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Review Article

# Effects of Metformin on Hepatic Steatosis in Adults with Nonalcoholic Fatty Liver Disease and Diabetes: Insights from the Cellular to Patient Levels

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Nonalcoholic fatty liver disease (NAFLD) patients with diabetes constitute a subgroup of patients with a high rate of liver-related complications. Currently, there are no specific drug recommendations for these patients. Metformin, a conventional insulin sensitizer agent, has been widely prescribed in patients with diabetes. Metformin treatment has been shown to be effective at alleviating hepatic lipogenesis in animal models of NAFLD, with a variety of mechanisms being deemed responsible. To date, most studies have enrolled diabetic patients who are treated with metformin, with the drug being taken continuously throughout the study. Although evidence exists regarding the benefits of metformin for NAFLD in preclinical studies, reports on the efficacy of metformin in adult NAFLD patients have had some discrepancies regarding changes in liver biochemistry and hepatic fat content. Evidence has also suggested possible effects of metformin as regards the prevention of hepatocellular carcinoma tumorigenesis. This review was performed to comprehensively summarize the available *in vitro*, *in vivo* and clinical studies regarding the effects of metformin on liver steatosis for the treatment of adult NAFLD patients with diabetes. Consistent reports as well as controversial findings are included in this review, and the mechanistic insights are also provided. In addition, this review focuses on the efficacy of metformin as a monotherapy and as a combined therapy with other antidiabetic medications. **(Gut Liver 2021;15:827-840)**

**Key Words:** Diabetes mellitus; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common disease with increasing incidence worldwide. A large cohort in the United States showed a 5-fold increase in incidence from 1997 to 2014.[1] Insulin resistance has a pivotal role in NAFLD development and progression[2] and NAFLD patients with diabetes are a subgroup of patients with a high rate of liver-related complications.[3] In response to insulin resistance, hyperinsulinemia occurs causing the augmentation of hepatic *de novo* lipogenesis pathways, resulting in hepatic steatosis and further hepatic inflammation.[4] Currently, the treatment of NAFLD is markedly under investigation. To date, no medication has been approved to treat NAFLD and nonalcoholic steatohepatitis (NASH) by the Food and Drug Administration in the United States and there is no specific drug recommended for treating the subgroup of NAFLD patients with diabetes.

Metformin, an insulin sensitizer agent in the biguanide subclass, is a widely used drug in diabetic patients with a good safety profile. Since it involves multiple molecular mechanisms in glucose metabolism and anti-inflammatory effects,[5] metformin is one of the most interesting medications for the possible treatment or control of NAFLD progression. A previous meta-analysis of randomized-controlled trials evaluated the treatment response of metformin in patients with NAFLD and NASH.[6] It was concluded that metformin was not associated with liver histologic

improvement in patients with histologic NASH. However, most of the patients enrolled in these studies were nondiabetic[7-11] or patients with insulin resistance without established diabetes.[12] Currently, reports on the effects of metformin among diabetic NAFLD patients have been inconsistent. Since most of the diabetes patients were receiving metformin, the beneficial effects of this treatment need to be elucidated.

In this review, we have comprehensively summarized findings from *in vivo*, *in vitro*, and clinical studies regarding metformin for the treatment of adult NAFLD patients with diabetes. Our review focuses on the efficacy of metformin in treating liver steatosis. Consistent and controversial reports regarding the mechanisms responsible for the effect of metformin on NAFLD development are also discussed. Relevant publications in the PubMed database were included in this review, the search terms used being "metformin" and "NAFLD," "NASH" and "diabetes." Only the articles published in English were reviewed.

