We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

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.

IntechOpen

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, –β and –γ, 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.

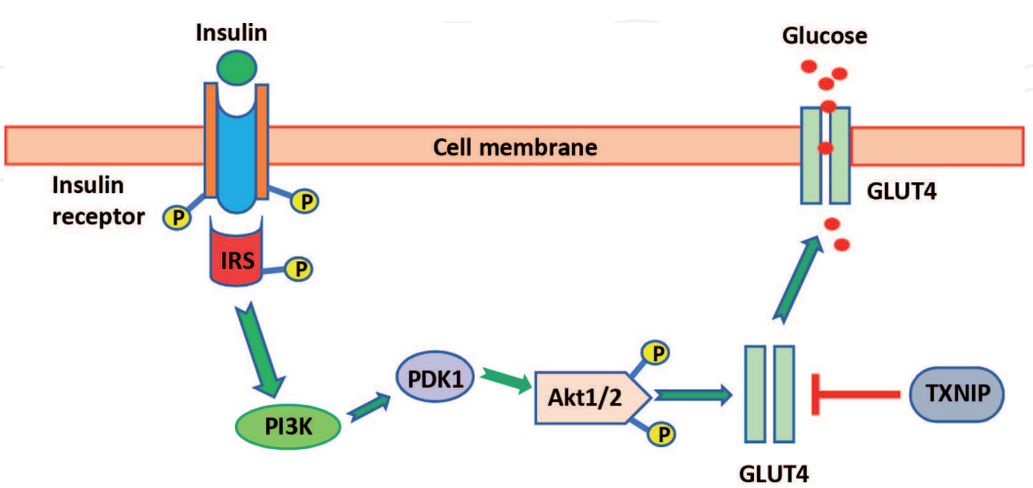

Figure 1.
Insulin binds to insulin receptor and induces its dimerization and auto phosphorylation of tyrosine residues in two transmembrane β subunits, which further lead to the phosphorylation of tyrosine residues on the IRS protein. These molecules can further activate PI3K, resulting in activation 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/m^{2,} 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/m^2, 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 antidiabetic 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 17α-hydroxylase/17,20 lyase (CYP17A1) and 3β-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].

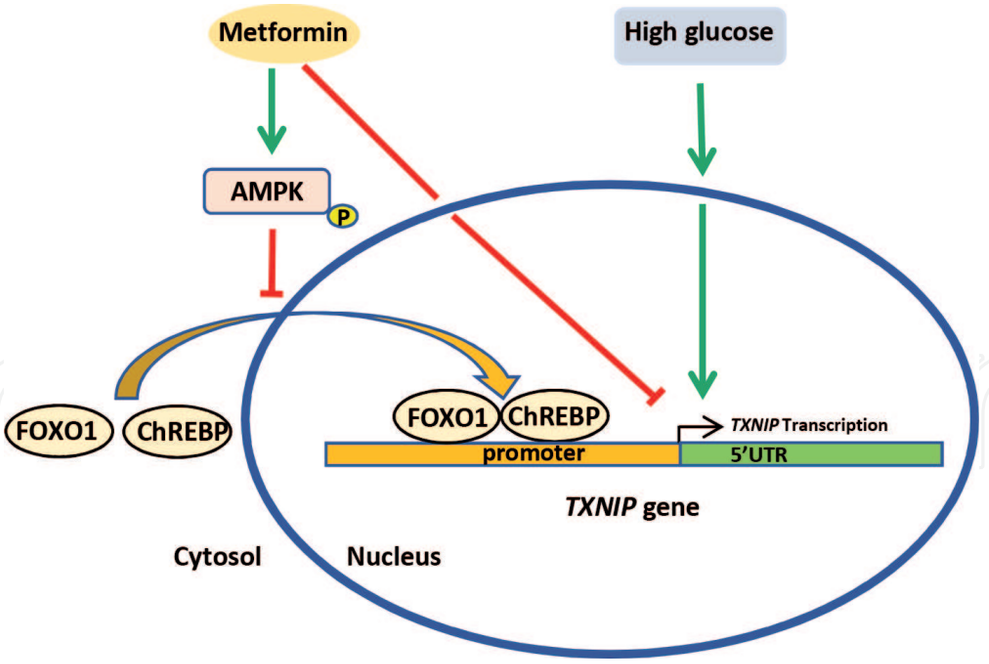

Figure 2.
Metformin inhibits the nuclear entry of ChREBP and FOXO1 from cytosol and their binding capacity to the TXNIP promoter, thus potently and effectively suppresses TXNIP transcription induced by high glucose at last. The inhibitory effect of metformin on nuclear translocation is AMPK-phosphorylation-dependent.

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

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] UKPDS G. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK prospective Diabetes study (UKPDS) group. Lancet. 1998;**352**:854-865. DOI: 10.1016/S0140-6736(98)07037-8

[2] Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of Clinical Investigation. 2001;**108**:1167-1174. DOI: 10.1172/JCI13505

[3] Coughlan KA, Valentine RJ, Ruderman NB, Saha AK. AMPK activation: A therapeutic target for type 2 diabetes? Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2014;**7**:241-253. DOI: 10.2147/DMSO.S43731

[4] Li X, Kover KL, Heruth DP, Watkins DJ, Moore WV, Jackson K, et al. New insight into metformin action: Regulation of ChREBP and FOXO1 activities in endothelial cells. Molecular Endocrinology. 2015;**29**:1184-1194. DOI: 10.1210/ME.2015-1090

[5] Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nature Cell Biology. 2011;**13**:1016-1023. DOI: 10.1038/ncb2329

[6] Wu N, Zheng B, Shaywitz A, Dagon Y, Tower C, Bellinger G, et al. AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. Molecular Cell. 2013;**49**:1167-1175. DOI: 10.1016/j.molcel.2013.01.035

[7] Chavez JA, Roach WG, Keller SR, Lane WS, Lienhard GE. Inhibition of GLUT4 translocation by Tbc1d1, a Rab GTPase-activating protein abundant in skeletal muscle, is partially relieved by AMP-activated protein kinase activation. The Journal of Biological Chemistry. 2008;**283**:9187-9195. DOI: 10.1074/jbc.M708934200

