Front. Med. 2015, 9(2): 173–186
DOI 10.1007/s11684-015-0384-0

REVIEW

Metformin and metabolic diseases: a focus on hepatic aspects

Juan Zheng (✉)[1,2,a], Shih-Lung Woo[1], Xiang Hu[1,2], Rachel Botchlett[1], Lulu Chen[2], Yuqing Huo[3], Chaodong Wu (✉)[1,b]

[1]*Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843, USA;* [2]*Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China;* [3]*Drug Discovery Center, Key Laboratory of Chemical Genomics, Peking University Shenzhen Graduate School, Shenzhen 518055, China*

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Abstract **Metformin has been widely used as a first-line anti-diabetic medicine for the treatment of type 2 diabetes (T2D). As a drug that primarily targets the liver, metformin suppresses hepatic glucose production (HGP), serving as the main mechanism by which metformin improves hyperglycemia of T2D. Biochemically, metformin suppresses gluconeogenesis and stimulates glycolysis. Metformin also inhibits glycogenolysis, which is a pathway that critically contributes to elevated HGP. While generating beneficial effects on hyperglycemia, metformin also improves insulin resistance and corrects dyslipidemia in patients with T2D. These beneficial effects of metformin implicate a role for metformin in managing non-alcoholic fatty liver disease. As supported by the results from both human and animal studies, metformin improves hepatic steatosis and suppresses liver inflammation. Mechanistically, the beneficial effects of metformin on hepatic aspects are mediated through both adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent pathways. In addition, metformin is generally safe and may also benefit patients with other chronic liver diseases.**

Keywords metformin; diabetes; hepatic steatosis; inflammatory response; insulin resistance

Introduction

Metformin is the most widely used first-line therapy for type 2 diabetes (T2D), and has numerous effects on human metabolism such as improvements in endothelial dysfunction, hemostasis and oxidative stress, insulin resistance, lipid profiles, and fat redistribution [1]. Recent advances reveal that metformin, in addition to its glucose-lowering action, is promising for specifically targeting metabolic differences between normal and abnormal metabolic signaling. Due to its insulin-sensitizing effect, metformin also is used for insulin resistance-related diseases such as non-alcoholic fatty liver disease (NAFLD) [2,3] and polycystic ovary syndrome (PCOS) [4].

Metformin exerts its metabolism-regulatory effects primarily on the liver, which plays a central role in controlling carbohydrate, lipid, and protein metabolism. Organic cation transporters (OCTs) of the SLC22 family play a pivotal role in the distribution and clearance of metformin. In support of this, OCTs mediate the intestinal absorption, hepatic uptake, and renal excretion of metformin [5]. Three OCT isoforms have been identified, and the expression of OCT1 and OCT2 is highly restricted to the liver and kidney, respectively; whereas OCT3 is more widely distributed [6]. The hepatic uptake of metformin is primarily mediated by OCT1(SLC22A1) and OCT3(SLC22A3), which are expressed on the basolateral membrane of hepatocytes [7]. Metformin has a preferential distribution in hepatocytes because of the high cellular uptake via the liver-enriched OCT1 [8]. In terms of improving hyperglycemia, metformin acts primarily through decreasing the expression of hepatic gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), thereby reducing hepatic glucose production (HGP). The molecular mechanisms underlying metformin actions appear to be complex and remain a topic of much debate. However, there is a general agreement that metformin administration results in activation of adenosine monophosphate-activated protein kinase (AMPK) in the liver, which in turn likely leads to a number of the pharmacologic effects of metformin including improvement of glucose and lipid metabolism [9]. Additionally, increasing

Received July 15, 2014; accepted October 24, 2014
Correspondence: [a]zhengjuan25@tamu.edu; [b]cdwu@tamu.edu

evidence suggests that metformin also acts via AMPK-independent mechanisms.

In this review, we focus on the hepatic aspects to describe the mechanisms of action (MOA) underlying metformin therapy for T2D and NAFLD. Furthermore, we compare metformin with other anti-diabetic agents and insulin sensitizers. Lastly, we summarize the few known side effects associated with metformin application. The knowledge gained from dissecting the principal mechanisms by which metformin generates beneficial effects can provide new inspiration for the prevention and/or cure of diabetes mellitus.

Metformin and diabetes

T2D is a major health problem associated with excess mortality and morbidity. Vascular complications are one of the most serious consequences of this disease. It has been shown that tight glycemic control contributes to reduction of the incidence of diabetes-associated complications. For this purpose, metformin is the first-line oral anti-diabetic drug for T2D recommended by international organizations with proven efficacy and cost-effectiveness [10–12]. This recommendation is based on the results of the UK Prospective Diabetes Study (UKPDS), a landmark clinical study, and several other clinical trials. The UKPDS reported that intensive glucose control with metformin appears to decrease the risk of diabetes-related endpoints and death in overweight diabetic patients, and is associated with less weight gain and fewer hypoglycemic attacks when compared with insulin and sulphonylureas [13]. Since then, much evidence demonstrates that metformin produces beneficial effects on glucose and lipid metabolism, and exhibits an excellent therapeutic index and a good safety profile with long-term treatment. In addition, metformin is generally considered weight-neutral with long-term use and does not increase the risk of hypoglycemia. Treatment with metformin limits myocardial infarction (MI) size in rodents [14,15], and also shows modest benefits on the risk of MI in humans [10]. This section documents the different MOA of metformin for the treatment of T2D. The main mechanisms underlying the anti-diabetic actions of metformin also are summarized in Fig. 1.

