamin B12 deficiency; patients who receive long-term metformin treatment (>6 months) at large doses have developed B12 deficiency [63, 64], so that annual screening of vitamin B12 level is recommended [65].

5.2 Adult T1D

Insulin resistance in T1D patients may contribute to poor glycemic control and is associated with increased insulin dose requirement [66]. Metformin treatment has been shown to increase insulin sensitivity, improve glycemic control, and reduce cardiovascular risk in patients with T1D [67]. The studies reported that metformin used as an adjunct therapy in T1D reduced insulin dose and body weight with no improvement in HbA1c and glycemic control [68, 69]. Another short term adjunct therapy with metformin demonstrated improved glycemic control, insulin sensitivity, and quality of life without weight gain, while long-term (2 years) metformin treatment was associated with decreased BMI [70]. A 1 year retrospective investigation reported an association between metformin as adjunct therapy and decreased glucose levels, decreased prevalence metabolic syndrome traits, and decreased insulin dose [71].

5.3 Pediatric T2D

Metformin was shown to be safe and effective for treatment of pediatric patients with T2D age 10 to 16 years old [72]. Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) recruited 699 youth and adolescents over a 4-year period. In this cohort study, metformin was used alone or in combination with life style modification or other antidiabetics drugs [73]. Metformin treatment was associated with decreased HbA1c and improved glycemic control in more than

half of the participants. Metformin plus rosiglitazone was significantly better than metformin monotherapy [74].

5.4 Pediatric T1D

Using metformin to improve glycemic control and insulin sensitivity in youth and adolescents with T1D has been reported in several clinical trials. Studies that report a positive association of metformin have reported: 1. Decreased insulin dose, BMI and waist circumference in adolescents with T1D [75]. 2. Lower daily insulin dose improved whole-body and peripheral insulin resistance in adolescents with T1D who were overweight/obese [76]. 3. Lower insulin dose and improved vascular smooth muscle function and HbA1c children with T1D [77]. 4. Decreased cardiovascular disease risk factors in youth with T1D [78]. 5. Improvement in HbA1c level in adolescents with T1D [79, 80]. In contrast, some trials did not observe improvement in HbA1c [76, 81], or glycemic control. As expected, there was an increased gastrointestinal adverse event in overweight adolescents with T1D [81].

6. Aging

Metformin has attracted interest for its potential effects on aging [82]. Metformin treatment has a positive association with reduction in the incidence of mortality from age-related diseases including diabetes, cancer, cardiovascular diseases, and neurodegenerative diseases. Metformin is reported to increase lifespan in several animal models. Cohort clinical trials, Metformin in Longevity Study (MILES) and Targeting Aging with Metformin (TAME), have been initiated to investigate metformin's anti-aging effects in human.

In several animal models, including nematodes and rodents, metformin has been shown to delay aging. Metformin treated female outbred mice (100 mg/kg in drinking water) showed an increased mean lifespan 37.8% [83]. The effects of metformin treatment were shown to be age dependent in mice. When treatment was started at the early stage of life, middle-age and late stages of life, the mean lifespan was increased by 21%, 7% and 13% respectively compared to the controls [84]. In a mouse breast cancer model, metformin delayed the onset of mammary adenocarcinoma and increased lifespan by a mean of 8% compared to the control group [85]. Metformin prolonged the survival time of male mice with Huntington's disease by 21.1%, but had no effects in female [86]. A recent study found that metformin reduced oxidative stress and inflammation, extended both lifespan and healthspan by 4–6% in different strains of mice, and attenuated the deleterious effects of aging in male mice [87].

Gut microbiota has been shown to affect health status and longevity and play a role in resistance to infection, inflammation, autoimmunity, and cancer, and the regulation of the brain-gut axis [88, 89]. Metformin acts directly on gut bacteria to decrease absorption of glucose, improve lipid metabolism and elevate agmatine production to extend host lifespan [10, 90].