[8] Bultot L, Guigas B, Von Wilamowitz-Moellendorff A, Maisin L, Vertommen D, Hussain N, et al. AMP-activated protein kinase phosphorylates and inactivates liver glycogen synthase. The Biochemical Journal. 2012;**443**:193-203. DOI: 10.1042/BJ20112026

[9] Miller RA, Birnbaum MJ. An energetic tale of AMPK-independent effects of metformin. The Journal of Clinical Investigation. 2010;**120**:2267-2270. DOI: 10.1172/JCI43661

[10] Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, et al. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut. 2014;**63**:727-735. DOI: 10.1136/gutjnl-2012-303839

[11] MacNeil LT, Schertzer JD, Steinberg GR. Bacteria transmit metformin-associated lifespan extension. Nature Reviews. Endocrinology. 2019;**16**:9-10. DOI: 10.1038/s41574-019-0278-3

[12] Collier CA, Bruce CR, Smith AC, Lopaschuk G, Dyck DJ. Metformin counters the insulin-induced suppression of fatty acid oxidation and stimulation of triacylglycerol storage in rodent skeletal muscle. American Journal of Physiology. Endocrinology and Metabolism. 2006;**291**:E182-E189. DOI: 10.1152/ajpendo.00272.2005

[13] Grapov D, Fiehn O, Campbell C, Chandler CJ, Burnett DJ, Souza EC, et al. Exercise plasma metabolomics and xenometabolomics in obese, sedentary, insulin-resistant women: Impact of a fitness and weight loss intervention. American Journal of Physiology.

Endocrinology and Metabolism. 2019;**317**:E999-E1014. DOI: 10.1152/ajpendo.00091.2019

[14] Zhao L, Fu Z, Wu J, Aylor KW, Barrett EJ, Cao W, et al. Inflammation-induced microvascular insulin resistance is an early event in diet-induced obesity. Clinical Science (London, England). 2015;**129**:1025-1036. DOI: 10.1042/CS20150143

[15] Sjoberg KA, Rattigan S, Jeppesen JF, Lundsgaard AM, Holst JJ, Kiens B. Differential effects of glucagon-like peptide-1 on microvascular recruitment and glucose metabolism in short- and long-term insulin resistance. The Journal of Physiology. 2015;**593**: 2185-2198. DOI: 10.1113/JP270129

[16] Bradley EA, Premilovac D, Betik AC, Hu D, Attrill E, Richards S, et al. Metformin improves vascular and metabolic insulin action in insulin resistant muscle. The Journal of Endocrinology. 2019;**243**:85-96. DOI: 10.1530/JOE-19-0067

[17] Giannarelli R, Aragona M, Coppelli A, Del Prato S. Reducing insulin resistance with metformin: The evidence today. Diabetes & Metabolism. 2003;**29**:6S28-6S35. DOI: 10.1016/s1262-3636(03)72785-2

[18] Adeva-Andany MM, Martinez-Rodriguez J, Gonzalez-Lucan M, Fernandez-Fernandez C, Castro-Quintela E. Insulin resistance is a cardiovascular risk factor in humans. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2019;**13**:1449-1455. DOI: 10.1016/j.dsx.2019.02.023

[19] Varvel SA, Voros S, Thiselton DL, Pottala JV, Dall T, Warnick GR, et al. Comprehensive biomarker testing of glycemia, insulin resistance, and beta cell function has greater sensitivity to detect diabetes risk than fasting glucose and HbA1c and is associated with improved glycemic control in clinical practice. Journal of Cardiovascular Translational Research. 2014;**7**:597-606. DOI: 10.1007/s12265-014-9577-1

[20] Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metabolism. 2009;**9**:311-326. DOI: 10.1016/j.cmet.2009.02.002

[21] Lee W, Lee HJ, Jang HB, Kim HJ, Ban HJ, Kim KY, et al. Asymmetric dimethylarginine (ADMA) is identified as a potential biomarker of insulin resistance in skeletal muscle. Scientific Reports. 2018;**8**:2133. DOI: 10.1038/s41598-018-20549-0

[22] Zemdegs J, Martin H, Pintana H, Bullich S, Manta S, Marques MA, et al. Metformin promotes anxiolytic and antidepressant-like responses in insulin-resistant mice by decreasing circulating branched-chain amino acids. The Journal of Neuroscience. 2019;**39**:5935-5948. DOI: 10.1523/JNEUROSCI.2904-18.2019

[23] Asagami T, Abbasi F, Stuelinger M, Lamendola C, McLaughlin T, Cooke JP, et al. Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. Metabolism. 2002;**51**:843-846. DOI: 10.1053/meta.2002.33349

[24] Granado M, Amor S, Martin-Carro B, Guerra-Menendez L, Tejera-Munoz A, Gonzalez-Hedstrom D, et al. Caloric restriction attenuates aging-induced cardiac insulin resistance in male Wistar rats through activation of PI3K/Akt pathway. Nutrition, Metabolism, and Cardiovascular Diseases. 2019;**29**:97-105. DOI: 10.1016/j.numecd.2018.09.005

[25] Fang P, Yu M, Zhang L, Wan D, Shi M, Zhu Y, et al. Baicalin against obesity and insulin resistance through

activation of AKT/AS160/GLUT4 pathway. Molecular and Cellular Endocrinology. 2017;**448**:77-86. DOI: 10.1016/j.mce.2017.03.027

[26] Ke B, Ke X, Wan X, Yang Y, Huang Y, Qin J, et al. Astragalus polysaccharides attenuates TNF-alpha-induced insulin resistance via suppression of miR-721 and activation of PPAR-gamma and PI3K/AKT in 3T3-L1 adipocytes. American Journal of Translational Research. 2017;**9**:2195-2206

[27] Ooi J, Adamu HA, Imam MU, Ithnin H, Ismail M. Polyphenol-rich ethyl acetate fraction isolated from *Molineria latifolia* ameliorates insulin resistance in experimental diabetic rats via IRS1/AKT activation. Biomedicine & Pharmacotherapy. 2018;**98**:125-133. DOI: 10.1016/j.biopha.2017.12.002

[28] Yang S, Chen Z, Cao M, Li R, Wang Z, Zhang M. Pioglitazone ameliorates Abeta42 deposition in rats with diet-induced insulin resistance associated with AKT/GSK3beta activation. Molecular Medicine Reports. 2017;**15**:2588-2594. DOI: 10.3892/mmr.2017.6342