Metabolic reprogramming

Increased HGP is a major cause of hyperglycemia in T2D. In contrast, reducing HGP accounts for, at least in part, the effects of anti-diabetic agents on lowering blood glucose levels. Metformin decreases HGP primarily through inhibiting gluconeogenesis [16]. Depending on nutritional status, metformin also has been shown to improve

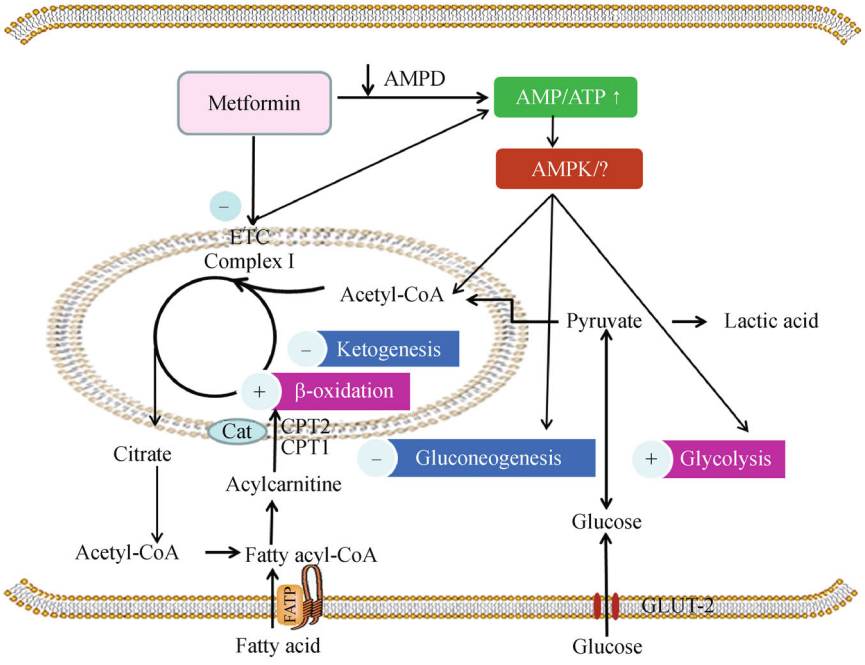

Fig. 1 MOA: metformin for type 2 diabetes. Metformin targets hepatocytes and acts through both AMPK-dependent and AMPK-independent pathways to suppress hepatic glucose production (HGP), thereby improving hyperglycemia of type 2 diabetes. Metformin also inhibits hepatic lipogensis and stimulates liver fatty acid oxidation, thereby correcting dyslipidemia and improving insulin resistance. See text for details.

hyperglycemia by decreasing hepatic glycogenolysis in the fasted states [17] and by increasing both glycolysis and glycogenesis in the fed state [18]. As additional evidence, the results from microarray analyses of global gene expression in the livers of obese diabetic *db/db* mice that were administered with a single dose of metformin (400 mg/kg) for 2 h show that metformin significantly alters the expression of genes involved in both glycolysis and gluconeogenesis [19]. A mechanistic study further indicates roles for metformin in increasing ser-436 phosphorylation of CREB binding protein (CBP) and in disrupting the formation of a complex among CBP, CREB, and the target of rapamycin-C2 (TORC2). This appears to account for the effect of metformin on suppressing the expression of gluconeogenic enzymes such as PEPCK and G6Pase via decreasing PPARγ-coactivator-1-α (PGC-1α) activities [20]. Consistent with the glucose-lowering effect of metformin, treatment with metformin stimulates glycolytic flux by increasing the activities of key glycolytic enzymes hexokinase (HKII) and 6-phosphofructo-1-kinase (PFK1) in diabetic mice [21].

