Metformin: from mechanisms of action to advanced clinical use

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Abstract

Metformin represents the-first line treatment and the most widely prescribed anti-hyperglycaemic drug for patients with type 2 diabetes. It can be used as monotherapy or in combination with other oral anti-hyperglycaemic drugs or insulin. Additionally, it is prescribed in type 1 diabetes; it proved to be effective in prediabetes and to provide beneficial effects in other insulin resistant states, such as polycystic ovary syndrome. However, the exact molecular mechanism of its action remains unknown. It was shown that it inhibits liver gluconeogenesis, facilitates glucose uptake into peripheral tissues, such as striated muscle and acts in the gut. In addition to anti-hyperglycaemic effects, metformin was shown to exert several beneficial, protective pleiotropic effects, particularly on the cardiovascular system, and that it is protective against cancer. Metformin has only a few side effects, the most serious one being metformin-associated lactic acidosis. The latter appears in rare clinical cases of pre-existent chronic kidney disease or advanced heart failure with tissue hypo-perfusion, which represent relative contraindications to metformin use. In the past the treatment with metformin was usually discontinued before iodinated contrast-enhanced imaging, but recently there is evidence of its safety even in patients with higher stages of chronic kidney disease. All in all, metformin is the drug with a long tradition and a promising future.

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1 Introduction

Metformin (1.1-dimethyl-biguanide) is the most widely used drug for the treatment of hyperglycaemia in type 2 diabetes. It is prescribed to more than 100 million patients worldwide yearly (1-4). Although it has been in clinical use for more than half a century, the exact mechanisms of its action have not yet been fully explained.

The beginnings of clinical use of metformin date back to 1957, when it was first prescribed to treat diabetes in humans (5); while the effectiveness of guanidine hydrochloride in an animal model was demonstrated by Wattanabe as early as 1918 (6). Despite the fact that metformin had been present on the UK market since 1958, it was approved by the US Food and Drug Administration (*FDA*) as late as 1995 (7). These reservations could be partially attributed to the fact that in 1970 another biguanide, phenformin, was withdrawn from the market because of the high incidence of lactic acidosis and cardiovascular complications (8).

2 The evidence-based options for metformin use

In international and Slovenian national guidelines, metformin is recommended as the first-choice drug to be used after failure of non-pharmacological measures in type 2 diabetes mellitus (1-4). It is also used as an add-on-drug to insulin in patients with type 1 diabetes mellitus (9,10) and as monotherapy in some other insulin-resistant conditions (e.g. polycystic ovary syndrome) (11). Metformin became the gold standard therapy in the treatment of type 2 diabetes mellitus because of its efficiency, its ability to be used in combination with other anti-hyperglycaemic agents, its safety profile, low price and favourable metabolic and cardiovascular effects (12). In the UKPDS multicenter prospective study (*UK Prospective Diabetes Study*)the subset of patients with newly diagnosed diabetes treated with metformin (UKPDS34) had a 32 % reduced risk for all outcomes associated with diabetes, a 42 % reduced risk of diabetes-related death and a 36 % reduction in the total mortality (13). The first results of this research were published in 1998 and after a 10-year observation period, a 21 % reduction in risk for any diabetes-related outcome, a 27 % reduction in overall mortality, and a 33 % lower incidence of myocardial infarction in the subgroup of obese patients treated with metformin (UKPDS80) were recorded (all statistically significant) (14). The results of some of the subsequent meta-analyses of randomized controlled trials, which included a small number of studies, did not support the findings of the UKPDS trial (15). A meta-analysis of more than 30 studies confirmed the reduced mortality in patients treated with metformin compared to those receiving placebo or any other diabetes drug, which was consistent with the results of the UKPDS trial (16).

3 Pharmacokinetics of metformin

Metformin is ingested orally in a daily dose of 500 mg to a maximum of 3000 mg, or 35 mg/kg of body weight per day. Absorption is slow and takes place in the proximal part of the small intestine, i.e. in the duodenum and jejunum. Higher doses of metformin tend to slow down its absorption and reduce its bioavailability. Slow absorption and subsequent accumulation in the digestive tract may lead to adverse effects (17). On the Slovenian market metformin is formulated as immediate release (IR) tablets usually taken 2 to 3 times daily. The advantages of metformin extended release formulation (XR), which will soon be available on the Slovenian market, are similar pharmacokinetic properties with once-daily dosing and a lower incidence of gastrointestinal tract side-effects (18). The use of a new formulation of metformin with delayed release (DR), revealed that most of its anti-hyperglycaemic effects were caused by metformin action in the gastrointestinal tract and were not dependent on its bioavailability. The results of studies using DR metformin promise greater safety, since with lower bioavailability most adverse events and contraindications could be avoided (19).

Metformin is a biguanide that is a strong base with a pKa of 12.4 and exists mainly as a cation in compartments at physiological pH. Metformin is a hydrophilic base and is unable to cross plasma membranes by passive diffusion (20). The membrane transport therefore needs to be facilitated by organic cationic transporters (*OCT).* OCT1 is expressed in the endothelium of the digestive tract, liver and red blood cells, a subset of OCT2 is expressed in kidney cells, and OCT3 is present in skeletal muscle, brain cells, placenta and other cells (17,21). Plasma con-

centrations of metformin normally reach values between 1 and 50 μM, with the highest concentration detected in the portal system and liver (22). Metformin remains mostly non-metabolized and 90 % of the drug is excreted in urine by tubular secretion via OCT2 in the kidneys (17,23). Because of metformin's inability to passively diffuse into intracellular space, therapeutic effectiveness of the drug may depend on the expression and various genetic forms of the membrane transporter OCT (1/2/3) (24,25). Metformin's absorption takes place slower than its excretion, therefore the former represents a rate limiting step in its metabolism. The half-life of metformin in plasma is usually 2 to 6 hours, but it may be extended up to 14 hours due to possible accumulation in the digestive tract and erythrocytes (17).

4 Pharmacodynamics of metformin

Metformin's lowers blood glucose levels by reducing hepatic glucose production (inhibition of gluconeogenesis) (26) and by lowering liver and skeletal muscle insulin resistance. Yet, some of its almost forgotten mechanisms of action that take place in the gastrointestinal tract before absorption into the blood flow should not to be neglected (27).

Metformin mechanisms of action on the molecular level are complex and not yet fully understood; a schematic summary of its action is shown in Figure 1. It is known that metformin acts through the AMP-activated protein kinase route (AMPK pathway) or by mechanism of action independent of AMPK.

4.1 Effects in the liver

4.1.1 AMPK-dependent effects

Upon entering the liver cell metformin inhibits gluconeogenesis primarily through its action in the mitochondria, where it inhibits complex 1 in mitochondrial respiratory chain. This prevents the formation of energy-rich ATP (*adenosine-5'-triphosphate*). As a result, the concentrations of both, ADP (*adenosine diphosphate*) and AMP (*adenosine monophosphate*) increase. Increased levels of ADP and AMP in comparison with ATP lead to a lack of energy needed for energy-consuming enzymatic processes of gluconeogenesis. Increased levels of ADP and AMP in the signaling pathway of metformin have two effects: the activation of (1) AMPK, and (2) liver kinase B1 (LKB1), which regulates AMPK (28).

AMPK is the most important enzyme in the regulation of the cellular energy balance. It is activated by the binding of ADP or AMP molecule on its subunit (γ subunit). LKB1 further increases activation of AMPK through the mechanism of positive feedback (29,30). Activated AMPK inhibits gluconeogenesis through actions targeting several key proteins involved in gluconeogenesis (31). Firstly, it inhibits core protein CRTC2 (*CREB-regulated transcription coactivator 2*), which has a key role in regulating the expression of enzymes of gluconeogenesis. The inhibited CRTC2 exits the cell nucleus. Further, AMPK indirectly stimulates an increase in liver sirtuin 1, which also inhibits CRTC2 and leads to its degradation. The inhibited and dislocated CRTC2 disables expression of enzymes involved in gluconeogenesis, which is further facilitated by AMPK-induced dissociation of the transcription complex CREB (*CAMP response element binding protein*)-CBP (*CREB binding protein*)-CRTC2 (29) (Figure 1).