[29] Zhang Z, Liu H, Liu J. Akt activation: A potential strategy to ameliorate insulin resistance. Diabetes Research and Clinical Practice. 2017;**156**:107092. DOI: 10.1016/j.diabres.2017.10.004

[30] Hajduch E, Litherland GJ, Hundal HS. Protein kinase B (PKB/Akt)—A key regulator of glucose transport? FEBS Letters. 2001;**492**:199-203. DOI: 10.1016/s0014-5793(01)02242-6

[31] Chan TO, Rittenhouse SE, Tsichlis PN. AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation. Annual Review of Biochemistry. 1999;**68**:965-1014. DOI: 10.1146/annurev.biochem.68.1.965

[32] Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, et al. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. The Journal of Clinical Investigation. 2003;**112**:197-208. DOI: 10.1172/JCI16885

[33] Thomas H, Reynold EM, Cinquino N, Gaugler M. Effects of aging on insulin action and AkT signaling is isoform specific AKT knockout mice. The FASEB Journal. 2011;**25**:supplement-Ib-1

[34] Tsuchiya A, Kanno T, Nishizaki T. PI3 kinase directly phosphorylates Akt1/2 at Ser473/474 in the insulin signal transduction pathway. The Journal of Endocrinology. 2014;**220**:49-59. DOI: 10.1530/JOE-13-0172

[35] Beg M, Abdullah N, Thowfeik FS, Altorki NK, McGraw TE. Distinct Akt phosphorylation states are required for insulin regulated Glut4 and Glut1-mediated glucose uptake. eLife. 2017;**6**:1-22. DOI: 10.7554/eLife.26896

[36] Wang Z, Wang Y, Han Y, Yin Q, Hu S, Zhao T, et al. Akt is a critical node of acute myocardial insulin resistance and cardiac dysfunction after cardiopulmonary bypass. Life Sciences. 2019;**234**:116734. DOI: 10.1016/j.lfs.2019.116734

[37] Garabadu D, Krishnamurthy S. Metformin attenuates hepatic insulin resistance in type-2 diabetic rats through PI3K/Akt/GLUT-4 signalling independent to bicuculline-sensitive GABAA receptor stimulation. Pharmaceutical Biology. 2017;**55**:722-728. DOI: 10.1080/13880209.2016.1268635

[38] Shen X, Wang L, Zhou N, Gai S, Liu X, Zhang S. Beneficial effects of combination therapy of phloretin and metformin in streptozotocin-induced

diabetic rats and improved insulin sensitivity in vitro. Food & Function. 2020;**11**:392-403. DOI: 10.1039/c9fo01326a

[39] Chutkow WA, Birkenfeld AL, Brown JD, Lee HY, Frederick DW, Yoshioka J, et al. Deletion of the alpha-arrestin protein Txnip in mice promotes adiposity and adipogenesis while preserving insulin sensitivity. Diabetes. 2010;**59**:1424-1434. DOI: 10.2337/db09-1212

[40] Nasoohi S, Parveen K, Ishrat T. Metabolic syndrome, brain insulin resistance, and Alzheimer's disease: Thioredoxin interacting protein (TXNIP) and Inflammasome as Core amplifiers. Journal of Alzheimer's Disease. 2018;**66**:857-885. DOI: 10.3233/JAD-180735

[41] Parikh H, Carlsson E, Chutkow WA, Johansson LE, Storgaard H, Poulsen P, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Medicine. 2007;**4**:e158. DOI: 10.1371/journal.pmed.0040158

[42] Li X, Kover KL, Heruth DP, Watkins DJ, Guo Y, Moore WV, et al. Thioredoxin-interacting protein promotes high-glucose-induced macrovascular endothelial dysfunction. Biochemical and Biophysical Research Communications. 2017;**493**:291-297. DOI: 10.1016/j.bbrc.2017.09.028

[43] Waldhart AN, Dykstra H, Peck AS, Boguslawski EA, Madaj ZB, Wen J, et al. Phosphorylation of TXNIP by AKT mediates acute influx of glucose in response to insulin. Cell Reports. 2017;**19**:2005-2013. DOI: 10.1016/j.celrep.2017.05.041

[44] Tao X, Cai L, Chen L, Ge S, Deng X. Effects of metformin and Exenatide on insulin resistance and AMPKalpha-SIRT1 molecular pathway in PCOS rats. Journal of Ovarian Research. 2019;**12**:86. DOI: 10.1186/s13048-019-0555-8

[45] American Diabetes A. 2. Classification and diagnosis of Diabetes: Standards of medical Care in Diabetes-2019. Diabetes Care. 2019;**42**:S13-S28. DOI: 10.2337/dc19-S002

[46] CDCP. Center for Disease Control and Prevention, Prediabetes: Your Chance to Prevent Type 2 Diabetes. 2020. Available from : http://cdc.gov/diabetes

[47] Metformin for Prediabetes. JAMA. 2017;**317**:1171. DOI: 10.1001/jama.2016.17844

[48] Hostalek U, Gwilt M, Hildemann S. Therapeutic use of metformin in prediabetes and diabetes prevention. Drugs. 2015;**75**:1071-1094. DOI: 10.1007/s40265-015-0416-8

[49] Naidoo P, Wing J, Rambiritch V. Effect of sitagliptin and metformin on prediabetes progression to type 2 Diabetes - a randomized, double-blind, double-arm, multicenter clinical trial: Protocol for the Sitagliptin and metformin in PreDiabetes (SiMePreD) study. JMIR Research Protocols. 2016;**5**:e145. DOI: 10.2196/resprot.5073

[50] Khokhar A, Umpaichitra V, Chin VL, Perez-Colon S. Metformin use in children and adolescents with Prediabetes. Pediatric Clinics of North America. 2017;**64**:1341-1353. DOI: 10.1016/j.pcl.2017.08.010

[51] Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. The New England Journal of Medicine. 2002;**346**:393-403. DOI: 10.1056/NEJMoa012512

[52] Diabetes Prevention Program Research G. Long-term effects of

lifestyle intervention or metformin on diabetes development and microvascular complications over 15-year follow-up: The Diabetes prevention program outcomes study. The Lancet Diabetes and Endocrinology. 2015;**3**:866-875. DOI: 10.1016/S2213-8587(15)00291-0