AMPK is considered a sensor of energy metabolism by "sensing" the cellular AMP:ATP ratio [22]. When activated, AMPK switches cells from an anabolic to a catabolic state, shutting down the ATP-consuming synthetic pathways and restoring energy balance. As a major intracellular energy sensor, AMPK is recognized as an important target for metabolic disorders such as T2D and liver diseases. Because of this, the glucose-lowering effect of metformin has been previously attributed to the activation of liver AMPK. As supporting evidence, genetic ablation of liver kinase B1 (LKB-1), which is upstream of AMPK, eliminates the ability of metformin to activate AMPK *in vivo* and results in hyperglycemia, as well as increased expression of genes for gluconeogenic enzymes [23]. As mentioned above, AMPK activity is important to the glucose-lowering effect of metformin. However, there also is increasing evidence indicating that metformin does not act directly on either LKB1 or AMPK. For example, mice lacking both AMPK catalytic subunits in the liver display blood glucose levels comparable with those of wild-type mice [24]. Of significance, the repression of G6Pase expression in response to metformin treatment is preserved in mouse primary hepatocytes in which AMPK or LKB1 had been depleted [24]. These findings, along with others, strongly suggest that metformin inhibits hepatic gluconeogenesis by decreasing hepatic energy state (reduction in intracellular ATP content) in an LKB1- and AMPK-independent manner [24–26]. Indeed, the primary site of metformin action appears to be the respiratory chain complex I, and the AMPK-activating effect of metformin is likely a consequence of metformin actions on the mitochondria [27]. Regardless of AMPK activation and the consequences of AMPK activation, inhibiting cellular respiration decreases gluconeogenesis in the liver [28]. Also, the AMP:ATP ratio may be crucial for the control of glycolytic activity; as ATP is a substrate of glucokinase. In fact, in response to metformin treatment, the cellular levels of ATP are decreased whereas the AMP levels in livers of fasted rats are increased [29]. Also, there is accumulating evidence suggesting that the AMPK/p70 ribosomal S6 kinase-1 (S6K1) pathway is of critical importance in fuel energy metabolism. Enhancing AMPK activity by pharmacologic agents has been shown to inhibit the mTORC1/S6K1 pathway in hepatocytes [30], whose role in the regulation of hepatic glucose production remains to be defined. S6K1 is a serine kinase downstream in the insulin signaling pathway that directly phosphorylates IRS-1 on multiple serine residues and serves to inhibit insulin signaling [16,31,32]. Metformin treatment is associated with the suppression of S6K activation. This could be one mechanism explaining the insulin sensitizing effect of metformin, thereby indirectly contributing to improvement of glucose homeostasis.

Recent studies support several novel alternative pathways that are likely involved in the control of glucose homeostasis by metformin. For example, metformin inhibits AMP deaminase (AMPD) activity [33]. Knockdown of AMPD obviated metformin stimulation of glucose transport [33]. Thus, metformin likely increases AMP through inhibition of AMPD [33]. In addition, metformin treatment results in the accumulation of AMP and related nucleotides. This, in turn, inhibits adenylate cyclase, reduces the levels of cyclic AMP and protein kinase A (PKA) activity, abrogates the phosphorylation of critical protein targets of PKA, and blocks glucagon-dependent glucose output from hepatocytes [34]. To be noted, metformin treatment also improves liver lipid metabolism [35]. Given the role of hepatic fat deposition in bringing about insulin resistance, improving hepatic lipid metabolism may contribute to the overall beneficial effects of metformin independent of metformin actions on HGP. The beneficial effects of metformin on hepatic lipid metabolism are further discussed below.

Oxidative stress and antioxidant reserve

It is now well accepted that hyperglycemia increases reactive oxygen species (ROS) production, which contributes to the development of diabetic complications. Excessive deposition of lipids (in particular saturated fatty acid-enriched lipids) in the liver also enhances the risk of T2D, and further increases the generation of oxidative stress. In a human study, long-term metformin treatment increases antioxidant enzymatic activities and serum glutathione levels, thereby improving the antioxidant status [36]. Additionally, metformin treatment significantly reduces advanced oxidation protein products (AOPP) and advanced glycation end products (AGEs) [37]. These effects of metformin are thought to not only contribute to

metformin actions on improving glucose metabolic homeostasis, but also account for metformin actions on reducing diabetic complications. Although the antioxidant properties of metformin are not fully characterized, results from both *in vitro* and *in vivo* studies suggest that metformin can scavenge ROS [38–40]. For example, metformin decreases ROS production in response to high glucose (HG) in HepG2 cells [41].

Regulation of circadian clock

Dysregulation of circadian clock functions is increasingly shown to underlie, at least in part, the development of insulin resistance and T2D. Based on the results of a recent study, it is proposed that metformin causes a dramatic shift in the circadian phase in the peripheral tissue in an AMPK-dependent manner [42]. In support of this, metformin-induced AMPK activation promotes the phosphorylation of Ser386 on casein kinase 1 (CK1), one of the key circadian regulators. This enhances the CK1-mediated phosphorylation of Period 2 (Per2), leading to the degradation of Per2 and ultimately the shortening of the period length in Rat-1 fibroblasts. Interestingly, assessment of circadian expression of the core clock and metabolic genes in the peripheral tissue reveals that metformin has tissue-specific effects. For example, metformin causes circadian phase advances in the liver and phase delays in the muscle in clock and metabolic genes and/or protein expression [43]. Also, the expressions of the core circadian components CLOCK and BMAL1 and AMPK activity are decreased in white adipose tissue of *db/db* and HFD-fed mice. Further, in response to metformin treatment, AMPK activity is increased in adipose tissue of *db/db* mice, which is accompanied with increased circadian component expression and a phenotypic shift away from lipid accretion [44]. However, the extent to which regulation of circadian clocks contributes to metformin actions remains to be determined.