The activation of cytoplasmic AMPK inhibits the action of acetyl-CoA carboxylase, thereby reducing the production of malonyl-CoA. The latter is an important precursor in the process of

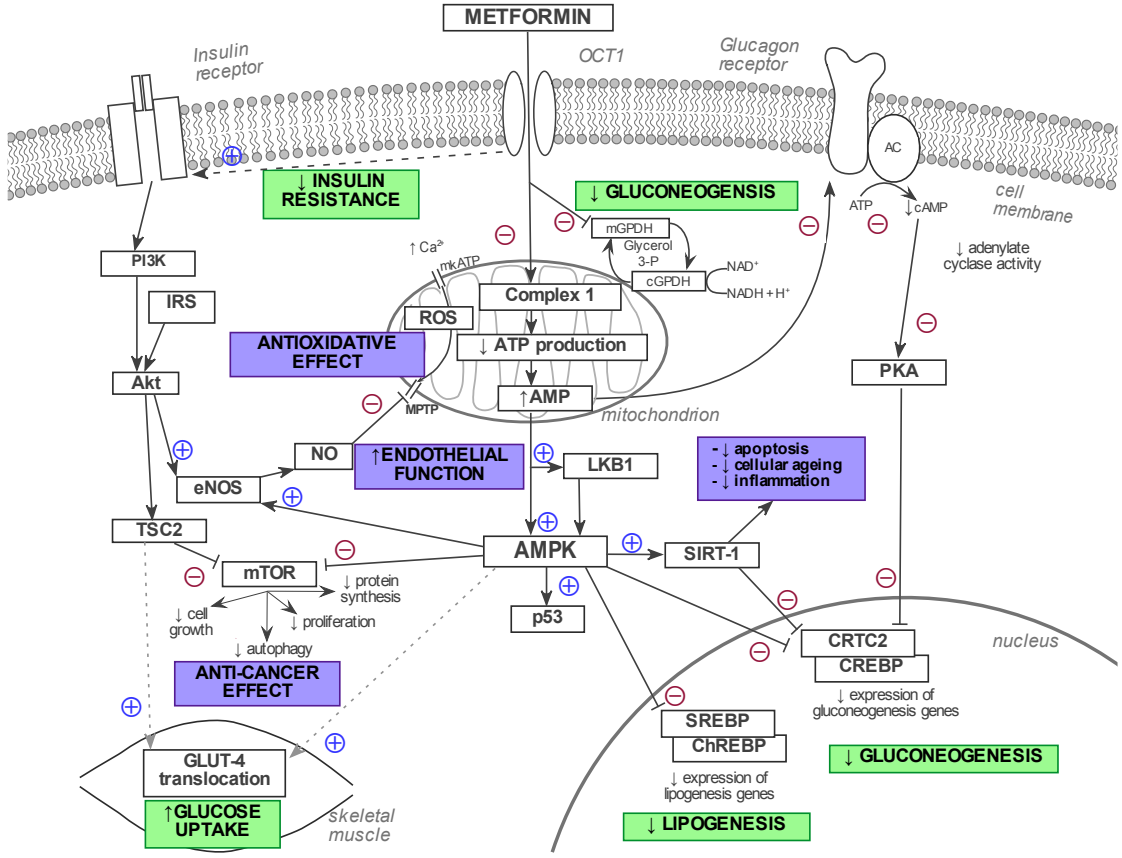

Figure 1: A schematic representation of metformin molecular mechanisms of action. (c)AMP – (cyclic) adenin monophosphate, AC – adenylate cyclase, AKT – protein kinase B, AMPK – 5-adenosine monophosphate-activated protein kinase, ATP – adenosine triphosphate, ChREBP – carbohydrate-responsive element-binding protein, CREB – cAMP response element binding protein, CRTC2 – CREB-regulated transcription coactivator 2, eNOS – endothelial nitric oxide synthase, GLUT 4 – glucose transporter type 4, IRS – insulin receptor substrate, LKB1 - liver kinase B1, m/cGPD(H) – guanosine diphoshate, mPTP – mitochondrial permeability transition pore, mTOR – mechanistic target of rapamycin, NAD(H) – nicotinamide adenine dinucleotide, NO – nitric oxide, OCT 1 – organic cation transporter 1, PI3K – phosphatidylinositol-4.5-bisphosphate 3-kinase, PKA – protein kinase A, ROS – reactive oxygen species, SIRT-1 – sirtuin 1, SREBP – sterol regulatory element-binding protein, TSC2 – tuberous sclerosis complex 2.

lipogenesis, and inhibits beta-oxidation of fatty acids. AMPK further inhibits the expression of several enzymes involved in lipogenesis indirectly by inhibiting the transcriptional activity of SREBP-1 (*sterol regulatory element binding protein-1*) and ChREBP (*carbohydrate-responsive element binding protein*) (29).

4.1.2 AMPK-independent effects

AMPK-independent effects of metformin are generated through several different pathways as follows:

1. Metformin increases the activity of the insulin receptor and its substrate, which increases the uptake of glucose in the liver cell (32).

2. Metformin opposes the effect of glucagon, thereby lowering fasting glycaemia. Indirectly, through increased formation of AMP, which binds directly to adenylate cyclase enzyme and inhibits its function, metformin also inhibits glucagon-mediated formation of cAMP, which inhibits glycogenolysis (33). Treatment with metformin does not give rise to hypoglycaemia which would be expected after the suppression of glucagon effects. It is believed that metformin-mediated inhibition of glucagon in humans is incomplete, or that compensatory mechanisms that prevent hypoglycaemia are simultaneously activated (32).
3. Metformin inhibits mitochondrial glycerophosphate dehydrogenase (*mGDP*), to which increasing importance has been attributed recently. Inhibition of mGDP prevents glycerol from entering into the process of gluconeogenesis, and changes the oxido-reduction state of the cell. This reduces the conversion of lactate into pyruvate and thus the entry of lactate into the gluconeogenesis (34).

4.2 Effects on skeletal muscle

Metformin increases the uptake and utilization of glucose in peripheral tissues (skeletal muscle), thereby reducing insulin resistance. It increases glucose uptake in skeletal muscle via increased translocation of GLUT4 (*glucose transporter type 4*) transporters to the plasma membrane, which may be carried out via two different signaling pathways: (1) via stimulation of signaling pathways of protein kinase C (PKC), also influenced by insulin action (35), and (2) through activation of AMPK pathway (Figure 1) (36).

4.3 Effects in the gastrointestinal tract

In the gastrointestinal tract, metformin slows the absorption of glucose. It also increases the production of intestinal hormones and peptides. It plays a particularly important role in the incretin axis: it increases the action of glucagon-like peptide 1 (GLP-1), which stimulates glucose-dependent insulin secretion and inhibits glucagon secretion (37). Metformin-treated patients showed lower activity of the enzyme dipeptidyl peptidase-4 (DDP-4), which otherwise degrades incretins. (38). Metformin is also assumed to have a favourable effect on the composition of intestinal microbiota (39).

5 The role of metformin in the treatment of hyperglycaemia

5.1 Type 2 diabetes mellitus

Metformin monotherapy as the first-line treatment in patients with type 2 diabetes mellitus is started after the non-pharmacological treatment, including lifestyle change, regular exercise, healthy diet and weight reduction, has failed to achieve appropriate glycaemic control. Metformin is introduced into the therapy if there are no contraindications for its use (as described further on) (4,40). It is usually started at a low dose (500 mg once or twice daily), and gradually increased (individually) over a few weeks to 850–1000 mg 2 to 3 times per day. In a patient with newly diagnosed type 2 diabetes it can be expected that after 2 months of metformin therapy (total dose of 2000 mg daily) the values of glycated haemoglobin (HbA1c) and fasting glucose will decrease on average by 1.4 % and 2.9 mmol/l, respectively (41).