[53] Ratner RE, Christophi CA, Metzger BE, Dabelea D, Bennett PH, Pi-Sunyer X, et al. Prevention of diabetes in women with a history of gestational diabetes: Effects of metformin and lifestyle interventions. The Journal of Clinical Endocrinology and Metabolism. 2008;**93**:4774-4779. DOI: 10.1210/jc.2008-0772

[54] Moin T, Li J, Duru OK, Ettner S, Turk N, Keckhafer A, et al. Metformin prescription for insured adults with prediabetes from 2010 to 2012: A retrospective cohort study. Annals of Internal Medicine. 2015;**162**:542-548. DOI: 10.7326/M14-1773

[55] American Diabetes A. 3. Prevention or delay of type 2 Diabetes: Standards of medical Care in Diabetes-2019. Diabetes Care. 2019;**42**:S29-S33. DOI: 10.2337/dc19-S003

[56] Diabetes Canada Clinical Practice Guidelines Expert C, Houlden RL. Introduction. Canadian Journal of Diabetes. 2018;**42**(Suppl 1):S1-S5. DOI: 10.1016/j.jcjd.2017.10.001

[57] Cahn A, Cernea S, Raz I. An update on DPP-4 inhibitors in the management of type 2 diabetes. Expert Opinion on Emerging Drugs. 2016;**21**:409-419. DOI: 10.1080/14728214.2016.1257608

[58] Marin-Penalver JJ, Martin-Timon I, Sevillano-Collantes C, Del Canizo-Gomez FJ. Update on the treatment of type 2 diabetes mellitus. World Journal of Diabetes. 2016;**7**:354-395. DOI: 10.4239/wjd.v7.i17.354

[59] Flory JH, Hennessy S, Bailey CJ, Inzucchi SE. Reports of lactic acidosis attributed to metformin, 2015-2018. Diabetes Care. 2019;**43**:244-246. DOI: 10.2337/dc19-0923

[60] Deden LN, Aarts EO, Aelfers SCW, van Borren M, Janssen IMC, Berends FJ, et al. Risk of metformin-associated lactic acidosis (MALA) in patients after gastric bypass surgery. Obesity Surgery. 2018;**28**:1080-1085. DOI: 10.1007/s11695-017-2974-1

[61] Angioi A, Cabiddu G, Conti M, Pili G, Atzeni A, Matta V, et al. Metformin associated lactic acidosis: A case series of 28 patients treated with sustained low-efficiency dialysis (SLED) and long-term follow-up. BMC Nephrology. 2018;**19**:77. DOI: 10.1186/s12882-018-0875-8

[62] Lipska KJ, Flory JH, Hennessy S, Inzucchi SE. Citizen petition to the US food and drug administration to change prescribing guidelines: The metformin experience. Circulation. 2016;**134**:1405-1408. DOI: 10.1161/CIRCULATIONAHA.116.023041

[63] Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, et al. Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. The Journal of Clinical Endocrinology and Metabolism. 2016;**101**:1754-1761. DOI: 10.1210/jc.2015-3754

[64] Alvarez M, Sierra OR, Saavedra G, Moreno S. Vitamin B12 deficiency and diabetic neuropathy in patients taking metformin: A cross-sectional study. Endocrine Connections. 2019;**8**:1324-1329. DOI: 10.1530/EC-19-0382

[65] Patel JJ, Mundi MS. Metformin for Type 2 Diabetes. Journal of the American Medical Association. 2019;**322**:1312-1313. DOI: 10.1001/jama.2019.11497

[66] Priya G, Kalra S. A review of insulin resistance in type 1 diabetes: Is there a place for adjunctive metformin?

Diabetes Therapy. 2018;**9**:349-361. DOI: 10.1007/s13300-017-0333-9

[67] George P, McCrimmon RJ. Potential role of non-insulin adjunct therapy in type 1 diabetes. Diabetic Medicine. 2013;**30**:179-188. DOI: 10.1111/j.1464-5491.2012.03744.x

[68] Lund SS, Tarnow L, Astrup AS, Hovind P, Jacobsen PK, Alibegovic AC, et al. Effect of adjunct metformin treatment in patients with type-1 diabetes and persistent inadequate glycaemic control. A randomized study. PLoS One. 2008;**3**:e3363. DOI: 10.1371/journal.pone.0003363

[69] Jacobsen IB, Henriksen JE, Beck-Nielsen H. The effect of metformin in overweight patients with type 1 diabetes and poor metabolic control. Basic & Clinical Pharmacology & Toxicology. 2009;**105**:145-149. DOI: 10.1111/j.1742-7843.2009.00380.x

[70] Moon RJ, Bascombe LA, Holt RI. The addition of metformin in type 1 diabetes improves insulin sensitivity, diabetic control, body composition and patient well-being. Diabetes, Obesity & Metabolism. 2007;**9**:143-145. DOI: 10.1111/j.1463-1326.2006.00599.x

[71] Beysel S, Unsal IO, Kizilgul M, Caliskan M, Ucan B, Cakal E. The effects of metformin in type 1 diabetes mellitus. BMC Endocrine Disorders. 2018;**18**:1. DOI: 10.1186/s12902-017-0228-9

[72] Jones KL, Arslanian S, Peterokova VA, Park JS, Tomlinson MJ. Effect of metformin in pediatric patients with type 2 diabetes: A randomized controlled trial. Diabetes Care. 2002;**25**:89-94. DOI: 10.2337/diacare.25.1.89

[73] Group TS, Zeitler P, Epstein L, Grey M, Hirst K, Kaufman F, et al. Treatment options for type 2 diabetes in adolescents and youth: A study of the comparative efficacy of metformin alone or in combination with rosiglitazone or lifestyle intervention in adolescents with type 2 diabetes. Pediatric Diabetes. 2007;**8**:74-87. DOI: 10.1111/j.1399-5448.2007.00237.x

[74] Group TS, Zeitler P, Hirst K, Pyle L, Linder B, Copeland K, et al. A clinical trial to maintain glycemic control in youth with type 2 diabetes. The New England Journal of Medicine. 2012;**366**:2247-2256. DOI: 10.1056/NEJMoa1109333