## EFFECTS OF METFORMIN ON LIPOGENESIS REDUCTION IN NAFLD: REPORTS FROM *IN VITRO* STUDIES

The findings from *in vitro* studies demonstrated that metformin could reduce lipid accumulation[13] and *de novo* fatty acid synthesis.[14-16] Several proteins have been shown to be essential to the regulation of hepatic *de novo* lipogenesis. For example, the enzyme acetyl-CoA carboxylase (ACC) catalyzes acetyl CoA into malonyl CoA, a precursor for fatty acid hepatic synthesis, ACC playing a vital role in a rate limiting step of lipogenesis.[17] Phosphorylation of ACC via AMP-activated protein kinase (AMPK) inhibits the action of ACC, leading to inhibition of lipogenesis.[18,19] Metformin increased inhibitory phosphorylation of ACC[13] and induced hepatic Rho-kinase 1 (ROCK1) inhibition,[16] resulting in AMPK activation and a decrease in lipogenic genes associated with *de novo* lipogenesis.[16] Autophagy restoration via the sirtuin 1 (SIRT1) dependent pathway[13] and signal transducer and activation of transcription 3 (STAT3) inhibition[20] by metformin has been demonstrated. Anti-apoptotic activity,[21] protection against lipid-induced necrotic cell death,[21] reduction of oxidative stress[21] and inflammatory markers[20] were also shown in metformin treated cells. These *in vitro* reports are summarized in Supplementary Table 1.

## EFFECTS OF METFORMIN ON LIPOGENESIS REDUCTION IN NAFLD: REPORTS FROM *IN VIVO* STUDIES

Several *in vivo* studies evaluated the effects of metformin on the reduction of hepatic fat content and the mechanism responsible. A variety of studies involving a range of dosages, routes, and durations of metformin treatment in genetically modified mice which exhibit features of hepatic steatosis, or dietary models of NAFLD rats or mice have been performed. Most of the studies demonstrate the effectiveness of intrahepatic lipid reduction by metformin.[13,14,16,20,22-28] However, there are a few contradictory reports showing ineffectiveness of metformin treatment.[15,29-31] The differences in the NAFLD models used causing the varying degrees of disease severity, and accompanying metabolic derangement could be responsible for the discrepancies. It is observable that among the studies showing negative effects, the models with more severe disease were used, including the use of mice feeding with higher percentage of fat in high fat diet (HFD),[15] methionine- and choline-deficient diet,[29] Zucker diabetic fatty rat,[30] and Goto-Kakizaki rat fed with HFD.[31] The dosing and route of metformin administration were also varied between studies, and this could potentially affect the drug absorption with all studies using intraperitoneal route administration showing positive effects.[13,14,20]

### 1. Effects of metformin on molecular mechanisms of hepatic steatosis (*de novo* lipogenesis reduction and increased fatty acid β-oxidation)

Metformin is known to activate AMPK.[32] The inhibition of phosphorylation of ACC by AMPK resulting in *de novo* lipogenesis reduction is one of the most widely mentioned responsible mechanisms.[14,16,22,24,30] An ACC knock-in mouse model had increased liver triglyceride (TG) content and increased liver fibrosis.[14] Metformin treatment decreased hepatic lipogenesis and liver TG content in wild-type mice but not in ACC knock-in mice. These findings suggested that inhibition of phosphorylation of ACC by AMPK was essential in metformin action.[14] Other studies added weight to this by demonstrating increasing AMPK activation and decreasing hepatic TG content in mice treated with metformin.[16,22,24,30] It has been proposed that AMPK activation was mediated by ROCK1.[16] Lipogenic gene expression of proteins involved in hepatic lipogenesis, including sterol regulatory element-binding protein 1 (SREBP-1c),[16,23] ACC,[23] fatty acid synthase (FAS)[16,23] and stearoyl-CoA desaturase-1 (SCD1)[16] were reduced with metformin treatment. It was speculated that these changes were related to the activation of AMPK.[16,33]

Leptin is an adipose tissue-produced peptide which decreases hepatic *de novo* fatty acid synthesis and promotes peroxisome proliferator-activated receptor gamma coactivator-1α (PPARα)-dependent fatty acid beta oxidation.[34] Circulating leptin levels were found to be higher in NAFLD patients than controls[35] and it was proposed that the blunted response of the liver to leptin action was related to hepatic steatosis.[36] An enhanced leptin sensitivity by metformin is one of potential mechanisms underlying its steatosis alleviation effect.[23] However, a study in Zucker diabetic fatty rats, those with missense mutation in the leptin receptor gene which develop early fatty liver, severe hyperlipidemia, and insulin resistance,[37] showed that metformin had no effect on NAFLD.[30] This finding may imply the leptin gene is essential for metformin treatment to be effective, or suggest that the extent of the effect of metformin was not enough in the case of a more severe and early onset of disease. Proteins involved in mitochondrial lipid oxidation were up-regulated following metformin treatment,[38] suggesting the potential effect of metformin in increasing mitochondrial lipid oxidation. All these reports are summarized in Table 1.