Metformin can be used in combination with all other anti-hyperglycaemic drugs. The drug to be combined with metformin is selected individually for each patient, based on the risk of hypoglycaemia, weight gain and other side effects and potential contraindications. Metformin is most often used in combination with sulfonylureas or with insulin. In the latter case, a 15–25 % reduction in insulin consumption, less weight gain and a lower incidence of hypoglycaemia than in insulin monotherapy can be expected (12). No significant differences in total or cardiovascular mortality were found between patients treated with the combination of metformin and insulin and patients treated with insulin alone. It is important to note that previous studies lasted for too short time period (approx. 6 months on average) to provide sufficient data to prove that the treatment would result in decreased mortality (42).

Metformin can also be used in combination with DDP-4 inhibitors. This combination improves glycaemic control in patients with type 2 diabetes, and is a promising option for providing cardiovascular protection, mainly due to the simultaneous and synchronous protective action of both drugs (43).

5.2 Type 1 diabetes mellitus

Adding metformin to insulin treatment in patients with type 1 diabetes mellitus reduces insulin consumption, however the incidence of hypoglycaemia with combined treatment varies (44,45). According to the meta-analysis by Vella and co-workers a combination of insulin and metformin decreases daily insulin consumption by 5.7–10.1 units/day, HbA1c by 0.6–0.9 % and total cholesterol levels by 0.3–0.41 mmol/l. This meta-analysis suggests that metformin reduces insulin consumption and decreases body weight in patients with type 1 diabetes, but that its effect on glycaemic control is relatively small. There is no evidence yet that the use of metformin in patients with type 1 diabetes improves survival or reduces the incidence of chronic diabetic complications (45). Guidelines of the American Diabetes Association (ADA) and the Canadian Diabetes Association (CDA) suggest an off-label indication for treatment with metformin in overweight or obese patients with type 1 diabetes mellitus (40,46).

5.2 Gestational diabetes

According to the current Slovenian guidelines, insulin represents the first-line therapy for gestational diabetes. Even though previous studies have not confirmed any risk of metformin for pregnant women or foetus, metformin can be prescribed for the treatment of gestational diabetes only in rare cases when treatment with insulin is not possible (4).

Metformin crosses the placenta, but its effects on the foetus have not yet been well studied, thus its use in pregnancy is limited (47). Meta-analyses of observational studies did not provide evidence of increased incidence of foetal malformations or neonatal mortality with the use of metformin in the first trimester of pregnancy. The Metformin in Gestational Diabetes (MiG) study did not show any differences in the incidence of foetal hypoglycaemia, respiratory distress, need for phototherapy, birth injuries, Apgar score value or prematurity rates between the group of pregnant women treated with metformin and the group receiving insulin. Women with gestational diabetes treated with metformin had significantly higher rates of preterm births, but the difference between the groups was not clinically important.

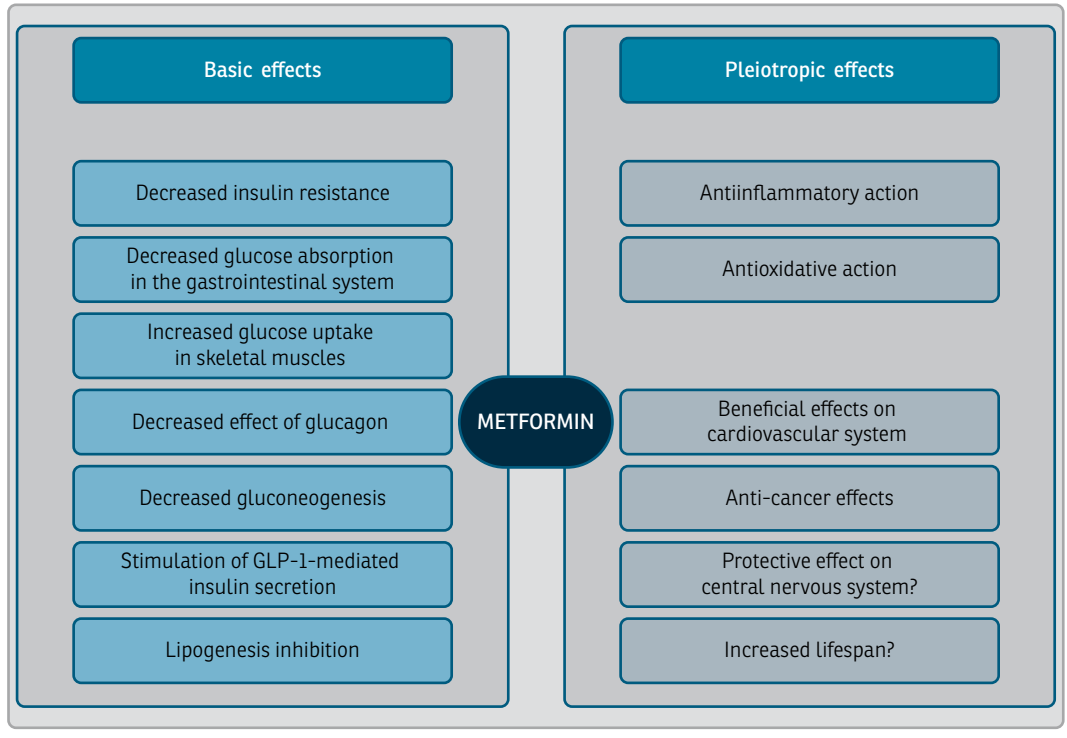

Figure 2: A schematic review of basic anti-hyperglicaemic and additional beneficial (pleiotropic) effects of metformin.

There is a hypothesis that treatment with metformin during pregnancy may have beneficial effects on the metabolic profile of offspring, but larger studies are needed to confirm this assertion (48).

5.4 Conditions associated with increased risk for the development of type 2 diabetes mellitus

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) carry an increased risk for the development of type 2 diabetes mellitus. While IGT is statistically significantly associated with an increased risk for cardiovascular events, this was not confirmed for IFG (49). The most convincing evidence that metformin prevents progression of previously described conditions to type 2 diabetes mellitus was provided by the *Diabetes Prevention Program* (DPP) study, which enrolled 3,234 subjects. Treatment with metformin reduced the incidence of type 2 diabetes mellitus by 31 % compared to placebo; this risk was additionally halved by lifestyle changes. Maximum effect of metformin was seen in the subgroup of individuals under 60 years of age and in those with body mass index over 35 kg/m^2 (50). A slightly lesser effect (risk reduction of 18 %) was seen 10 years after the end of the intervention. Later meta-analyses of randomized trials revealed beneficial effects of metformin on slowing the progression of conditions associated with increased risk for the development of type 2 diabetes mellitus to a clinically overt disease, even when using metformin at low doses (250 mg twice daily) (51).

6 The role of metformin in decreasing insulin resistance

It was shown that metformin decreases insulin resistance in the liver and skeletal muscles. This beneficial effect of

metformin is also important in the treatment of non-diabetic patients.

6.2 Polycystic ovary syndrome

Insulin resistance is present in polycystic ovary syndrome (PCOS). In Slovenia, metformin is used to treat PCOS although it has not yet been officially approved for the treatment of this condition (52). A meta-analysis of 31 clinical trials showed that treatment with metformin in overweight women with PCOS increases the probability of ovulation, improves the regularity of menstrual cycles and reduces the level of serum androgens, but there was no convincing evidence that it increased live birth rates (53). Its effects are due to the decreased influence of insulin excess on the ovarian cells, direct effects of metformin on theca and granulosa ovarian cells, diminished oxidation of free fatty acids and reduced secretion of androgens from ovaries and adrenal glands (11).

7 Pleiotropic effects of metformin

In addition to its basic anti-hyperglycaemic action, metformin has also additional pleiotropic effects, that are not mediated directly through the influence on the glucose metabolism (12,29,54,55). These include benefits for cardiovascular system and other organs, as well as anti-cancer effects and positive effects on non-alcoholic fatty liver disease. Basic and pleiotropic effects of metformin are summarized in Figure 2.