Alteration of autophagy

Defective autophagic pathways have been implicated in the pathophysiology of T2D. Autophagy activity and the expression of some key autophagy genes are suppressed in the presence of insulin resistance and hyperinsulinemia [45]. Also, hepatic autophagy is found to regulate fat deposition and insulin resistance, the latter two events usually form a vicious cycle during the development of T2D and NAFLD. As supporting evidence, the insulin-sensitizing effect of metformin is associated with induction of autophagy in diabetic mice [35]. Further, metformin treatment recovers autophagy in ethanol-treated hepatocytes via AMPK/mTOR-mediated signaling [46]. Although these findings suggest that metformin is capable of altering autophagy, further investigations are required to clarify the underlying mechanisms.

Metformin and NAFLD

Non-alcoholic fatty liver disease (NAFLD) is a clinical manifestation which encompasses the whole spectrum of liver diseases including hepatic steatosis, non-alcoholic steatohepatitis (NASH), and cirrhosis without significant alcohol consumption [47]. While simple steatosis is generally considered as histologically benign, it could progress to NASH during overt liver necroinflammation, and could eventually progress to cirrhosis, liver failure and liver cancer [47,48]. The estimated prevalence of NAFLD ranges from 6% to 35% with a median of 20% worldwide in the general population [47,49]. It is reported that NASH is becoming a more common cause for liver transplantation in the United States, and is on the path of becoming the most common [50].

Although the pathogenesis of NAFLD is not fully understood, NAFLD could be represented by a “two hits” model that was first proposed by Day and James [51]. The first “hit” requires the production of hepatic steatosis. Factors that contribute to hepatic steatosis include increased hepatic *de novo* lipogenesis, decreased hepatic β-oxidation, increased free fatty acid supply from adipose, and decreased very-low density lipoprotein (VLDL) triglyceride output [48,52,53]. The second “hit” requires a source of oxidative stress capable of initiating significant lipid peroxidation, leading to histological damage [51]; though nowadays, there is more and more evidence showing that the second “hit” could be promoted by a chronic proinflammatory environment induced by obesity-related adipose tissue dysfunction and obesity-induced insulin resistance. This is important, as adipose dysfunction is a critical source of adipocytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) that could promote liver inflammation (NASH) [54]. However, the sequence of these “two hits” has been challenged in the sense that inflammation could precede hepatic steatosis and the metabolic events present in NAFLD are suggested to occur in a parallel rather than a consecutive manner. In addition, emerging evidence suggests that there are multiple factors contributing to NAFLD concurrently. These factors that lead to liver inflammation include gut-derived mediators, adipose-derived mediators, and endoplasmic reticulum stress. Therefore, a “multiple parallel hits” concept might be a more precise reflection of the current knowledge of NAFLD [48].

At present, there has yet to be a standard treatment for managing NAFLD. Since NAFLD is a hepatic manifestation that is highly prevalent in obese and type 2 diabetic individuals [49,55], suggested approaches in managing

NAFLD aim at improving insulin sensitivity, and include metformin treatment. There is numerous evidence showing beneficial effects of metformin on improving NAFLD phenotypes through improving hepatic steatosis and suppressing liver inflammation. Overall, the main mechanisms underlying the beneficial effects of metformin on NAFLD are discussed below and summarized in Fig. 2.

Improvement of hepatic steatosis

Much evidence suggests that the main molecular mediator on which metformin acts to improve NAFLD is AMPK [35,52,56,57]. Interestingly, AMPK was originally discovered by its ability to inhibit fatty acid synthesis [58] and cholesterol synthesis [59] through decreasing activities of acetyl-CoA carboxylase (ACC) and HMG-CoA reductase, respectively. Metformin is able to activate AMPK, leading to the activation of downstream cascades, which results in improved hepatic lipid metabolism and decreased steatosis levels [35,60,61]. For example, treatment of *ob/ob* mice with metformin showed a marked decrease in liver size and hepatic steatosis level [62]. Also, both rat hepatocytes [9,56] and human HepG2 cultures [63] metformin treatment leads to a decrease in hepatic acetyl-coA carboxylase (ACC) activation dependent on hepatic AMPK activation, as well as an increase in hepatic fatty acid oxidation (FAO). Furthermore, concurrent treatment with metformin and an AMPK inhibitor brings about an increase in ACC activity, along with attenuated metformin actions on suppressing hepatic lipogenesis and on increasing FAO [9]. These findings demonstrate a critical role for AMPK in mediating metformin actions. In addition, AMPK is known to interact with sterol regulatory element binding protein 1-c (SREBP 1-c), a transcription factor known to induce the expression of target lipogenic genes including fatty acid synthase (FAS) [9,64]. This explains, at least in part, how metformin improves hepatic steatosis. Consistently, metformin treatment significantly decreases the hepatic mRNA expressions for SREBP-1c and FAS, and concurrently increases AMPK activation [9,56].