[75] Nadeau KJ, Chow K, Alam S, Lindquist K, Campbell S, McFann K, et al. Effects of low dose metformin in adolescents with type I diabetes mellitus: A randomized, double-blinded placebo-controlled study. Pediatric Diabetes. 2015;**16**:196-203. DOI: 10.1111/pedi.12140

[76] Cree-Green M, Bergman BC, Cengiz E, Fox LA, Hannon TS, Miller K, et al. Metformin improves peripheral insulin sensitivity in youth with type 1 diabetes. The Journal of Clinical Endocrinology and Metabolism. 2019;**104**:3265-3278. DOI: 10.1210/jc.2019-00129

[77] Anderson JJA, Couper JJ, Giles LC, Leggett CE, Gent R, Coppin B, et al. Effect of metformin on vascular function in children with type 1 diabetes: A 12-month randomized controlled trial. The Journal of Clinical Endocrinology and Metabolism. 2017;**102**:4448-4456. DOI: 10.1210/jc.2017-00781

[78] Bjornstad P, Schafer M, Truong U, Cree-Green M, Pyle L, Baumgartner A, et al. Metformin improves insulin sensitivity and vascular health in youth with type 1 diabetes mellitus. Circulation. 2018;**138**:2895-2907. DOI: 10.1161/CIRCULATIONAHA.118.035525

[79] Sarnblad S, Kroon M, Aman J. Metformin as additional therapy in adolescents with poorly

controlled type 1 diabetes: Randomised placebo-controlled trial with aspects on insulin sensitivity. European Journal of Endocrinology. 2003;**149**:323-329. DOI: 10.1530/eje.0.1490323

[80] Urakami T, Morimoto S, Owada M, Harada K. Usefulness of the addition of metformin to insulin in pediatric patients with type 1 diabetes mellitus. Pediatrics International. 2005;**47**:430-433. DOI: 10.1111/j.1442-200x.2005.02075.x

[81] Libman IM, Miller KM, DiMeglio LA, Bethin KE, Katz ML, Shah A, et al. Effect of metformin added to insulin on glycemic control among overweight/obese adolescents with type 1 diabetes: A randomized clinical trial. JAMA. 2015;**314**:2241-2250. DOI: 10.1001/jama.2015.16174

[82] Soukas AA, Hao H, Wu L. Metformin as anti-aging therapy: Is it for everyone? Trends in Endocrinology and Metabolism. 2019;**30**:745-755. DOI: 10.1016/j.tem.2019.07.015

[83] Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, et al. Metformin slows down aging and extends life span of female SHR mice. Cell Cycle. 2008;**7**:2769-2773. DOI: 10.4161/cc.7.17.6625

[84] Anisimov VN, Berstein LM, Popovich IG, Zabezhinski MA, Egormin PA, Piskunova TS, et al. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY). 2011;**3**:148-157. DOI: 10.18632/aging.100273

[85] Anisimov VN, Egormin PA, Piskunova TS, Popovich IG, Tyndyk ML, Yurova MN, et al. Metformin extends life span of HER-2/neu transgenic mice and in combination with melatonin inhibits growth of transplantable tumors in vivo. Cell Cycle. 2010;**9**:188-197. DOI: 10.4161/cc.9.1.10407

[86] Pigazzi M, Ricotti E, Germano G, Faggian D, Arico M, Basso G. cAMP response element binding protein (CREB) overexpression CREB has been described as critical for leukemia progression. Haematologica. 2007;**92**:1435-1437. DOI: 10.3324/haematol.11122

[87] Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, Scheibye-Knudsen M, et al. Metformin improves healthspan and lifespan in mice. Nature Communications. 2013;**4**:2192. DOI: 10.1038/ncomms3192

[88] Konturek PC, Haziri D, Brzozowski T, Hess T, Heyman S, Kwiecien S, et al. Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases. Journal of Physiology and Pharmacology. 2015;**66**:483-491

[89] Vaiserman AM, Koliada AK, Marotta F. Gut microbiota: A player in aging and a target for anti-aging intervention. Ageing Research Reviews. 2017;**35**:36-45. DOI: 10.1016/j.arr.2017.01.001

[90] Pryor R, Norvaisas P, Marinos G, Best L, Thingholm LB, Quintaneiro LM, et al. Host-microbe-drug-nutrient screen identifies bacterial effectors of metformin therapy. Cell. 2019;**178**:1299-1312.e29. DOI: 10.1016/j.cell.2019.08.003

[91] Kulkarni AS, Brutsaert EF, Anghel V, Zhang K, Bloomgarden N, Pollak M, et al. Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. Aging Cell. 2018;**17**:e12713. DOI: 10.1111/acel.12723

[92] March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under

contrasting diagnostic criteria. Human Reproduction. 2010;**25**:544-551. DOI: 10.1093/humrep/dep399

[93] Morley LC, Tang T, Yasmin E, Norman RJ, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database of Systematic Reviews. 2017;**11**:CD003053. DOI: 10.1002/14651858.CD003053.pub6

[94] Nandi A, Chen Z, Patel R, Poretsky L. Polycystic ovary syndrome. Endocrinology and Metabolism Clinics of North America. 2014;**43**:123-147. DOI: 10.1016/j.ecl.2013.10.003

[95] Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism. 1994;**43**:647-654. DOI: 10.1016/0026-0495(94)90209-7

[96] Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, et al. Metformin effects on clinical features, endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: A randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. The Journal of Clinical Endocrinology and Metabolism. 2000;**85**:139-146. DOI: 10.1210/jcem.85.1.6293

[97] Hirsch A, Hahn D, Kempna P, Hofer G, Nuoffer JM, Mullis PE, et al. Metformin inhibits human androgen production by regulating steroidogenic enzymes HSD3B2 and CYP17A1 and complex I activity of the respiratory chain. Endocrinology. 2012;**153**: 4354-4366. DOI: 10.1210/en.2012-1145

[98] Zheng J, Shan PF, Gu W. The efficacy of metformin in pregnant women with polycystic ovary syndrome: A meta-analysis of clinical trials. Journal of Endocrinological Investigation. 2013;**36**:797-802. DOI: 10.3275/8932

[99] Glueck CJ, Goldenberg N, Pranikoff J, Khan Z, Padda J, Wang P. Effects of metformin-diet intervention before and throughout pregnancy on obstetric and neonatal outcomes in patients with polycystic ovary syndrome. Current Medical Research and Opinion. 2013;**29**:55-62. DOI: 10.1185/03007995.2012.755121