The reported effects of metformin on microbiota and animals have promoted interest in evaluating its effects on human longevity. In 2014, Metformin in Longevity Study (MILES, NCT02432287) clinical trial was initiated to examine the effects of metformin treatment on the biology of aging in humans, and to determine if treatment with metformin (1700 mg/day) could restore more youthful gene expression in elderly people with impaired glucose tolerance. Results from MILES showed that 6-weeks of metformin treatment in older adults (~70-year-old participants) improved age-associated gene expression, and significantly influenced metabolic and non-metabolic pathways in skeletal muscle and subcutaneous

adipose tissue [91]. Currently, MILES has progressed to a phase 4 trial. Targeting Aging with Metformin (TAME) is managed by America Federation for Aging Research (AFAR) to investigate metformin's ability to delay the onset of comorbidities related to aging. The plan is to recruit 3000 older adults (aged 65–79 years old) without diabetes who will be randomly assigned to 1500 mg metformin daily or placebo for 6 years, with a mean follow-up time of more than 3–5 years (<https://www.afar.org/research/TAME>). These ongoing trials are expected to further evaluate and update the roles of metformin in antiaging.

7. PCOS

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting about 5–15% of reproductive age women [92, 93]. PCOS is associated with insulin resistance and hyperinsulinemia, even in lean women. The condition puts women at risk for infertility, obesity, diabetes, as well as cardiovascular disease [94]. Metformin has been used to treat PCOS for 25 years and is currently recommended in combination with other therapy.

Clinically, metformin was first reported as a treatment for PCOS in 1994 [95]. A 6-month trial of metformin or placebo in women with PCOS found that metformin improved menstruation and insulin sensitivity, and reduced hyperinsulinemia and hyperandrogenemia [96]. In addition, metformin has been found to inhibit androgen production by repressing the steroidogenic enzymatic activities of 17 α -hydroxylase/17,20 lyase (CYP17A1) and 3 β -hydroxysteroid dehydrogenase type 2 (HSD3B2) in the theca cells taken from the ovaries of women with PCOS [97].

Women treated with metformin had increased rates of ovulation and pregnancy [93], reduced rates of early pregnancy loss, preterm delivery, preeclampsia, and fetal growth restriction [98, 99], and improved live birth rates [93]. There were no serious adverse effects in pregnant women with PCOS treated with metformin or their offspring [98–100]. These results indicate that the roles of metformin are not only in glucose metabolism, but also in regulating ovarian hormonal activities and functions in women with PCOS.

There is not enough evidence to recommend metformin as first-line therapy for women with PCOS but adding metformin to other PCOS treatment seems an optimal option. Gastrointestinal side effects were more common in metformin combined with clomiphene citrate than clomiphene citrate alone, but the combined therapy may have beneficial effects in the rates of ovulation and pregnancy [93, 101]. Combination of metformin with clomiphene citrate can be considered as the first line therapy in anovulatory PCOS women without other infertility factors [102]. Metformin was less effective than clomiphene citrate in obese women with PCOS [93, 102]. Combined therapy of metformin and spironolactone showed greater improvement in menstrual cycles and hyperinsulinemia. Adding metformin to ethinyl estradiol-cyproterone acetate treatment in non-obese women with PCOS resulted in significant decreases in androgen levels and increases sex hormone-binding globulin level, which confirmed that metformin also, has some beneficial effects in non-obese women with PCOS [103]. In a DHEA-induced PCOS rat animal model, metformin treatment restored ovarian angiogenesis and follicular development [104].

8. Cardiovascular diseases

Cardiovascular diseases (CVD) are the leading cause of death and disability in the world. Metformin might have sustained beneficial role on reducing CVD risk

and mortality [105, 106]. The cardioprotective effects include reduction of weight gain and hyperinsulinemia, improvement of endothelial function and fibrinolysis, and reduction of low-grade inflammation, oxidative stress, and glycation.

Recent clinical studies have shown that metformin has protective effects on vascular endothelial function and angiogenesis in patients with T2D [107]. Several clinical trials have reported that metformin treatment reduced CVD risk in T2D [1, 108]. Recently the efficacy of metformin in modifying CVD outcomes has been challenged [109–111] but updated evidence support that metformin is cardiovascular protective [112]. A meta-analysis that included 40 clinical trials comprising 1,066,408 patients has shown that metformin reduced cardiovascular mortality, all-cause mortality and cardiovascular events in coronary artery disease [105].