Suppression of liver inflammation

As mentioned above, simple steatosis is a more benign form of NAFLD whereas NASH is the more severe form. Whether hepatic steatosis precedes NASH or NASH precedes hepatic steatosis, liver inflammation is a key factor that leads to histological damage and the progression of NASH, leading to terminal liver diseases such as cirrhosis, hepatocellular carcinoma, and liver failure. This involves a complex interaction that includes cross-talk among residing hepatic populations and extrahepatic systems. In other words, liver inflammation could originate from several sources such as hepatocyte inflammatory responses, macrophage/Kupffer cell proinflammatory activation, and/or adipose tissue inflammation [56,65–69]. Here, we focus on the levels of hepatocytes and Kupffer cells, the inflammatory factors that could contribute to inflammatory damage, as evidenced in NASH, and the beneficial effects of metformin in this aspect.

In hepatocytes, fat deposition is sufficient to trigger inflammatory responses. In particular, hepatic exposure to excess free fatty acids (FFAs) is able to trigger inflammatory pathways such as c-jun N-terminal protein kinase 1 (JNK) and nuclear factor-κB (NF-κB) which increase the production of proinflammatory cytokines such as interleukin-8 (IL-8), and induces hepatocyte apoptosis, another characteristic of NASH. Kupffer cells are liver-specific macrophages that reside in the liver sinusoids and constitute approximately 20% of the liver non-parenchymal cells [70]. Much evidence suggests that Kupffer cells are critical in the pathogenesis of NAFLD [71–73]. For example, Kupffer cell ablation in methionine-choline deficient (MCD) mice shows a decrease in toll-like

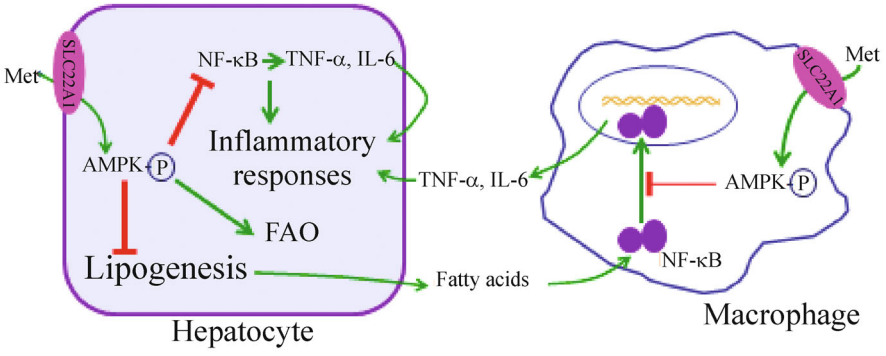

Fig. 2 MOA: metformin for NAFLD. In hepatocytes, metformin suppresses lipogenesis and stimulates fatty acid oxidation, thereby decreasing hepatocyte production of palmitate. This improves hepatic steatosis and, in turn, decreases fat deposition-associated macrophage (Kupffer cell) proinflammatory activation. In both hepatocytes and macrophages, metformin inhibits inflammatory signaling to suppress the production of proinflammatory cytokines. This contributes to suppression of liver inflammation. See text for details.

receptor-4 (TLR-4) and TNF-α mRNA expressions, followed by attenuation of histological appearance of hepatic steatosis, inflammation, and necrosis [73]. Given this, metformin action on suppression of liver inflammation [57] appears to be attributable to the effects of metformin on decreasing hepatocyte and macrophage inflammatory responses.

The results of a recent study show that metformin has a direct effect on inhibiting hepatocyte and macrophage inflammatory responses in both rat hepatoma H4IIE cells and bone marrow-derived macrophages (BMDMs), respectively [56]. This study is paramount first by providing evidence that metformin ameliorates liver inflammation in obese mice. The subsequent experiments using H4IIE cells and BMDMs show that metformin treatment suppresses the inflammatory responses, evidenced by blunted JNK1 and NF-κB signaling under the stimulation of lipopolysaccharide (LPS). In addition, metformin treatment markedly decreases the effect of LPS on stimulating mRNA expression levels of IL-1β, IL-6, and TNF-α in BMDMs. To be noted, AMPK signaling in hepatocytes is markedly increased within the same study. This suggests a potential link between hepatocyte AMPK and liver inflammation given that AMPK has been widely discussed as an upstream inhibitor of the inflammatory NF-κB cascade [74]. Thus, metformin has a direct effect on suppressing hepatocyte and macrophage inflammatory responses during the pathology of liver inflammation, as evidenced in NAFLD. However, the extent to which metformin action on hepatocytes versus macrophages/Kupffer cells contributes to suppression of liver inflammation during NAFLD remains to be determined. Also, it is worth noting that AMPK activation may not directly mediate metformin actions [35]; although much evidence shows that metformin actions on NAFLD are highly associated with AMPK signaling. Instead, the probable target of metformin is inhibiting the respiratory chain complex I of liver mitochondria [27,75], leading to the inhibition of ATP synthesis and causing a rise in the AMP:ATP ratio and thus, the activation of AMPK.