### 2. Effects of metformin on hepatic inflammation, oxidative stress, and fibrosis

Tumor necrosis factor-α (TNF-α) is known to be a mediator of apoptosis and hepatotoxicity.[39] It is also involved in NAFLD development and NASH progression.[40] The results regarding TNF-α level upon metformin treatment are inconsistent, showing both reduction in[20,25,26,31] and neutral TNF-α levels.[28,29] Other inflammatory markers, including, inducible nitric oxide synthase (iNOS),[25,26] interleukin-1β,[20] transforming growth factor β (TGF-β),[28] and CD68[22] decreased upon metformin treatment. While the markers of inflammation decreased, the oxidative stress parameters glutathione and superoxide dismutase (SOD),[25] and the antioxidant protein peroxiredoxin 6 (PRDX-6)[38] increased after metformin treatment. These findings provide a potential mechanism for metformin in treatment of NAFLD by alleviating inflammation in the liver and decreasing oxidative stress. All these reports are summarized in Table 2.

### 3. Direct degradation of intracellular lipid by autophagy induction

The induction of autophagy enables cells to reutilize their own constituents for energy, one of the approaches for NAFLD treatment.[41] The downregulation of SIRT1 expression and autophagy induction in the liver of *ob/ob* mice were restored following treatment with metformin.[13] Additionally, metformin was shown to inhibit the STAT3 pathway,[20] the pathway in which inhibition also induced autophagy.[42] All these reports are summarized in Table 3.

### 4. Other proposed mechanisms of metformin

Apolipoprotein A-I (ApoA-I) may be involved in the treatment effects of metformin as its deficiency increased mice sensitivity to diet-induced obesity[43] and blunted the beneficial effect of metformin on liver lipid content.[24] Metformin alters the enzymes and genes associated with NAFLD development. Deficiency of the enzyme glycine N-methyltransferase (GNMT) which has a crucial role in NAFLD development,[44] was up-regulated upon treatment.[38] It also induced transcriptome alteration which is negatively correlated with liver disease and injuries,[22] and induced changes in gene expression associated with the NAFLD phenotypes.[22]

Intestinal dysbiosis and gut barrier function were found to be associated with the development of NAFLD.[45] The mechanism behind the protective effects of metformin against NAFLD development could be partly due to the modulation of the population of intestinal microbiota,[27] protection against tight junction protein loss,[26] and the reduction of bacterial endotoxins.[26,27] All these reports are summarized in Supplementary Table 2. A summary of *in vivo* and *in vitro* reports, regarding the mechanisms behind the action of metformin and lipogenesis reduction in the NAFLD model is also shown in Fig. 1.

## EFFECTS OF METFORMIN ON THE LIVER IN NAFLD PATIENTS WITH DIABETES

Preclinical studies showed remarkable improvement in liver histology and in the reduction of hepatic fat content following treatment with metformin as mentioned earlier. Therefore, metformin was expected to be a promising medication against NASH. However, metformin had limited impact in clinical studies among NAFLD or NASH patients without diabetes.[7,8,12] The question remained to be answered is whether metformin treatment in NAFLD patients with diabetes could provide any clinical benefit since it is accepted as a safe medication and is widely used. Here we summarized available clinical reports on NAFLD patients with diabetes, including the effects of metformin as a monotherapy, comparison of metformin to other antidiabetic medications and as part of a combination treatment.

### 1. Effects of metformin as a monotherapy in NAFLD patients with diabetes

The efficacy of metformin monotherapy in the NAFLD population with diabetes has rarely been evaluated in a randomized-controlled study. The majority of the clinical

**Table 1.** Effects of Metformin on the Molecular Mechanisms of Hepatic Steatosis (*De Novo* Lipogenesis Reduction and Increased Fatty Acid β-Oxidation): Evidence from *In Vivo* Reports