7.1 Mechanisms of pleiotropic effects

Similarly to the basic anti-hyperglycaemic action of metformin, its pleiotropic effects are AMPK-dependent and -independent. Through the above described pathways, metformin activates AMPK, which not only controls or inhibits gluconeogenesis and lipogenesis, but also affects the enzymes and proteins involved in cell cycle and protein metabolism. The AMPK-dependent pathway plays an important role in cell proliferation and differentiation (56). Signalisation via this pathway is important for the basic anti-hyperglicaemic effects of metformin; it causes phosphorylation of endothelial nitric oxide synthase (eNOS), which leads to an increase in nitric oxide (NO) synthesis. Metformin also phosphorylates eNOS independently of AMPK, via increased activity of the insulin receptor and consequent activation of phosphatidylinositol-4.5-bisphosphate 3-kinase/protein kinase B (PI3K/Akt) pathway. The increased NO synthesis prevents the opening of the mitochondrial permeability transition pore (mPTP) on the mitochondrial membrane and the release of reactive oxygen species (ROS) into the cell – anti-oxidative activity (54).

Additionally, metformin inhibits the mechanistic target of rapamycin (mTOR), which is involved in the process of cell growth via two pathways: (1) directly through AMPK, or (2) through the activation of the PI3K/Akt signaling pathway, which is AMPK-independent. The signaling pathway PI3K/Akt activates the tumour suppressor complex of the tuberous sclerosis complex 2 (TSC2), which inhibits the mTOR (Figure 1) (55,56).

7.2 Pleiotropic cardiovascular effects

Since cardiovascular diseases remain the leading cause of morbidity and mortality in patients with diabetes mel-

litus (52 %), the beneficial cardioprotective activity of metformin represents one of its most important pleiotropic effects (29,54). The UKPDS study and some other studies have shown that metformin reduces the risk of cardiovascular disease and total mortality (13,29,57). This is probably not only due to the direct effect of metformin on glycaemia, but also to its beneficial pleiotropic activity.

Pleiotropic effects of metformin on the cardiovascular system are mediated through the following mechanisms:

1. *Improvement of the endothelial function:* metformin improves endothelium-dependent vasorelaxation (58) by increasing eNOS and NO and decreasing sVCAM-1 and E-selectin (59). Metformin also decreases the endothelial and vascular smooth muscle cell death through reduced expression of angiotensin II type 1 receptor gene (60).
2. *Effects on the lipid profile:* metformin has beneficial effects on serum lipid concentration, since it decreases the concentration of free fatty acids, triglycerides, total cholesterol and LDL cholesterol. Some studies also found that metformin could increase HDL cholesterol levels (61).
3. *Effects on haemostats:* metformin inhibits coagulation and stimulates fibrinolysis. In patients with type 2 diabetes metformin decreased the levels of several coagulation factors, such as von Willebrand's factor (vWF) and factor VII. In addition it reduced the levels of plasminogen-activator-inhibitor-1 (PAI-1), which inhibits fibrinolysis (62). Also, metformin directly affects fibrin structure and function by decreasing factor XIII activity and changing fibrin structure (12). Metformin also inhibits thrombocyte aggregation (29).
4. *Anti-inflammatory activity:* metformin may inhibit pro-inflammatory responses through direct inhibition of the nuclear factor κB (NF-κB) pathway (63). Its anti-inflammatory activity may be mediated through sirtuin 1, a protein important in intracellular pathways associated with metabolism, stress response, cell cycle and ageing (12). Metformin acts anti-inflammatory by inhibiting tumour necrosis factor α (TNFα) in human monocytes and by decreasing C-reactive protein (CRP) (64).
5. *Anti-oxidative activity:* metformin decreases the production of reactive oxygen species (ROS) in mitochondria, inhibits the production of advanced glycosylation end products (AGE) in arterial wall (12,56,65), and decreases the concentration of reduced glutathione (12).
6. *Blood pressure decrease:* the influence of metformin on blood pressure has not yet been fully explored. In the BIGPRO1 trial, carried out in overweight subjects, blood pressure decreased in the group treated with metformin. Other studies found no-significant effect of metformin on blood pressure. Metformin probably influences blood pressure through its action on vascular smooth muscle cells (12,66).
7. *Decreased myocardial ischaemic-reperfusion injury:* metformin decreases the extent of myocardial ischaemic-reperfusion injury through activation of protein kinase B (Akt) signaling pathway or through AMPK activation. The latter activates eNOS, which prevents the mPTP pore opening on the mitochondrial membrane, thus acting in a cardioprotective way (29,54).
8. *Effects on the cardiac function*: metformin decreases the incidence of

chronic heart failure (mostly through AMPK activation and myocardial fibrosis inhibition). Therefore, it is suggested that chronic heart failure should not be an absolute contraindication for metformin treatment (see further text) (67).

7.3 Pleiotropic effects on cancer

It was found that patients with type 2 diabetes mellitus may have an increased risk of various types of cancer, particularly of liver, pancreas, endometrium, colon, breast and bladder cancer (12,29,55,68), but a decreased risk for prostate cancer (69). Compared to the general population, patients with diabetes mellitus have increased mortality due to malignant diseases (12). Treatment with metformin may lower mortality due to malignant diseases and decrease cancer incidence (by 25–30 %), this effect being more pronounced with higher metformin dosages (55,68,70). *In vivo* studies showed greater cytotoxicity with the combination of metformin and different cytostatic agents. Radiotherapy was more effective in patients treated with metformin (56).

Increased incidence of malignant diseases in diabetic patients is probably the consequence of increased insulin concentration, increased insulin resistance, hyperglycaemia and increased concentration of IGF-1 (and indirect activation of mTOR via PI3K/Akt pathway) (12,71). The proposed mechanisms of metformin anti-cancer properties are not fully understood. They are mainly mediated through the AMPK-dependent and AMPK-independent pathways (12,29,55,56). Through the AMPK pathway, metformin inhibits the synthesis of fatty acids, cholesterol, mTOR activity, stimulates the tumour suppressor gene p53 activity and decreases the concentration of c-myc oncogene, which is one of the crucial factors for the growth of malignant cells. Independently of AMPK, metformin also inhibits mTOR, chronic inflammation and the formation of oxygen free radicals that would otherwise stimulate the development of malignant cells (29,55). Some studies have shown that the anti-cancer effect of metformin is mediated through microRNAs (miRNA), key regulators of many biological processes, such as cell proliferation, differentiation, apoptosis, stress response and angiogenesis (56). Modulation of miRNAs may play an important role in the above mentioned processes. MiRNAs often behave as tumour suppressors or oncogenes, thereby either promoting or inhibiting the progression of malignant cells. Anti-cancer activity of metformin could be mediated through the stimulation or inhibition of different miRNA subtypes (56,72). The body of available data is large, so we would refer only to the following findings: metformin stimulated the expression of miRNA-33a in breast cancer and therefore inhibited cell proliferation through the inhibition of c-myc oncogene (73); metformin increased the expression of miRNA-192 and miRNA-26a, which resulted in the apoptosis of malignant cells in pancreatic tumour (74); by inhibiting the miRNA-222 expression in lung cancer, metformin increased the activity of p27 and p57 tumour suppressor genes (75). Some evidence exists to support anti-cancer effects of metformin via miRNAs in other types of cancer (56,72), but a detailed description would go beyond the scope of this manuscript.

Several studies have shown that metformin decreases the incidence of various types of cancer (76-80). In patients with type 2 diabetes treatment with metformin reduced the incidence and mortality of colorectal carcinoma (79,81). In female

patients with type 2 diabetes metformin lowered the incidence of breast cancer. In women with breast cancer metformin decreased the incidence of metastases (77,78). In addition, women with breast cancer who received metformin plus neoadjuvant chemotherapy had a higher disease remission rate than women not treated with metformin (82). Metformin extended the survival rate in patients with pancreatic cancer (83). Metformin inhibited the growth of pancreatic cancer cells by its direct influence on fatty acid synthesis (84). It also decreased the progression of renal cell carcinoma (85,86). Treatment with metformin reduced the incidence and progression of prostate cancer, (87) primarily by reducing c-myc protein (88) levels and through IGF-1 reduced the formation of androgens. It may act synergistically with anti-androgen drugs commonly prescribed for the treatment of metastatic prostate carcinoma (56). Despite extensive evidence of metformin effectiveness in diabetic patients with malignant diseases, the use of metformin for this population has not yet been established in clinical practice.