Clinical implications of metformin on NAFLD

Numerous clinical studies have investigated the effectiveness of metformin on patients with NAFLD and NASH [76–86]. The majority of the studies show that metformin improves biochemical and metabolic parameters of NAFLD, but not liver histology. For example, a recent meta-analysis [87] indicates that metformin improves insulin sensitivity of metformin-treated patients with hepatic steatosis, but not in NASH patients, based on HOMA-IR assessment. Also, metformin improves aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in NASH patients. In contrast, metformin treatment does not significantly improve liver histological variables for steatosis, inflammation, hepatocellular ballooning, and fibrosis. These results are consistent with other published systematic studies [88–90]. Based on these studies, the American Association for the Study of Liver Diseases (AASLD) practice guideline does not recommend metformin as a specific treatment for NASH [47]. However, considering that the beneficial effects of metformin on insulin resistance are well-established, and that NAFLD relates closely to insulin resistance, metformin may therefore be used for the management of NAFLD/NASH with concurrent diabetes or insulin resistance. Due to the limited number of large clinical trials and the heterogeneity of available data, more randomized controlled trials with larger sample sizes and a longer follow-up duration need to be conducted to address the optimum dosage and duration of therapy to achieve sustainable effects of metformin.

Metformin versus other agents

It is clear that metformin brings about beneficial effects largely by targeting the liver. In this section, metformin is compared with other agents that have similar effects on metabolic diseases such as insulin resistance and NAFLD. These agents include thiazolidinediones (TZDs) and berberine.

Metformin vs. TZDs

TZDs, a structural class of compounds, are ligands of the peroxisome proliferator-activated receptor (PPAR) γ, which is intricately involved in insulin signaling [91]. TZDs are considered as the first drugs that directly target insulin resistance [91] and have been widely used as efficacious diabetes preventive and therapeutic pharmacological agents for more than a decade [92,93]. TZDs decrease insulin resistance and hyperglycemia by improving hepatic and peripheral tissue utilization of glucose [91]. It is reported that TZDs also have multiple effects on insulin secretion, lipid metabolism, body fat distribution, adipose tissue function, hepatic steatosis, vascular endothelial function, microalbuminuria, hypertension, inflammation, and the pro-coagulant profile [91,94,95]. Rosiglitazone, pioglitazone, and troglitazone are members of the TZDs class [96]. Currently, rosiglitazone and pioglitazone are available in the United States whereas troglitazone was withdrawn from the market in the beginning of this century because of its drug-induced liver injury [91]. The European Medicines Agency (EMA) suspended the market authorization of rosiglitazone in 2010, and the United States Food and Drug Administration (FDA) restricts its use, due to its possible association with an increased risk of ischemic heart disease [97]. As for pioglitazone, the Government of India suddenly suspended

it though no definitive cause and effect association was shown with any of the adverse events namely bladder cancer, anemia, fractures and heart failure [98]. Moreover, the concerns on its possible adverse effects are increasing and the agent is becoming more and more controversial [94,99]. Overall, it is thought that the current TZDs are first-generation, non-specific activators of PPAR γ, which may be the key point for TZDs resulting in a wide array of deleterious side effects and why there is currently a limitation on their use. The development of highly targeted selective PPAR γ modulators and dual PPAR γ/α agonists might be new cues for their present dilemma [100].

Metformin has been widely used to lower blood glucose of patients with T2D by improving insulin sensitivity, which is similar to pioglitazone. However, metformin is reported to mainly improve the ability of insulin to stimulate glucose uptake in muscle and suppress HGP [101], through its involvement with mitochondria and AMP-activated protein kinase, not PPARγ. In addition to its efficacy at lowering glucose levels, metformin is widely considered to produce mild weight loss and delay or prevent diabetes [102]. It has only a minimal risk of hypoglycemia and causes lactic acidosis very rarely, although it is more commonly associated with gastrointestinal side effects [103]. Many studies support the efficacy and safety of metformin even during pregnancy with respect to immediate pregnancy outcomes [104]. Therefore, metformin used for diabetes treatment and prevention is deemed safe and well tolerated and, unlike TZDs, is not encumbered by weight gain or potential hepatotoxicity [102,105].