| Author (year) | Model (age) | Method | Metformin (dose/route/duration) | Effects of metformin | Interpretations |
|---|---|---|---|---|---|
| Studies showing effective intrahepatic lipid reduction by metformin | | | | | |
| Fullerton *et al.* (2013)[14] | Male C57BL/6J mice (6 weeks old) | - HFD | - 50 mg/kg/day/IP/6 weeks<br>- Starting after HFD for 12 weeks | ↓ Hepatic lipogenesis, TG<br>↓ Malonyl CoA | Inhibitory phosphorylation of ACC by AMPK was essential for controlling lipid metabolism and metformin action. |
| | | - ACC-DKI<br>- HFD | - 50 mg/kg/day/IP/6 weeks<br>- Starting after HFD for 12 weeks | ↔ Hepatic lipogenesis, TG<br>↔ Malonyl CoA | |
| Karavia *et al.* (2015)[24] | Male C57BL/6J mice (10–12 weeks old) | - Western diet | - 300 mg/kg/day/PO/18 weeks | ↓ Hepatic TG, histologic steatosis<br>↑ P-AMPK/total AMPK | Metformin induced phosphorylation of AMPK. |
| Guo *et al.* (2018)[22] | Male C57BL/6J mice (3 weeks old) | - HFD | - 3 mg/kg/day/PO/5 weeks<br>- Starting after HFD for 12 weeks | ↓ Hepatic TG, TC<br>↑ p-AMPK/AMPK<br>↓ p-ACC/ACC | Metformin activated AMPK *in vivo*. |
| Huang *et al.* (2018)[16] | Male C57BL/6J mice (6 weeks old) | - HFD | - 250 mg/kg/day/PO/4 weeks then 500 mg/kg/day/PO/8 weeks<br>- Starting after HFD for 14 weeks | ↓ Hepatic TG, TC<br>↑ AMPK activity<br>↓ FAS, SCD1, SREBP-1c<br>↓ Hepatic ROCK1 activity | Metformin inactivated ROCK1, resulting in activation of AMPK signaling. |
| | | - L-ROCK1$^{-/-}$<br>- HFD | | ↔ AMPK activity<br>↔ FAS, SCD1, SREBP-1c | |
| Tang *et al.* (2016)[23] | Male C57BL/6J mice (4–6 weeks old) | - HFD | - 50 mg/kg/day/PO/15 days<br>- Starting after HFD for 5 months | ↓ Hepatic TG<br>↓ SREBP-1c, FAS, ACC-1<br>↑ Hepatic LepR | Metformin dose-dependently enhanced hepatic leptin sensitivity. |
| | | | - 200 mg/kg/day PO/15 days<br>- Starting after HFD for 5 months | ↓ Hepatic TG<br>↓ SREBP-1c, FAS, ACC-1<br>↑ ↑ Hepatic LepR | |
| Stachowicz *et al.* (2012)[38] | Female C57BL/6J mice (8 weeks old) | - apoE$^{-/-}$ | - 10 mg/kg/day/PO/16 weeks | ↑ SCAD, ECHD3, IPYR | Metformin up-regulated protein related fatty acid beta-oxidation. |
| Brandt *et al.* (2019)[26] | Female C57BL/6J mice (6–8 weeks old) | - Fat-fructose-and cholesterol-rich diet | - 300 mg/kg/day/PO/4 days | ↓ Hepatic TG<br>↓ Liver NAS<br>↓ SREBP-1c<br>↔ FAS, ACC | Metformin improved liver histology and delayed the development of NAFLD. |
| | | | - 300 mg/kg/day/PO/6 weeks | ↔ Hepatic TG<br>↓ Liver NAS<br>↔ SREBP-1c, FAS, ACC | |
| Studies showing ineffective intrahepatic lipid reduction by metformin | | | | | |
| Ford *et al.* (2015)[15] | Male C57BL/6J mice (8 weeks old) | - HFD | - 2.5 g/kg/day/PO/5 weeks<br>- Starting after HFD for 5 weeks | ↔ Hepatic TG<br>↔ p-ACC/ACC | Metformin had no effect on hepatic TG content and AMPK activation. |
| Sui *et al.* (2019)[30] | Male Zucker diabetic fatty rats (4–8 weeks old) | - fa/fa rats | - 50 mg/kg/day/PO/24 weeks | ↑ Histologic fatty changes<br>↓ Total AMPK<br>↑ p-AMPK<br>↔ SCD1<br>↓ G6PDX, HMGCS1, IGFBP1 | Metformin promoted AMPK activation and correction of gene expression associated with LepR mutation. |