7.4 Pleiotropic effects in non-alcoholic fatty liver disease

Currently, the best treatment options for non-alcoholic fatty liver disease (NAFLD) include weight reduction, regular physical activity and healthy diet (89). The effectiveness of metformin in decreasing the content of liver fat was shown in a mice model with NAFLD, while clinical findings are opposing. One of the possible reasons is the small number of patients included in the research. However, even in the larger TONIC clinical trial, the efficacy of metformin in patients with NAFLD without concomitant diabetes mellitus was not confirmed (90).

7.5 Pleiotropic effects on other organs

Metformin was shown to decrease serum levels of thyrotropin (TSH) (91). It influences redistribution and decrease of body fat (12).

Non-clinical studies on nematodes and rodents have confirmed that metformin extended their life span (92,93). There are no clinical studies that would confirm the direct effect of metformin on ageing or on increasing life span. The UKPDS study showed that treatment with metformin reduced the risk of death from diabetes by 42 %, and decreased total mortality by 36 % (13).

Moreover, in *vitro* studies showed that metformin could increase neurogenesis, improve neuron activity and decrease their degradation (94). Further clinical research in this field is required.

8 Metformin adverse reactions

8.1 Gastrointestinal adverse reactions

The most common adverse effects of metformin occur in the gastrointestinal system, and include flatulence, cramps, diarrhoea, nausea and vomiting. Rarely, it causes a metallic taste in the mouth. Gastrointestinal adverse reactions are usually caused by fast titration of the drug or initial high dose regimens. Usually, these effects disappear with time. They usually disappear with dose reduction or after changing drug formulation to XR or DR option (12).

8.2 Vitamin B12-associated adverse reactions

In addition to causing gastrointestinal adverse reactions, metformin may

diminish vitamin B12 absorption in the gut. Consequently, patients with type 2 diabetes treated with metformin may have diminished levels of vitamin B12. Vitamin B12 levels reduction is dependent on the cumulative metformin dose (95). Clinical significance of these findings is still a matter of debate as only several studies in this field showed association with the development of megaloblastic anaemia (96), cognitive decline and advancement of peripheral diabetic neuropathy (97). Because of the high vitamin B12 availability in the organism, B12 deficiency usually becomes apparent only after several years of metformin use. Monitoring vitamin B12 levels and substituting B12 in case of diminished availability are recommended. Preventive substitution is not recommended at the moment (95).

8.3 Metformin-associated lactic acidosis

Metformin-associated lactic acidosis (MALA) is a rare, but potentially fatal adverse effect associated with metformin treatment. Its incidence is estimated to be around 1/23,000–30,000 patient-years. Interestingly, the incidence of lactic acidosis in diabetic patients treated with other oral anti-hyperglycaemics without metformin, is estimated at 1/18,000–21,000 patient-years, and is significantly higher than with metformin (98).

Metformin-associated lactic acidosis is called high anion gap acidosis with lactate levels > 5 mmol/l and pH ≤ 7.35. Severe acidosis is associated with multiorgan failure, characterized by neurologic signs (stupor, coma, convulsions) and cardiovascular manifestations (hy-

Table 1: Recommended metformin dose adjustments according to the stage of chronic kidney disease (4,104,105).

Chronic kidney disease stage	eGFR (ml/min/1,73 m^2)	Maximum total daily metformin dose (mg)	Other recommendations
1	≥90	3000	
2	60–89	3000	
3a	45–59	2000–3000	• Safe use at full dose, unless worsening of kidney function suspected • Frequent follow-up of kidney function advised
3b	30–44	1000	• Safe use in adjusted dose • Continue already initiated drug use • Cessation of metformin use, if worsening of kidney function suspected • Very frequent follow-up of kidney function recommended
4		metformin contraindicated	
5		metformin contraindicated	

eGFR – estimated glomerular filtration rate

potension, heart rhythm disturbances). Mortality is very high, and is estimated at 30–50 % (99).

Pathophysiologically, there are two types of lactic acidosis. Type A lactic acidosis is due to overproduction of lactate, occurring to restore cellular energy levels (in the form of ATP) under anaerobic conditions (without oxygen). This type of lactic acidosis can be seen in states of circulatory collapse, such as severe heart failure, sepsis, and other states of shock. Type B lactic acidosis, on the other hand, is a consequence of underutilisation of lactate due to its abnormal removal through oxidation and gluconeogenesis under aerobic conditions. Type B lactic acidosis is seen in liver failure, diabetes mellitus, cancer, and intoxications with alcohol or metformin. A combination of both type A and B lactic acidosis also exists (99,100).

The exact mechanism of MALA is not clear yet. It can usually be seen in patients with pre-existent chronic kidney disease, advanced heart failure, or other chronic conditions, that could lead to the lactate acid forming state. In mitochondria, lactate is oxidised into carbon monoxide and water, resulting in cellular energy generation. Additionally, lactate can be metabolised back to glucose through gluconeogenesis in the liver and, to a lower extent, in the kidneys. Metformin inhibits the mitochondrial respiratory chain in the liver and muscles, responsible for lactate removal. Therefore, this inhibition leads to accelerated lactate formation and, on the other hand, diminishes its removal (99,100).

MALA is primarily treated with haemodialysis that allows excess drug removal and acid-base balance regulation (99,100).

9 Risk factors for the development of metformin-associated lactic acidosis and the use of metformin in states predisposing to increased lactic acid production

9.1 Chronic kidney disease

Ninety percent of metformin is excreted unchanged by the kidneys. Therefore in chronic kidney disease (CKD) it can start accumulating in the body, which leads to unacceptable over-therapeutic levels in plasma. This causes increased inhibition of gluconeogenesis and mitochondrial respiratory chain, leading to a higher risk of lactic acidosis. Therefore, CKD stage 3 or higher (eGFR ≤ 60 ml/min/1.73 m^2) represents a relative contraindication for metformin use. Nevertheless, evidence is accumulating that metformin treatment in CKD patients is safe (101). The Cochrane analysis of 347 controlled trials with more than 70,490 patient-years of metformin use reported no MALA or significant increase in lactate levels. In 43 % of those trials, CKD was not a contraindication for metformin treatment (102). Another analysis of the Swedish registry of patients with type 2 diabetes including more than 50,000 patients showed that metformin treatment was safe even in patients with eGFR as low as 30 ml/min/1.73 m^2 (103). Table 1 presents the recommended metformin dose adjustments based on these data (4,104,105).

9.2 Heart failure

In view of the accumulating evidence on a very low incidence of lactic acidosis in patients with both type 2 diabetes

and heart failure treated with metformin (102), FDA dismissed heart failure as an absolute contraindication for metformin treatment (106). At least five randomized trials showed favourable outcome in heart failure in diabetic patients treated with metformin (107-109). Consequently, metformin treatment is recommended in patients with stable heart failure regardless of its stage (NYHA I-IV), as long as their kidney function is stable or they do not have additional risk factors for acute deterioration of heart failure or lactic acidosis (110).

9.3 Stable coronary artery disease and acute coronary syndrome

Treatment with metformin may have beneficial cardiovascular effects in diabetic patients with stable coronary disease, as revealed by several trials (13,29,57,111). Similar findings were reported for diabetic patients with acute coronary syndrome. In the latter, treatment with metformin was associated with better short- and long-term prognosis, as compared to patients treated with other anti-hyperglycaemic drugs. Consequently, treatment with metformin is not contraindicated in diabetic patients with either stable coronary disease or acute coronary syndrome, as long as they are at low risk for cardiogenic shock in case of an acute event (112).

9.4 Liver failure

Advanced decompensated liver cirrhosis and severe liver hypo-perfusion represent absolute contraindications for metformin treatment in diabetic patients, even though there has been no research in this field (112). Yet in 100 patients with type 2 diabetes and liver cirrhosis due to hepatitis C followed up for 5.7 years, a statistically significant reduction in the incidence of hepatocellular carcinoma and mortality from liver disease was found (113). These results suggest that metformin can be used in diabetic patients with liver cirrhosis. Additionally, treatment with metformin is possible in patients with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis, although no significant reduction of liver fat content can be expected in these patient groups (112).