[100] Lautatzis ME, Goulis DG, Vrontakis M. Efficacy and safety of metformin during pregnancy in women with gestational diabetes mellitus or polycystic ovary syndrome: A systematic review. Metabolism. 2013;**62**:1522-1534. DOI: 10.1016/j.metabol.2013.06.006

[101] Ganie MA, Khurana ML, Nisar S, Shah PA, Shah ZA, Kulshrestha B, et al. Improved efficacy of low-dose spironolactone and metformin combination than either drug alone in the management of women with polycystic ovary syndrome (PCOS): A six-month, open-label randomized study. The Journal of Clinical Endocrinology and Metabolism. 2013;**98**:3599-3607. DOI: 10.1210/jc.2013-1040

[102] Costello M, Garad R, Hart R, Homer H, Johnson L, Jordan C, et al. A review of first line infertility treatments and supporting evidence in women with polycystic ovary syndrome. Medical Sciences (Basel). 2019;**7**:1-10. DOI: 10.3390/medsci7090095

[103] Elter K, Imir G, Durmusoglu F. Clinical, endocrine and metabolic effects of metformin added to ethinyl estradiol-cyproterone acetate in non-obese women with polycystic ovarian syndrome: A randomized controlled study. Human Reproduction. 2002;**17**:1729-1737. DOI: 10.1093/humrep/17.7.1729

[104] Di Pietro M, Parborell F, Irusta G, Pascuali N, Bas D, Bianchi MS, et al. Metformin regulates ovarian angiogenesis and follicular development in a female polycystic ovary syndrome rat model. Endocrinology. 2015;**156**:1453-1463. DOI: 10.1210/en.2014-1765

[105] Han Y, Xie H, Liu Y, Gao P, Yang X, Shen Z. Effect of metformin on all-cause and cardiovascular mortality in patients with coronary artery diseases: A systematic review and an updated meta-analysis. Cardiovascular Diabetology. 2019;**18**:96. DOI: 10.1186/s12933-019-0900-7

[106] Rena G, Lang CC. Repurposing metformin for cardiovascular disease. Circulation. 2018;**137**:422-424. DOI: 10.1161/CIRCULATIONAHA.117.031735

[107] Dore FJ, Domingues CC, Ahmadi N, Kundu N, Kropotova Y, Houston S, et al. The synergistic effects of saxagliptin and metformin on CD34+ endothelial progenitor cells in early type 2 diabetes patients: A randomized clinical trial. Cardiovascular Diabetology. 2018;**17**:65. DOI: 10.1186/s12933-018-0709-9

[108] Kooy A, de Jager J, Lehert P, Bets D, Wulffele MG, Donker AJ, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. Archives of Internal Medicine. 2009;**169**:616-625. DOI: 10.1001/archinternmed.2009.20

[109] Griffin SJ, Leaver JK, Irving GJ. I mpact of metformin on cardiovascular disease: A meta-analysis of randomised trials among people with type 2 diabetes. Diabetologia. 2017;**60**:1620-1629. DOI: 10.1007/s00125-017-4337-9

[110] Boussageon R, Gueyffier F, Cornu C. Metformin as firstline treatment for type 2 diabetes: Are we sure? BMJ. 2016;**352**:h6748. DOI: 10.1136/bmj.h6748

[111] Boussageon R, Supper I, Bejan-Angoulvant T, Kellou N, Cucherat M, Boissel JP, et al. Reappraisal of metformin efficacy in the treatment of type 2 diabetes: A meta-analysis of randomised controlled trials. PLoS Medicine. 2012;**9**:e1001204. DOI: 10.1371/journal.pmed.1001204

[112] Loi H, Boal F, Tronchere H, Cinato M, Kramar S, Oleshchuk O, et al. Metformin protects the heart against hypertrophic and apoptotic remodeling after myocardial infarction. Frontiers in Pharmacology. 2019;**10**:154. DOI: 10.3389/fphar.2019.00154

[113] Bonow RO, Gheorghiade M. The diabetes epidemic: A national and global crisis. The American Journal of Medicine. 2004;**116**(Suppl 5A):2S-10S. DOI: 10.1016/j.amjmed.2003.10.014

[114] Bashier A, Bin Hussain A, Abdelgadir E, Alawadi F, Sabbour H, Chilton R. Consensus recommendations for management of patients with type 2 diabetes mellitus and cardiovascular diseases. Diabetology and Metabolic Syndrome. 2019;**11**:80. DOI: 10.1186/s13098-019-0476-0

[115] Harrington JL, de Albuquerque Rocha N, Patel KV, Verma S, McGuire DK. Should metformin remain first-line medical therapy for patients with type 2 diabetes mellitus and atherosclerotic cardiovascular disease? An alternative approach. Current Diabetes Reports. 2018;**18**:64. DOI: 10.1007/s11892-018-1035-z

[116] Wong AK, Symon R, AlZadjali MA, Ang DS, Ogston S, Choy A, et al. The effect of metformin on insulin resistance and exercise parameters in patients with heart failure. European Journal of Heart Failure. 2012;**14**:1303-1310. DOI: 10.1093/eurjhf/hfs106

[117] Petrie JR, Chaturvedi N, Ford I, Brouwers M, Greenlaw N, Tillin T, et al. Cardiovascular and metabolic effects

of metformin in patients with type 1 diabetes (REMOVAL): A double-blind, randomised, placebo-controlled trial. The Lancet Diabetes and Endocrinology. 2017;**5**:597-609. DOI: 10.1016/S2213-8587(17)30194-8

[118] Jadhav S, Ferrell W, Greer IA, Petrie JR, Cobbe SM, Sattar N. Effects of metformin on microvascular function and exercise tolerance in women with angina and normal coronary arteries: A randomized, double-blind, placebo-controlled study. Journal of the American College of Cardiology. 2006;**48**:956-963. DOI: 10.1016/j.jacc.2006.04.088

[119] Griffin SJ, Bethel MA, Holman RR, Khunti K, Wareham N, Brierley G, et al. Metformin in non-diabetic hyperglycaemia: The GLINT feasibility RCT. Health Technology Assessment. 2018;**22**:1-64. DOI: 10.3310/hta22180