ACC, acetyl-CoA carboxylase; ACC-DKI, ACC double knock-in mutation; AMPK, AMP-activated protein kinase; apoE$^{-/-}$, apolipoprotein E knock-out mutation; ECHD3, enoyl-CoA hydratase domain-containing protein 3; FAS, fatty acid synthase; G6PDX, glucose-6-phosphate dehydrogenase X-linked; HFD, high fat diet; HMGCS1, 3-hydroxy-3-methylglutaryl-coA synthase 1; IGFBP1, insulin-like growth factor-binding protein-1; IP, intraperitoneal route; IPYR, inorganic pyrophosphatase; LepR, leptin receptor; L-ROCK1$^{-/-}$, liver-specific ROCK1 deficient mice; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; p-ACC, phosphorylation of acetyl-CoA carboxylase; p-AMPK, phosphorylation of AMPK; PO, per os; ROCK1, Rho-kinase 1; SCAD, short-chain specific acyl-Co A dehydrogenase; SCD1, stearoyl-CoA desaturase-1; SREBP-1c, sterol regulatory element-binding protein 1; TC, total cholesterol contents; TG, triglyceride content; ↓, significant decrease; ↑, significant increase; ↔, no significant change.

**Table 2.** Metformin Effects on Hepatic Inflammation, Oxidative Stress, and Fibrosis: Evidence from *In Vivo* Reports

| Author (year) | Model (age) | Method | Metformin (dose/route/duration) | Effects of metformin | Interpretations |
|---|---|---|---|---|---|
| Studies showing effective intrahepatic lipid reduction by metformin | | | | | |
| Stachowicz *et al.* (2012)[38] | Female C57BL/6J mice (8 weeks old) | - apoE$^{-/-}$ | - 10 mg/kg/day/PO/16 weeks | ↑ PRDX-6 | Metformin up-regulated antioxidant protein. |
| Guo *et al.* (2018)[22] | Male C57BL/6J mice (3 weeks old) | - HFD | - 3 mg/kg/day/PO/5 weeks<br>- Starting after HFD for 12 weeks | ↓ Hepatic TG, TC<br>↓ Hepatic CD68+ IHC staining | Metformin reduced macrophage content in the liver. |
| Khalaf *et al.* (2019)[25] | Male albino rats (200–250 g weight) | - 10% fructose in drinking water | - 300 mg/kg/day/PO/4 weeks | ↓ Hepatic TG<br>↑ GSH, SOD<br>↓ MDA<br>↓ TNF-α<br>↓ Histologic steatosis<br>↔ Histologic inflammation<br>↓ iNOS stained cell | Metformin reduced oxidative stress and inflammatory mediators. |
| Li *et al.* (2019)[20] | Male C57BL/6J mice (6 weeks old) | - MCD diet | - 250 mg/kg/day/IP/4 weeks | ↓ Histologic steatosis and inflammatory cell infiltration<br>↓ TNF-α, IL-1β, IL-6 | Metformin reduced hepatic inflammatory markers. |
| de Jesús Acosta-Cota *et al.* (2019)[28] | Male Wistar rats (4 weeks old) | - 50% sucrose (wt/vol) in drinking water | - 200 mg/kg/day/PO/5 weeks<br>- Starting after sucrose for 16 weeks | ↔ Hepatic TG<br>↓ Hepatic TC<br>↔ TNF-α, IL-6, IL-10<br>↓ TGF-β<br>↓ Number of rats with fibrosis | Metformin decreased hepatic cholesterol contents and TGF-β. |
| Brandt *et al.* (2019)[26] | Female C57BL/6J mice (6–8 weeks old) | - Fat/fructose-and cholesterol-rich diet | - 300 mg/kg/day/PO/4 days<br><br>- 300 mg/kg/day/PO/6 weeks | ↓ Hepatic TG<br>↓ Neutrophilic granulocytes<br>↔ TNF-α<br>↔ Hepatic TG<br>↓ Neutrophilic granulocytes<br>↓ TNF-α | Metformin treatment decreased inflammatory cell infiltration and TNF-α in the liver. |
| Studies showing ineffective intrahepatic lipid reduction by metformin | | | | | |
| Matafome *et al.* (2011)[31] | Goto-Kakizaki rats (8 weeks old) | - HFD | - 60 mg/kg/day/PO/4 weeks<br>- Starting after HFD for 12 weeks | ↔ Hepatic TG, TC<br>↓ TNF-α<br>↔ IL-6, protein carbonyl, serum CRP | Metformin decreased TNF-α in liver. |
| Mahzari *et al.* (2019)[29] | Male C57BL/6J mice (10 weeks old) | - MCD diet | - 250 mg/kg/day/PO/6 weeks | ↔ Hepatic TG<br>↔ TNF-α, CD68 mRNA<br>↔ Collagen 1, TGF-β, Smad3<br>↔ Liver fibrosis area | Metformin did not improve NASH features and proteins associated with profibrotic pathways. |