9.5 Chronic respiratory failure

Advanced respiratory failure may lead to hypoxic states and consequent accelerated glycolysis and lactic acid formation. In this field, there have been no trials that would identify the risk of MALA development in patients with diabetes. Consequently, metformin treatment is contraindicated in patients with diabetes mellitus and advanced chronic respiratory failure (112). Metformin treatment is not started in this patient population, but patients already on chronic metformin therapy can continue receiving this medication (110).

9.6 Elderly patients

The use of metformin in diabetic patients of advanced age has proved effective and well tolerated by the patients. Moreover, elderly patients treated with metformin had no hypoglycaemic episodes. Yet caution with the use of metformin is advised in frail diabetic patients with low body/muscle mass or with other relative contraindications for treatment with this drug (114).

10 Use of metformin in patients undergoing intravascular iodinated contrast –enhanced imaging investigations

Exposure of patients to intravascular iodinated contrast agents used for imaging investigations may lead to acute kidney injury, i.e. the so-called contrast-induced nephropathy. Its incidence is dependent of the stage of the pre-existent CKD and the amount of the iodinated contrast used. Also, the latter can accumulate in case of acute kidney injury occurring in patients treated with metformin. Since MALA is a relatively rare phenomenon, the previously strict guidelines on interruption of metformin therapy before intravascular administration of iodinated contrast agent were softened. The European guidelines issued by the European Society of Urogenital Radiology in 2013 recommend that in metformin-treated diabetic patients with CKD the following protocol should be followed before performing intravascular iodinated contrast-enhanced imaging (115):

- Patients with eGFR ≥ 60 ml/min/1.73 m^2 (CKD stages 1 and 2) can continue taking metformin, i.e. stopping the metformin treatment before and after the investigation is not necessary. In patients with eGFR 30–59 ml/min/1.73 m^2 (CKD stage 3), decision on metformin use is based on the following: (a) with intravenous administration of iodinated contrast and if the patients have eGFR ≥ 45 ml/min/1.73 m^2, metformin treatment discontinuation is not necessary; (b) with intra-arterial administration of iodinated contrast or if the patients have eGFR 30–44 ml/min/1.73 m^2, metformin is stopped 48 hours before the investigation. In these patients, metformin should be restarted at least 48 hours after the investigation, if the kidney function remains stable.
- In patients with eGFR ≤ 30 ml/min/1.73 m^2 (CKD stages 4 and 5) or with a disease that may lead to acute liver failure or a hypoxic state, metformin treatment is contraindicated. In emergency situations, metformin treatment is discontinued before intravascular iodinated contrast administration; after the investigation, the patient is monitored for possible MALA development. In case of stable kidney function and absence of MALA, metformin can be reintroduced 48 hours after the investigation.

With intravascular use of gadolinium contrast agent metformin discontinuation is not necessary at any CKD stage (115).

In addition to the above described guidelines, all additional measures used in the prevention of contrast-induced nephropathy should be followed, in particular good hydration of patients with CKD.

11 Conclusion

Metformin has been on the market for almost 60 years, and thanks to its efficacy and safety it remains the gold standard and the first-choice therapy for patients with type 2 diabetes mellitus. There is a plethora of evidence for its anti-hyperglycaemic action and its beneficial effects in other diabetes mellitus-associated and insulin-resistant states. Metformin provides additional, beneficial pleiotropic effects, the best described being cardioprotective and anti-cancer effects. Additionally, it shows promise in treating degenerative diseases of the central nervous system and, perhaps, has a role in slowing the ageing. Metformin

has a relatively low incidence of adverse reactions, the most common being gastrointestinal side effects. A relatively rare, but most dangerous complication is the development of metformin-associated lactic acidosis associated with pre-existent chronic kidney disease or advanced heart failure with tissue hypo-perfusion. Recently, there has been evidence that metformin can safely be used in patients undergoing intravascular iodinated contrast-enhanced imaging studies even in those with advanced chronic kidney disease. Thanks to the above described anti-hyperglycaemic and other beneficial effects, along with its proven efficacy and safety, metformin has a bright future.

References

1. Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, et al. Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Comprehensive Type 2 Diabetes Management Algorithm–2016 Executive Summary. Endocr Pract. 2016;22(1):84–113.
2. International Diabetes Federation Guideline Development G. Global guideline for type 2 diabetes. Diabetes Res Clin Pract. 2014;104(1):1–52.
3. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015;38(1):140–9.
4. Zaletel J, Ravnik-Oblak M, Zavratnik A, eds. Slovenske smernice za klinično obravnavo sladkorne bolezni tipa 2. 3 ed. Ljubljana: Diabetološko združenje Slovenije; 2016.
5. Sterne J. Du nouveau dans les antidiabetiques. La NN dimethylamin guanyl guanide. Maroc Med. 1957;36(388):1295–6.
6. Watanabe CK. Studies in the metabolic changes by administration of guanidine hydrochloride on blood sugar content. J Biol Chem. 1918;33(2):253–65.
7. Bailey CJ, Day C. Metformin: its botanical background. Practical Diabetes Int. 2004;21(3):115–7.
8. Misbin RI. The phantom of lactic acidosis due to metformin in patients with diabetes. Diabetes Care. 2004;27(7):1791–3.
9. Liu C, Wu D, Zheng X, Li P, Li L. Efficacy and safety of metformin for patients with type 1 diabetes mellitus: a meta-analysis. Diabetes Technol Ther. 2015;17(2):142–8.
10. Ludvigsson J. The latest pharmacotherapy options for type 1 diabetes. Expert Opin Pharmacother. 2014;15(1):37–49.
11. Palomba S, Falbo A, Zullo F, Orio F, Jr. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 2009;30(1):1–50.
12. Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr. 2013;5(1):6.
13. 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(9131):854–65.
14. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med. 2008;359(15):1577–89.
15. 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 Med. 2012;9(4):e1001204.
16. Lamanna C, Monami M, Marchionni N, Mannucci E. Effect of metformin on cardiovascular events and mortality: a meta-analysis of randomized clinical trials. Diabetes Obes Metab. 2011;13(3):221–8.
17. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 1996;30(5):359–71.
18. Ali S, Fonseca V. Overview of metformin: special focus on metformin extended release. Expert Opin Pharmacother. 2012;13(12):1797–805.
19. Buse JB, DeFronzo RA, Rosenstock J, Kim T, Burns C, Skare S, et al. The Primary Glucose-Lowering Effect of Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic and 12-Week Dose-Ranging Studies. Diabetes Care. 2016;39(2):198–205.
20. Detaille D, Guigas B, Leverve X, Wiernsperger N, Devos P. Obligatory role of membrane events in the regulatory effect of metformin on the respiratory chain function. Biochem Pharmacol. 2002;63(7):1259–72.
21. Dresser MJ, Leabman MK, Giacomini KM. Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. J Pharm Sci. 2001 90(4):397–421.
22. Wilcock C, Bailey CJ. Accumulation of metformin by tissues of the normal and diabetic mouse. Xenobiotica. 1994;24(1):49–57.
23. Hacker K, Maas R, Kornhuber J, Fromm MF, Zolk O. Substrate-Dependent Inhibition of the Human Organic Cation Transporter OCT2: A Comparison of Metformin with Experimental Substrates. PLoS One. 2015;10(9):e0136451.