[120] Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: A consensus report. CA: a Cancer Journal for Clinicians. 2010;**60**:207-221. DOI: 10.3322/caac.20078

[121] Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;**330**:1304-1305. DOI: 10.1136/bmj.38415.708634.F7

[122] Hosono K, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, et al. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. Cancer Prevention Research (Philadelphia, Pa.). 2010;**3**:1077-1083. DOI: 10.1158/1940-6207.CAPR-10-0186

[123] Hadad S, Iwamoto T, Jordan L, Purdie C, Bray S, Baker L, et al. Evidence for biological effects of metformin in operable breast cancer: A pre-operative, window-of-opportunity, randomized trial. Breast Cancer Research and Treatment. 2011;**128**:783-794. DOI: 10.1007/s10549-011-1612-1

[124] Camacho L, Dasgupta A, Jiralerspong S. Metformin in breast cancer - an evolving mystery. Breast Cancer Research. 2015;**17**:88. DOI: 10.1186/s13058-015-0598-8

[125] Coyle C, Cafferty FH, Vale C, Langley RE. Metformin as an adjuvant treatment for cancer: A systematic review and meta-analysis. Annals of Oncology. 2016;**27**:2184-2195. DOI: 10.1093/annonc/mdw410

[126] Saraei P, Asadi I, Kakar MA, Moradi-Kor N. The beneficial effects of metformin on cancer prevention and therapy: A comprehensive review of recent advances. Cancer Management and Research. 2019;**11**:3295-3313. DOI: 10.2147/CMAR.S200059

[127] Dulskas A, Patasius A, Linkeviciute-Ulinskiene D, Zabuliene L, Urbonas V, Smailyte G. Metformin increases cancer specific survival in colorectal cancer patients-national cohort study. Cancer Epidemiology. 2019;**62**:101587. DOI: 10.1016/j.canep.2019.101587

[128] Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Metformin as an anticancer agent. Trends in Pharmacological Sciences. 2018;**39**:867-878. DOI: 10.1016/j.tips.2018.07.006

[129] Bridges HR, Jones AJ, Pollak MN, Hirst J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. The Biochemical Journal. 2014;**462**:475-487. DOI: 10.1042/BJ20140620

[130] El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. The Journal of Biological

Chemistry. 2000;**275**:223-228. DOI: 10.1074/jbc.275.1.223

[131] Pernicova I, Korbonits M. Metformin--mode of action and clinical implications for diabetes and cancer. Nature Reviews. Endocrinology. 2014;**10**:143-156. DOI: 10.1038/nrendo.2013.256

[132] Herzig S, Shaw RJ. AMPK: Guardian of metabolism and mitochondrial homeostasis. Nature Reviews. Molecular Cell Biology. 2018;**19**:121-135. DOI: 10.1038/nrm.2017.95

[133] Huang S, Houghton PJ. Targeting mTOR signaling for cancer therapy. Current Opinion in Pharmacology. 2003;**3**:371-377

[134] Vogt PK. PI 3-kinase, mTOR, protein synthesis and cancer. Trends in Molecular Medicine. 2001;**7**:482-484. DOI: 10.1616/s1471-4914(01)0216-x

[135] Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell. 2000;**103**:253-262. DOI: 10.1016/s0092-8674(00)00117-3

[136] Muller PA, Vousden KH. p53 mutations in cancer. Nature Cell Biology. 2013;**15**:2-8. DOI: 10.1038/ncb2641

[137] Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Molecular Cell. 2005;**18**:283-293. DOI: 10.1016/j.molcel.2005.03.027

[138] Cerezo M, Tichet M, Abbe P, Ohanna M, Lehraiki A, Rouaud F, et al. Metformin blocks melanoma invasion and metastasis development in AMPK/p53-dependent manner. Molecular Cancer Therapeutics. 2013;**12**:1605-1615. DOI: 10.1158/1535-7163.MCT-12-1226-T

[139] Juan J, Gu QZ, Mavis C, Czuczman MS, Hernandezollizaliturri FJ. Metformin induces p53-dependent mitochondrial stress in therapy-sensitive and -resistant lymphoma pre-clinical model and primary patients sample with B-cell non-Hodgkin lymphoma (NHL). Blood. 2015;**126**:4008. DOI: 10.1181/blood.v126.23.4008.4008

[140] Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, Gounon P, et al. Targeting cancer cell metabolism: The combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. Cancer Research. 2010;**70**:2465-2475. DOI: 10.1158/0008-5472.CAN-09-2782

[141] Yudhani RD, Astuti I, Mustofa M, Indarto D, Muthmainah M. Metformin modulates cyclin D1 and P53 expression to inhibit cell proliferation and to induce apoptosis in cervical cancer cell lines. Asian Pacific Journal of Cancer Prevention. 2019;**20**:1667-1673. DOI: 10.31557/APJCP.2019.20.6.1667

[142] Jeong YK, Kim MS, Lee JY, Kim EH, Ha H. Metformin Radiosensitizes p53-deficient colorectal Cancer cells through induction of G2/M arrest and inhibition of DNA repair proteins. PLoS One. 2015;**10**:e0143596. DOI: 10.1371/journal.pone.0143596

[143] Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Research. 2007;**67**:6745-6752. DOI: 10.1158/0008-5472.CAN-06-4447

[144] Dotsch V, Bernassola F, Coutandin D, Candi E, Melino G. p63 and p73, the ancestors of p53. Cold Spring Harbor Perspectives in Biology. 2010;**2**:a004887. DOI: 10.1101/cshperspect.a004887

[145] Moses MA, George AL, Sakakibara N, Mahmood K, Ponnamperuma RM, King KE, et al. Molecular mechanisms of p63-mediated

squamous Cancer pathogenesis. International Journal of Molecular Sciences. 2019;**20**:3590. DOI: 10.3390/ijms20143590

[146] Yi Y, Chen D, Ao J, Sun S, Wu M, Li X, et al. Metformin promotes AMP-activated protein kinase-independent suppression of DeltaNp63alpha protein expression and inhibits cancer cell viability. The Journal of Biological Chemistry. 2017;**292**:5253-5261. DOI: 10.1074/jbc.M116.769141