Metformin vs. berberine

Due to the potential side effects of current pharmacotherapies for metabolic syndrome, many research efforts are increasingly focusing on exploring the healing potential of natural products. Berberine is an alkaloid of the protoberberine type, and is present in an array of plants such as *Coptis chinensis* [106,107]. It is reported that this plant has been used for medicinal purposes for more than 2500 years in Ayurvedic and Chinese medicine [108]. Traditionally, berberine is used as an antimicrobial and antiprotozoal agent, which has been employed in Chinese medicine for many decades [107]. Currently, berberine is an over-the-counter pharmaceutical item in China for microbial diarrhea treatment and sold in the US as a dietary supplement [109]. Remarkably, berberine has been recently shown to exhibit multiple biological activities including antimalarial, anti-HIV, antifungal, immunoregulatory, anti-inflammatory, antitumor, anti-depression, anti-obesity, anti-diabetic, anti-hyperlipidemia and cholesterol-lowering effects [106,109].

Although, the exact mechanisms of berberine effects remain poorly understood, AMPK [110], PPAR γ [111], PKC [112], glucagon-like peptide 2 [113], antioxidant and anti-inflammatory activities [114], increased insulin receptor expression [115], and 11β-hydroxysteroid dehydrogenase [108] are likely involved in the underlying beneficial effects of berberine. Additionally, it is hypothesized that modulating gut microbiota may be another anti-diabetic mechanism for berberine actions [116].

Recent studies have revealed novel pharmacological properties and therapeutic applications of berberine, mainly concerning metabolic diseases, such as obesity and T2D [107]. It is demonstrated by the existing evidence that berberine appears to be beneficial for treating hyperglycemia in T2D and exhibits efficacy comparable with that of conventional oral hypoglycemic agents such as metformin [117]. In fact, beneficial effects of berberine in experimentally-induced diabetic animals are likely mediated by improved glucose homeostasis, increased in insulin expression and pancreatic β-cells regeneration, as well as decreased in lipid peroxidation [118]. Further, there is evidence that berberine is able to inhibit hepatic gluconeogenesis and increase glycolysis in diabetic rats [119,120]. It seems that berberine and metformin possess similar effects in regulating AMPK activation as well. In one study, Turner *et al.* reported that the efficacy of berberine on glucose metabolism is achieved by activation of AMPK and improvement in insulin action through inhibiting the mitochondrial respiratory complex I [121]. In the same study, they also found that treatment with dihydroberberine, a more biologically active form of berberine, is able to decrease liver triglyceride content. In fact, there is evidence that berberine is able to decrease lipogenesis and increase lipid oxidation in the liver. However, the evidence of berberine for treating T2D should be cautiously interpreted due to the lack of high quality clinical trials. Large and well-designed randomized controlled trials should be performed and it may be a little early to recommend berberine for routine clinical use against T2D [117]. Although having a botanical background similar to berberine, metformin, in contrast, has been used for the therapeutic management of T2D for several decades and was approved by the United States Food and Drug Administration (FDA) in 1995 after many years of use in Europe [104,122]. It has now been recommended as the first-line drug in oral diabetes treatment for several years [123,124].

Additional aspects

Metformin is usually well tolerated. However, transient mild gastrointestinal adverse effects such as nausea, vomiting, abdominal pain, flatulence, and diarrhea are common, especially during the initiation of metformin therapy [125,126]. Although gastrointestinal intolerance often happens, metformin-induced hepatotoxicity is rare.

Metformin does not appear to cause or exacerbate liver injury and indeed, may be beneficial in patients with NAFLD, chronic hepatitis B and C viral infection.

Metformin-related hepatotoxicity

Metformin does not undergo hepatic metabolism (no metabolites have been identified in humans) or biliary excretion. Thus, metformin is considered safe from a hepatic standpoint [8,127,128]. Minor enzyme elevations have been reported to occur during metformin therapy in less than 1% of patients. Clinically, metformin-induced hepatotoxicity is very rare, with less than 20 cases having been described in the literature despite widespread use of metformin for several decades. When it occurred, liver injury usually appeared after 1 to 8 weeks [129], of metformin therapy, typically with symptoms of nausea, vomiting, weakness and fatigue followed by jaundice, with marked elevations in serum liver transaminases and intrahepatic cholestasis [130,131]. The mechanisms of metformin-induced hepatotoxicity are unknown, and appear to be direct, idiosyncratic, or a drug-drug interaction leading to acute hepatocellular and/or cholestatic jaundice. Reports suggest that metformin can induce acute portal and parenchymal inflammation [132]. There has been no reported specific treatment for metformin-associated hepatotoxicity. However, after discontinuation of metformin, symptoms resolve rapidly and liver enzymes return to normal values within a few weeks [132].