24. Dujic T, Causevic A, Bego T, Malenica M, Velija-Asimi Z, Pearson ER, et al. Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with Type 2 diabetes. Diabet Med. 2015;33(4):511–4.
25. Ornik V, Ferk P. Raznolikost v farmakološkem odzivu na zdravljenje z metforminom. Zdrav Vestn. 2013;82(7):487–96.
26. Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, et al. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes. 2000;49(12):2063–9.
27. Song R. Mechanism of Metformin: A Tale of Two Sites. Diabetes Care. 2016;39(2):187–9.
28. Hardie DG. Neither LKB1 nor AMPK are the direct targets of metformin. Gastroenterology. 2006;131(3):973; author reply 4–5.
29. Pryor R, Cabreiro F. Repurposing metformin: an old drug with new tricks in its binding pockets. Biochem J. 2015;471(3):307–22.
30. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012;13(4):251–62.
31. Foretz M, Viollet B. Regulation of hepatic metabolism by AMPK. J Hepatol. 2011;54(4):827–9.
32. Pernicova I, Korbonits M. Metformin—mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014;10(3):143–56.
33. Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ. Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature. 2013,494(7436):256–60.
34. Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature. 2014;510(7506):542–6.
35. Turban S, Stretton C, Drouin O, Green CJ, Watson ML, Gray A, et al. Defining the contribution of AMP-activated protein kinase (AMPK) and protein kinase C (PKC) in regulation of glucose uptake by metformin in skeletal muscle cells. J Biol Chem. 2012;287(24):20088–99.
36. Kristensen JM, Treebak JT, Schjerling P, Goodyear L, Wojtaszewski JF. Two weeks of metformin treatment induces AMPK-dependent enhancement of insulin-stimulated glucose uptake in mouse soleus muscle. Am J Physiol Endocrinol Metab. 2014;306(10):E1099–109.
37. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. Endocrinology. 2011;152(12):4610–9.
38. Lindsay JR, Duffy NA, McKillop AM, Ardill J, O'Harte FP, Flatt PR, et al. Inhibition of dipeptidyl peptidase IV activity by oral metformin in Type 2 diabetes. Diabet Med. 2005;22(5):654–7.
39. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. Appl Environ Microbiol. 2014;80(19):5935–43.
40. Professional Practice Committee for the Standards of Medical Care in Diabetes-2016. Diabetes Care. 2016;39 Suppl 1:S107–8.
41. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N Engl J Med. 1995;333(9):541–9.
42. Hemmingsen B, Christensen LL, Wetterslev J, Vaag A, Gluud C, Lund SS, et al. Comparison of metformin and insulin versus insulin alone for type 2 diabetes: systematic review of randomised clinical trials with meta-analyses and trial sequential analyses. BMJ. 2012;344:e1771.
43. Goldstein BJ, Feinglos MN, Lunceford JK, Johnson J, Williams-Herman DE, Sitagliptin 036 Study G. Effect of initial combination therapy with sitagliptin, a dipeptidyl peptidase-4 inhibitor, and metformin on glycemic control in patients with type 2 diabetes. Diabetes Care. 2007;30(8):1979–87.
44. Nwosu BU, Maranda L, Cullen K, Greenman L, Fleshman J, McShea N, et al. A Randomized, Double-Blind, Placebo-Controlled Trial of Adjunctive Metformin Therapy in Overweight/Obese Youth with Type 1 Diabetes. PLoS One. 2015;10(9):e0137525.
45. Vella S, Buetow L, Royle P, Livingstone S, Colhoun HM, Petrie JR. The use of metformin in type 1 diabetes: a systematic review of efficacy. Diabetologia. 2010;53(5):809–20.
46. Canadian Diabetes Association Clinical Practice Guidelines Expert C, McGibbon A, Richardson C, Hernandez C, Dornan J. Pharmacotherapy in type 1 diabetes. Can J Diabetes. 2013;37 Suppl 1:S56–60.
47. Charles B, Norris R, Xiao X, Hague W. Population pharmacokinetics of metformin in late pregnancy. Ther Drug Monit. 2006;28(1):67–72.
48. Rowan JA, Rush EC, Obolonkin V, Battin M, Wouldes T, Hague WM. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition at 2 years of age. Diabetes Care. 2011;34(10):2279–84.
49. Petersen JL, McGuire DK. Impaired glucose tolerance and impaired fasting glucose—a review of diagnosis, clinical implications and management. Diab Vasc Dis Res. 2005;2(1):9–15.
50. 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. N Engl J Med. 2002;346(6):393–403.
51. Lily M, Godwin M. Treating prediabetes with metformin: systematic review and meta-analysis. Can Fam Physician. 2009;55(4):363–9.
52. Košir R, Meden-Vrtovec H. Vloga metformina pri obravnavi bolnic s sindromom policističnih jajčnikov. Zdrav Vestn. 2009;78(3):137–41.
53. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. 2010;(1):CD003053.

54. Bromage DI, Yellon DM. The pleiotropic effects of metformin: time for prospective studies. Cardiovasc Diabetol. 2015;14:109.
55. Kasznicki J, Sliwinska A, Drzewoski J. Metformin in cancer prevention and therapy. Ann Transl Med. 2014;2(6):57.
56. Pulito C, Donzelli S, Muti P, Puzzo L, Strano S, Blandino G. microRNAs and cancer metabolism reprogramming: the paradigm of metformin. Ann Transl Med. 2014;2(6):58.
57. Gosmanova EO, Canada RB, Mangold TA, Rawls WN, Wall BM. Effect of metformin-containing antidiabetic regimens on all-cause mortality in veterans with type 2 diabetes mellitus. Am J Med Sci. 2008;336(3):241–7.
58. Romualdi D, Costantini B, Selvaggi L, Giuliani M, Cristello F, Macri F, et al. Metformin improves endothelial function in normoinsulinemic PCOS patients: a new prospective. Hum Reprod. 2008;23(9):2127–33.
59. de Jager J, Kooy A, Schalkwijk C, van der Kolk J, Lehert P, Bets D, et al. Long-term effects of metformin on endothelial function in type 2 diabetes: a randomized controlled trial. J Intern Med. 2014;275(1):59–70.
60. Forouzandeh F, Salazar G, Patrushev N, Xiong S, Hilenski L, Fei B, et al. Metformin beyond diabetes: pleiotropic benefits of metformin in attenuation of atherosclerosis. J Am Heart Assoc. 2014;3(6):e001202.
61. Wulffele MG, Kooy A, de Zeeuw D, Stehouwer CD, Gansevoort RT. The effect of metformin on blood pressure, plasma cholesterol and triglycerides in type 2 diabetes mellitus: a systematic review. J Intern Med. 2004;256(1):1–14.
62. Grant PJ. Beneficial effects of metformin on haemostasis and vascular function in man. Diabetes Metab. 2003;29(4 Pt 2):6S44–52.
63. Isoda K, Young JL, Zirlik A, MacFarlane LA, Tsuboi N, Gerdes N, et al. Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. Arterioscler Thromb Vasc Biol. 2006;26(3):611–7.
64. Carter AM, Bennett CE, Bostock JA, Grant PJ. Metformin reduces C-reactive protein but not complement factor C3 in overweight patients with Type 2 diabetes mellitus. Diabet Med. 2005;22(9):1282–4.
65. Beisswenger P, Ruggiero-Lopez D. Metformin inhibition of glycation processes. Diabetes Metab. 2003;29(4 Pt 2):6S95–103.
66. Fontbonne A, Diouf I, Baccara-Dinet M, Eschwege E, Charles MA. Effects of 1-year treatment with metformin on metabolic and cardiovascular risk factors in non-diabetic upper-body obese subjects with mild glucose anomalies: a post-hoc analysis of the BIGPRO1 trial. Diabetes Metab. 2009;35(5):385–91.
67. Papanas N, Maltezos E, Mikhailidis DP. Metformin and heart failure: never say never again. Expert Opin Pharmacother. 2012;13(1):1–8.
68. Inoue M, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. Arch Intern Med. 2006;166(17):1871–7.
69. Dankner R, Boffetta P, Balicer RD, Boker LK, Sadeh M, Berlin A, et al. Time-Dependent Risk of Cancer After a Diabetes Diagnosis in a Cohort of 2.3 Million Adults. Am J Epidemiol. 2016;183(12):1098–106.
70. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330(7503):1304–5.
71. Smith U, Gale EA. Cancer and diabetes: are we ready for prime time? Diabetologia. 2010;53(8):1541–4.
72. Bao B, Azmi AS, Ali S, Zaiem F, Sarkar FH. Metformin may function as anti-cancer agent via targeting cancer stem cells: the potential biological significance of tumor-associated miRNAs in breast and pancreatic cancers. Ann Transl Med. 2014;2(6):59.
73. Cioce M, Valerio M, Casadei L, Pulito C, Sacconi A, Mori F, et al. Metformin-induced metabolic reprogramming of chemoresistant ALDHbright breast cancer cells. Oncotarget. 2014;5(12):4129–43.
74. Li W, Yuan Y, Huang L, Qiao M, Zhang Y. Metformin alters the expression profiles of microRNAs in human pancreatic cancer cells. Diabetes Res Clin Pract. 2012;96(2):187–95.
75. Wang Y, Dai W, Chu X, Yang B, Zhao M, Sun Y. Metformin inhibits lung cancer cells proliferation through repressing microRNA-222. Biotechnol Lett. 2013;35(12):2013–9.
76. Amin S, Mhango G, Lin J, Aronson A, Wisnivesky J, Boffetta P, et al. Metformin Improves Survival in Patients with Pancreatic Ductal Adenocarcinoma and Pre-Existing Diabetes: A Propensity Score Analysis. Am J Gastroenterol. 2016;111(9):1350–7.
77. Chlebowski RT, McTiernan A, Aragaki AK, Rohan T, Wactawski-Wende J, Ipp E, et al. Metformin and breast cancer incidence in postmenopausal diabetic women in the Women's Health Initiative (WHI). J Clin Oncol. 2011;29(12):1503–4.
78. Jacob L, Kostev K, Rathmann W, Kalder M. Impact of metformin on metastases in patients with breast cancer and type 2 diabetes. J Diabetes Complications. 2016;30(6):1056–9.
79. Liu F, Yan L, Wang Z, Lu Y, Chu Y, Li X, et al. Metformin therapy and risk of colorectal adenomas and colorectal cancer in type 2 diabetes mellitus patients: A systematic review and meta-analysis. Oncotarget. 2016;8(9):16017–16026.
80. Rosato V, Tavani A, Gracia-Lavedan E, Guino E, Castano-Vinyals G, Villanueva CM, et al. Type 2 Diabetes, Antidiabetic Medications, and Colorectal Cancer Risk: Two Case-Control Studies from Italy and Spain. Front Oncol. 2016;6:210.
81. Lee JH, Kim TI, Jeon SM, Hong SP, Cheon JH, Kim WH. The effects of metformin on the survival of colorectal cancer patients with diabetes mellitus. Int J Cancer. 2012;131(3):752–9.
82. Xu H, Chen K, Jia X, Tian Y, Dai Y, Li D, et al. Metformin Use Is Associated With Better Survival of Breast Cancer Patients With Diabetes: A Meta-Analysis. Oncologist. 2015;20(11):1236–44.
83. Lee SH, Yoon SH, Lee HS, Chung MJ, Park JY, Park SW, et al. Can metformin change the prognosis of pancreatic cancer? Retrospective study for pan-