[147] Yoon MK, Ha JH, Lee MS, Chi SW. Structure and apoptotic function of p73. BMB Reports. 2015;**48**:81-90. DOI: 10.5483/bmbrep.2015.48.2.255

[148] Adamovich Y, Adler J, Meltser V, Reuven N, Shaul Y. AMPK couples p73 with p53 in cell fate decision. Cell Death and Differentiation. 2014;**21**:1451-1459. DOI: 10.1038/cdd.2014.60

[149] Schuler KM, Rambally BS, DiFurio MJ, Sampey BP, Gehrig PA, Makowski L, et al. Antiproliferative and metabolic effects of metformin in a preoperative window clinical trial for endometrial cancer. Cancer Medicine. 2015;**4**:161-173. DOI: 10.1002/cam4.353

[150] Cha JH, Yang WH, Xia W, Wei Y, Chan LC, Lim SO, et al. Metformin promotes antitumor immunity via endoplasmic-reticulum-associated degradation of PD-L1. Molecular Cell. 2018;**71**:606-620 .e7. DOI: 10.1016/j.molcel.2018.07.030

[151] Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, et al. Metformin: A review of its potential indications. Drug Design, Development and Therapy. 2017;**11**:2421-2429. DOI: 10.2147/DDDT.S141675

[152] Campbell JM, Stephenson MD, de Courten B, Chapman I, Bellman SM, Aromataris E. Metformin use associated with reduced risk of dementia in patients with Diabetes: A systematic review and meta-analysis. Journal of Alzheimer's Disease. 2018;**65**:1225-1236. DOI: 10.3233/JAD-180263

[153] Moreira RO, Campos SC, Soldera AL. Type 2 Diabetes mellitus and Alzheimer's disease: From physiopathology to treatment implications. Diabetes/Metabolism Research and Reviews. 2013;**71**:365-376. DOI: 10.1002/dmrr.2442

[154] Gudala K, Bansal D, Schifano F, Bhansali A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. Journal of Diabetes Investigation. 2013;**4**:640-650. DOI: 10.1111/jdi.12087

[155] Xu W, Caracciolo B, Wang HX, Winblad B, Backman L, Qiu C, et al. Accelerated progression from mild cognitive impairment to dementia in people with diabetes. Diabetes. 2010;**59**:2928-2935. DOI: 10.2337/db10-0539

[156] Hsu CC, Wahlqvist ML, Lee MS, Tsai HN. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. Journal of Alzheimer's Disease. 2011;**24**:485-493. DOI: 10.3233/JAD-2011-101524

[157] Shi Q, Liu S, Fonseca VA, Thethi TK, Shi L. Effect of metformin on neurodegenerative disease among elderly adult US veterans with type 2 diabetes mellitus. BMJ Open. 2019;**9**:e024954. DOI: 10.1136/bmjopen-2018-024954

[158] Wahlqvist ML, Lee MS, Hsu CC, Chuang SY, Lee JT, Tsai HN. Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with type 2 diabetes in a Taiwanese population cohort. Parkinsonism & Related Disorders. 2012;**18**:753-758. DOI: 10.1016/j.parkreldis.2012.03.010

[159] Ng TP, Feng L, Yap KB, Lee TS, Tan CH, Winblad B. Long-term metformin usage and cognitive function among older adults with diabetes. Journal of Alzheimer's Disease. 2014;**41**:61-68. DOI: 10.3233/JAD-131901

[160] Imfeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: A population-based case-control study. Journal of the American Geriatrics Society. 2012;**60**:916-921. DOI: 10.1111/j.1532-5415.2012.03916.x

[161] Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H, et al. Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care. 2013;**36**:2981-2987. DOI: 10.2337/dc13-0229

[162] Huang CC, Chung CM, Leu HB, Lin LY, Chiu CC, Hsu CY, et al. Diabetes mellitus and the risk of Alzheimer's disease: A nationwide population-based study. PLoS One. 2014;**9**:e87095. DOI: 10.1371/journal.pone.0087095

[163] Kuhla A, Brichmann E, Ruhlmann C, Thiele R, Meuth L, Vollmar B. Metformin therapy aggravates neurodegenerative processes in ApoE−/− mice. Journal of Alzheimer's Disease. 2019;**68**:1415-1427. DOI: 10.3233/JAD-181017

[164] Curry DW, Stutz B, Andrews ZB, Elsworth JD. Targeting AMPK signaling as a Neuroprotective strategy in Parkinson's disease. Journal of Parkinson's Disease. 2018;**8**:161-181. DOI: 10.3233/JPD-171296

[165] Kickstein E, Krauss S, Thornhill P, Rutschow D, Zeller R, Sharkey J, et al. Biguanide metformin acts on tau phosphorylation via mTOR/protein phosphatase 2A (PP2A) signaling. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:21830-21835. DOI: 10.1073/pnas.0912793107

[166] Paseban M, Mohebbati R, Niazmand S, Sathyapalan T, Sahebkar A. Comparison of the neuroprotective effects of aspirin, atorvastatin, captopril and metformin in diabetes mellitus. Biomolecules. 2019;**9**:1-12. DOI: 10.3390/biom9040118

[167] Alzoubi KH, Khabour OF, Al-Azzam SI, Tashtoush MH, Mhaidat NM. Metformin eased cognitive impairment induced by chronic L-methionine administration: Potential role of oxidative stress. Current Neuropharmacology. 2014;**12**:186-192. DOI: 10.2174/1570159X1166613112022 3201

[168] Jiang T, Yu JT, Zhu XC, Wang HF, Tan MS, Cao L, et al. Acute metformin preconditioning confers neuroprotection against focal cerebral ischaemia by pre-activation of AMPK-dependent autophagy. British Journal of Pharmacology. 2014;**171**:3146-3157. DOI: 10.1111/bph.12655

[169] Venna VR, Li J, Hammond MD, Mancini NS, McCullough LD. Chronic metformin treatment improves post-stroke angiogenesis and recovery after experimental stroke. The European Journal of Neuroscience. 2014;**39**:2129-2138. DOI: 10.1111/ejn.12556

[170] Jin Q, Cheng J, Liu Y, Wu J, Wang X, Wei S, et al. Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. Brain, Behavior, and Immunity. 2014;**40**: 131-142. DOI: 10.1016/j.bbi.2014.03.003