Diabetes increases CVD risk and mortality. More than 75% of male and more than 57% female T2D patients died from cardiovascular disease. The mortality of CVD with T2D patients is twice those without T2D [113]. Patients with chronic cardiovascular disease (CVD) comorbidity are likely to benefit from metformin treatment [1, 105, 108]. Metformin is recommended to be used alone or in combination with other drugs as the first line therapy in T2D patients with high risk of CVD, including atherosclerotic cardiovascular disease [114, 115].

Several clinical trials for metformin on participants with or without T1D diabetes have been completed [106]. Trials Metformin in Insulin Resistant Left Ventricular Dysfunction (TAYSIDE, NCT00473876) and Reducing with Metformin Vascular Adverse Lesions of Type 1 Diabetes (REMOVAL, NCT01483560) have promising data. TAYSIDE found that metformin had a beneficial effect in participants with nondiabetic chronic heart failure and insulin resistance, significantly improved the secondary endpoint of the slope of the ratio of minute ventilation to carbon dioxide production, fasting insulin resistance and weight loss [116]. REMOVAL showed that metformin reduced the prespecified tertiary end point of carotid artery intima-media thickness in T1D suggesting a cardiovascular protective effect [117]. In an 8-week period of metformin treatment for nondiabetic participants with cardiac syndrome X, metformin improved endothelium-dependent microvascular response, maximal ST-segment depression, Duke score, and chest pain incidence, which suggested that metformin may improve vascular function and decrease myocardial ischemia [118]. However, several studies reported that metformin was not found to be effective in their participants [106].

Investigation of Metformin in Pre-diabetes on Atherosclerotic Cardiovascular Outcomes (VA-IMPACT, NCT02915198) and Glucose Lowering in Non-diabetic Hyperglycemia Trial (GLINT, ISRCTN34875079) are current ongoing studies to further evaluate the effects of metformin on CVD [119]. The trials will evaluate the incidence of cardiovascular death and non-fatal myocardial infarction events. Their data will provide more insight on the association of metformin treatment on CVD.

The role of metformin in inhibiting mitochondrial enzymes and activating AMPK pathway are the most likely cellular mechanisms in cardiovascular protection. We have demonstrated that AMPK activated by metformin improved cellular function, decreased apoptosis, and reduced inflammation in vascular endothelial cells [4, 42]. TXNIP is a key regulator of cellular redox state induced by high glucose and promotes high-glucose-induced macrovascular endothelial dysfunction. We have also reported that metformin down-regulated high-glucose-induced TXNIP expression by inactivating ChREBP and Forkhead box O1 (FOXO1) through AMPK pathway (**Figure 2**) [4].