creatic cancer patients with pre-existing diabetes mellitus type 2. Dig Liver Dis. 2016;48(4):435–40.
84. Cantoria MJ, Boros LG, Meuillet EJ. Contextual inhibition of fatty acid synthesis by metformin involves glucose-derived acetyl-CoA and cholesterol in pancreatic tumor cells. Metabolomics. 2014;10(1):91–104.
85. Hamieh L, McKay RR, Lin X, Moreira RB, Simantov R, Choueiri TK. Effect of Metformin Use on Survival Outcomes in Patients With Metastatic Renal Cell Carcinoma. Clin Genitourin Cancer. 2016. In press.
86. Tseng CH. Use of metformin and risk of kidney cancer in patients with type 2 diabetes. Eur J Cancer. 2016;52:19–25.
87. Hankinson SJ, Fam M, Patel NN. A review for clinicians: Prostate cancer and the antineoplastic properties of metformin. Urol Oncol. 2017;35(1):21–9.
88. Akinyeke T, Matsumura S, Wang X, Wu Y, Schalfer ED, Saxena A, et al. Metformin targets c-MYC oncogene to prevent prostate cancer. Carcinogenesis. 2013;34(12):2823–32.
89. Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics. 2006;118(4):1388–93.
90. Lavine JE, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA. 2011;305(16):1659–68.
91. Cappelli C, Rotondi M, Pirola I, Agosti B, Formenti A, Zarra E, et al. Thyreotropin levels in diabetic patients on metformin treatment. Eur J Endocrinol. 2012;167(2):261–5.
92. 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(2):148–57.
93. Cabreiro F, Au C, Leung KY, Vergara-Irigaray N, Cocheme HM, Noori T, et al. Metformin retards aging in C. elegans by altering microbial folate and methionine metabolism. Cell. 2013;153(1):228–39.
94. Chung MM, Chen YL, Pei D, Cheng YC, Sun B, Nicol CJ, et al. The neuroprotective role of metformin in advanced glycation end product treated human neural stem cells is AMPK-dependent. Biochim Biophys Acta. 2015;1852(5):720–31.
95. Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. PLoS One. 2014;9(6):e100379.
96. Buvat DR. Use of metformin is a cause of vitamin B12 deficiency. Am Fam Physician. 2004;69(2):264; author reply 264, 266.
97. 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(10):2981–7.
98. Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. Metformin, sulfonylureas, or other antidiabetes drugs and the risk of lactic acidosis or hypoglycemia: a nested case-control analysis. Diabetes Care. 2008;31(11):2086–91.
99. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. Metabolism. 2016;65(2):20–9.
100. Kraut JA, Madias NE. Lactic acidosis. N Engl J Med. 2014;371(24):2309–19.
101. Miles JM, Rule AD, Borlaug BA. Use of metformin in diseases of aging. Curr Diab Rep. 2014;14(6):490.
102. Salpeter SR, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev. 2010;(4):CD002967.
103. Ekstrom N, Schioler L, Svensson AM, Eeg-Olofsson K, Miao Jonasson J, Zethelius B, et al. Effectiveness and safety of metformin in 51 675 patients with type 2 diabetes and different levels of renal function: a cohort study from the Swedish National Diabetes Register. BMJ Open. 2012;2(4).
104. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2012;35(6):1364–79.
105. Inzucchi SE, Lipska KJ, Mayo H, Bailey CJ, McGuire DK. Metformin in patients with type 2 diabetes and kidney disease: a systematic review. JAMA. 2014;312(24):2668–75.
106. Inzucchi SE, Masoudi FA, McGuire DK. Metformin in heart failure. Diabetes Care. 2007;30(12):e129.
107. Eurich DT, Majumdar SR, McAlister FA, Tsuyuki RT, Johnson JA. Improved clinical outcomes associated with metformin in patients with diabetes and heart failure. Diabetes Care. 2005;28(10):2345–51.
108. Pantalone KM, Kattan MW, Yu C, Wells BJ, Arrigain S, Jain A, et al. The risk of developing coronary artery disease or congestive heart failure, and overall mortality, in type 2 diabetic patients receiving rosiglitazone, pioglitazone, metformin, or sulfonylureas: a retrospective analysis. Acta Diabetol. 2009;46(2):145–54.
109. Roussel R, Travert F, Pasquet B, Wilson PW, Smith SC, Jr, Goto S, et al. Metformin use and mortality among patients with diabetes and atherothrombosis. Arch Intern Med. 2010;170(21):1892–9.
110. Goldberg T, Kroehl ME, Suddarth KH, Trinkley KE. Variations in Metformin Prescribing for Type 2 Diabetes. J Am Board Fam Med. 2015;28(6):777–84.
111. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with sulfonylurea monotherapy in type 2 diabetes. Diabetes Care. 2002;25(12):2244–8.
112. Scheen AJ, Paquot N. Metformin revisited: a critical review of the benefit-risk balance in at-risk patients with type 2 diabetes. Diabetes Metab. 2013;39(3):179–90.
113. Popov VB, Lim JK. Impact of insulin-sensitizing agents on risk for liver cancer and liver-related death in diabetic patients with compensated hepatitis C cirrhosis. J Clin Endocrinol Metab. 2011;96(8):2398–400.

114. Neumiller JJ, Setter SM. Pharmacologic management of the older patient with type 2 diabetes mellitus. Am J Geriatr Pharmacother. 2009;7(6):324–42.

115. Thomsen H, Webb J, Contrast Media. Safety Issues and ESUR Guidelines. Heidelberg: Springer; 014.