Metformin treatment for patients with viral hepatitis

Metformin may actually be beneficial for some forms of liver diseases, such as NAFLD, chronic hepatitis B and C viral infection. NAFLD frequently presents mild transaminase elevations, but these kinds of preexisting serum enzyme abnormalities should not be considered a contraindication to metformin use [133]. Because metformin is not considered intrinsically hepatotoxic, withholding metformin from patients with abnormal transaminases, or routinely monitoring transaminases before or during metformin treatment, is also not supported. A recent review shows that metformin may provide benefits in the treatment of viral hepatitis C, and in reducing the risk of hepatocellular carcinoma (HCC) in patients with T2D and hepatic C virus (HCV) [134]. In particular, metformin treatment reduces hepatitis C virus (HCV)-related insulin resistance [134]. Additionally, metformin treatment inhibits hepatitis B virus (HBV) protein production and replication in human hepatoma cells [135]. These results suggest that metformin also provides benefits in the treatment of viral hepatitis B. However, further investigations are needed to validate the beneficial effects of metformin treatment in patients with viral hepatitis.

Metformin treatment for patients with hepatocellular carcinoma

Metformin has also emerged as an agent with the potential to protect against cancer. Several recent studies show that metformin treatment of diabetes is associated with a reduced risk of HCC [136–138]. In addition, metformin use is associated with lower cancer-related mortality. However, the mechanisms underlying the protective potential of metformin are not well understood. Several reports indicate that the anti-cancer effects are mediated mainly through the LKB1-AMPK pathway [139]. In tumor suppressor phosphatase and tensin homolog-deleted on chromosome 10 (PTEN) knockout mice, metformin induces the activation of the LKB1-AMPK pathway, inhibits mTOR signaling, and significantly delays tumor onset [139]. Similarly, it has been demonstrated that AMPK activation by metformin induces p53-dependent autophagy [140]. Metformin is selectively toxic to p53-deficient cells, which provides a potential mechanism for the reduced incidence of tumors [140]. Additionally, recent evidence suggests that metformin also exerts anti-cancer effects through AMPK independent pathways. For example, metformin prevents liver tumorigenesis by inhibiting lipid synthesis in the liver without increasing AMPK activation [137]. Although further investigations are needed, there is no doubt that metformin can benefit patients with HCC.

Metformin application and lactic acidosis

Although circumstantial evidence shows that treatment with metformin may be linked to lactic acidosis, no causal relation has been proven. The pathogenesis of metformin-associated lactic acidosis is not completely understood. Lactate levels in the blood result from the balance between production and clearance. It is thought that metformin increases the levels of lactate through two potential mechanisms. First, metformin binds mitochondrial membrane and inhibits complex I of the respiratory chain, thereby inhibiting oxidative metabolism, which results in a shift toward anaerobic metabolism and potentially augments lactate production [141]. Second, metformin suppresses HGP from lactate, functionally decreasing lactate clearance [142]. However, metformin, unlike the earlier biguanide (phenformin), actually has poor adherence to the mitochondrial membrane [143] and is thought to enhance glucose oxidation without substantially affecting fasting lactate production in peripheral tissue [144]. Thus, metformin has a much lower risk of lactic acidosis than phenformin. In fact, a substantial meta-analysis of randomized controlled trials, which included 36 893 patients, concludes that treatment with metformin is not associated with an increased risk of lactic acidosis. Also,

there is no difference in the levels of lactate between metformin and placebo or other treated groups [145]. A recent study reported that diabetics on metformin have a 25-fold increased risk for hyperlactacidemia at the emergency room. However, metformin shows no apparent increase in the risk for lactic acidosis [146].

The incidence of metformin-associated lactic acidosis (MALA) is rare. The estimated rate of MALA is 2 to 9 cases per 100 000 person-years [147]. Although metformin is considered safe, it still carries a warning for use in patients with serious hepatic disease because of an increased risk of lactic acidosis. MALA has rarely been reported to cause mortality without other precipitating factors: predominantly renal or liver failure, congestive heart failure, pulmonary disease, peripheral vascular disease, or age older than 65, as these conditions may increase the risk of tissue anoxia and therefore the development of lactic acidosis. Literature evidence of liver disease being associated with MALA is largely represented by case reports [148]. Most such patients had cirrhosis [149] and/or chronic or excessive alcohol intake [150]. Patients with cirrhosis, particularly those with encephalopathy, may have arterial hypoxemia, which increases the risk of developing lactic acidosis [149,151]. For this reason, identifying patients with cirrhosis and particularly those with encephalopathy before initiating metformin seems prudent.

Acknowledgements

This work was supported, in whole or in part, by National Natural Science Foundation of China (81100562/H0711) (to J.Z.), and National Key Basic Research Program of China (2012CB910402) (to Y. H.), ADA grant 1-13-BS-214-BR, AHA 12BGIA9050003, NIH/NIDDK grants (1R01DK095828; 1R01DK095862) (to C.W.).

Compliance with ethics guidelines

Juan Zheng, Shih-Lung Woo, Xiang Hu, Rachel Botchlett, Lulu Chen, Yuqing Huo, and Chaodong Wu declare no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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