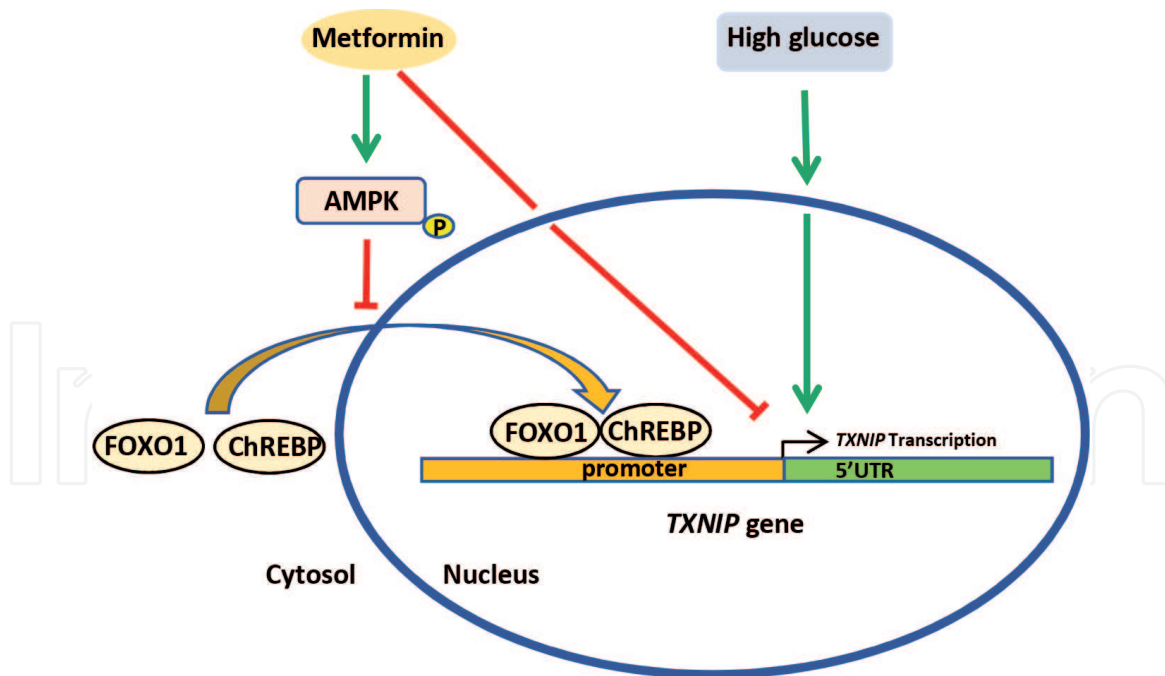


Figure 2.
Metformin inhibits the nuclear entry of ChREBP and FOXO1 from cytosol and their binding capacity to the TXNIP promoter, thus potently and effectively suppresses TXNIP transcription induced by high glucose at last. The inhibitory effect of metformin on nuclear translocation is AMPK-phosphorylation-dependent.

9. Cancer

Preexisting diabetes is a risk factor for cancers, including liver, pancreas, endometrium, colon, breast, and bladder cancers [120]. Epidemiological studies show that the incidence of cancer is decreased in patients with T2D treated with metformin [121]. Metformin has shown to inhibit cancer cell growth in clinical trials including cancer patients without diabetes [122–124]. Based on <http://ClinicalTrials.gov> in January 2020, there are more than 300 clinical trials investigating metformin in cancer treatment, more than 100 of them have been completed. The results were published or posted on <http://ClinicalTrials.gov>. These trials included patients with or without diabetes with different cancers using metformin treatment or combination of metformin with other anticancer drugs. Accumulating evidence from clinical trials and a national cohort study suggest that metformin treatment may improve therapeutic response and have potential beneficial effects on cancer prevention and therapy [125–127].

The effect of metformin on inhibiting cell proliferation can be classified as AMPK independent and AMPK dependent [128]. Metformin inhibits the electron transport chain, resulting in an elevated NADH/NAD⁺ ratio and decrease of ATP production in mitochondrial complex I ATP as well as activation of AMPK [129, 130]. AMPK activated by metformin subsequently regulates cell growth and survival by targeting metabolic enzymes and transporters [131, 132]. AMPK downregulates mTOR activity that plays a central role in the regulation of cell proliferation, growth, differentiation, migration, and survival [133–135].

Tumor protein 53 (p53) plays a central role in the cellular responses to repair of DNA damage, cell survival and apoptosis. p53 mutations occur in almost every type of human cancer cells and more than 50% of human cancers have a somatic p53 mutation [136]. AMPK activation induced phosphorylation at Ser15 of p53, leading to cell-cycle arrest [137].

Metformin was reported to inhibit melanoma cell invasion and metastasis via an AMPK/p53 dependent manner [138]. In a pre-clinical lymphoma model, metformin

treatment resulted in activation of p53, leading to cell apoptosis [139]. In the prostate cancer cells, the combination of metformin and 2-deoxyglucose resulted in p53-dependent cell apoptosis [140]. Metformin has been found to inhibit human cervical cancer cell proliferation and induce apoptosis via modulating p53 and cyclin D1 expression [141].

The effect of metformin on anti-cancer also has a p53-independent mechanism. Metformin has been shown to induce G2M arrest in p53-deficient colorectal cancer cells and tumors. When combined with ionizing radiation metformin therapy enhanced antitumor effects in radioresistant p53-deficient colorectal cancer cells [142]. Treatment with metformin increased apoptosis in p53-deficient human colon cancer cell and reduced tumor growth in xenografts of p53-deficient human colon cancer cells [143].

The p53 homologs, P63 and p73 have overlapping function in tumorigenesis and development [144]. P63 and P73 mutations are rare in human tumors, but they can be overexpressed. P63 plays a critical role in development of squamous epithelium and is overexpressed in squamous cell carcinoma [145]. Metformin inhibited p63 protein expression in squamous carcinoma cell, resulting in decreased cell viability and xenographic tumor growth [146]. P73 overexpression induces apoptosis and cell cycle arrest of tumor cells [147]. AMPK activated by metformin phosphorylated Ser426 of p73 leading to p73 accumulation and cell apoptosis in human colon cancer cells [148].

Metformin may prevent tumorigenesis by inhibiting the insulin like growth factor (IGF)-1 signaling pathway and increasing insulin sensitivity. The proliferation marker Ki-67 was significantly decreased in patients with endometrial cancer cell after metformin treatment [149]. Metformin enhances cytotoxic T lymphocyte (CTL) antitumor activity via activating AMPK to phosphorylate Ser195 of PDL-1 in a murine model of breast cancer which is consistent with the finding that tumor tissues from metformin-treated breast cancer patients exhibited reduced PDL-1 level with AMPK activation [150].

These findings suggest that metformin could be a useful adjuvant agent and has therapeutic benefits in several tumor types, including colorectal, prostate and breast cancers. However, there is limited evidence in other tumor types, and further clinical investigations are needed to evaluate metformin effects in cancer therapy.

10. Neurodegenerative diseases

Metformin is described to have a beneficial effect in neurodegenerative diseases (ND), including dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease and mild cognitive impairment [151, 152].

Population-based studies support an association between the elevated risk of ND in patients with T2D [153–155]. A large population cohort study used Taiwan's National Health Insurance Database to investigate the relationship between dementia, T2D, and metformin treatment. They found that the prevalence of dementia was increased in patients with T2D and that metformin therapy was associated with a 24% decrease in the incidence of dementia in patients with T2D. The combination treatment of metformin with sulfonylureas was associated with a 35% decrease in the risk of dementia in T2D patients over 8 years of observation [156]. In a recent study, long-term (>2 years) metformin therapy was associated with lower incidence of dementia among elderly adults with T2D. Longer term treatment (>4 years) was associated with reduced risk of Alzheimer's and Parkinson's diseases, and none with mild cognitive impairment [157]. A large T2D population cohort study found that sulfonylureas therapy increased the risk of Parkinson's disease, but adding metformin as a co-therapy significantly reduced the risk of Parkinson's disease in T2D

[158]. Long-term (>6 years) metformin treatment significantly reduced the risk of cognitive impairment among older adults with T2D [159].

In contrast, other studies have shown that the metformin therapy of T2D is associated with: 1. a slightly higher risk of Alzheimer's disease [160], 2. increased risk for cognitive impairment [161], and 3. no beneficial effects on preventing development of Alzheimer's disease after adjusting for underlying risk factors and the duration of diabetes since diagnosis [162]. In addition, metformin treatment aggravated neurodegenerative process in ApoE knockout mice [163].

The current evidence suggests that the neuroprotective effects of metformin occur via activation of AMPK/mTOR pathway and inhibition of tau phosphorylation [164, 165]. In addition, it is known that metformin enhances angiogenesis and neurogenesis, induces autophagy, reduces oxidative stress, and improves neurological deficits [166–170].

Despite the different findings from these studies, a recent meta-analysis suggests that metformin may prevent development of dementia in patients with diabetes indicating that metformin should be continued in patients with T2D patients at risk of the dementia or Alzheimer's disease. Use of metformin to prevent neurodegenerative diseases in people without diabetes is not supported by current evidence [152].

11. Conclusions

Metformin is currently approved and widely prescribed for patients with T2D and PCOS. The clinical trial data and clinical experience over several decades have demonstrated its safety and efficacy. The interest in metformin therapy has dramatically increased as the population-based cohort studies indicate that metformin can decrease the risk of cancer, cardiovascular and cerebral disease. Current studies indicate that metformin has potential for treatment of T1D, cancer, aging, cardiovascular and neurodegenerative diseases. Translational and clinical trials need to be continued and expanded to determine if there are indications for metformin therapy in diseases other than T2D.

Conflict of interest

The authors declare no conflict of interest.

Author details

Yun Yan*, Karen L. Kover and Wayne V. Moore
Department of Pediatrics, Division of Endocrinology, Children's Mercy Kansas City,
School of Medicine, University of Missouri Kansas City, Kansas City, USA

*Address all correspondence to: yyan@cmh.edu

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