apoE$^{-/-}$, apolipoprotein E knock-out mutation; CRP, C-reactive protein; GSH, glutathione; HFD, high fat diet; IHC, immunohistochemical staining; IL, interleukin; IP, intraperitoneal route; iNOS, inducible nitric oxide synthase; MCD, methionine-and choline-deficient diet; MDA, malondialdehyde; NASH, nonalcoholic steatohepatitis; PRDX-6, peroxiredoxin 6; PO, per os; SOD, superoxide dismutase; TC, total cholesterol contents; TG, triglyceride content; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; ↓, significant decrease; ↑, significant increase; ↔, no significant change.

**Table 3.** Metformin Effects on the Direct Degradation of Intracellular Lipid by Autophagy Induction: Evidence from *In Vivo* Reports

| Author (year) | Model (age) | Method | Metformin (dose/route/duration) | Effects of metformin | Interpretations |
|---|---|---|---|---|---|
| Studies showing effective intrahepatic lipid reduction by metformin | | | | | |
| Song *et al.* (2015)[13] | C57BL/6J mice (8 weeks old) | - *Ob/Ob* mice<br>- Chow diet | - 300 mg/kg/day/IP/4 weeks | ↓ Hepatic TG<br>↑ SIRT1 expression<br>↑ Autophagy | Metformin restored SIRT1 expression and induced autophagy. |
| Li *et al.* (2019)[20] | Male C57BL/6J mice (6 weeks old) | - MCD diet | - 250 mg/kg/day/IP/4 weeks | ↓ Histologic steatosis<br>↓ STAT3 protein and mRNA expression<br>↑ Autophagy | Metformin inactivated STAT3 signaling pathway and reversed autophagy inhibition. |

IP, intraperitoneal route; MCD, methionine-and choline-deficient diet; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3; TG, triglyceride content; ↓, significant decrease; ↑, significant increase.

studies were conducted with the primary aim of comparing the effects of metformin with other antidiabetic medications. No placebo-controlled study has been conducted at this time. In eight studies that reported the effects of metformin in diabetic NAFLD patients compared to the pretreatment baseline condition, the patients were treated with metformin at dosages ranging from 1,000 to 2,000 mg/day for 12 to 48 weeks.[46-53] These studies had various methods of NAFLD diagnosis, including ultrasonographic assessment of hepatic steatosis, a quantitative ultrasonographic method, or a liver/spleen computed tomography ratio (L/S CT ratio) of less than 1. One study included the patients who underwent liver biopsy and were diagnosed as NASH.[51]

Metformin was shown to be beneficial in patients with NAFLD compared to baseline. Five studies showed that metformin treatment for 12 to 24 weeks reduced the body mass index (BMI), liver fat content, liver enzymes, and hemoglobin A1c (HbA1c) and improved insulin resistance in NAFLD patients with type 2 diabetes mellitus (T2DM).[46-50] A prospective study in 11 patients with new-onset T2DM showed lower amounts of fat in the liver after 16 weeks of metformin treatment.[53] However, one study reported inconsistent findings. In this small study with 16 participants treated with metformin for 24 weeks, increased liver fat content was demonstrated, and no beneficial effects of metformin were found on the BMI, transaminase level, and HbA1c.[52] The possible cause of this conflicting result could be due partly to the limited number and type of patients enrolled. In this study, the enrolled patients were older than other studies (mean age of 60 years) with slightly lower baseline HbA1c compared to other studies (mean HbA1c of 7.4%). These patients' characteristics suggest longer NAFLD disease duration and less insulin resistance in the enrolled patients. Future studies are needed to test this hypothesis.

Metformin treatment significantly decreased liver fibrosis evaluated by noninvasive measurement in NAFLD patients.[46] However, inconsistent report exists. No significant improvement of fibrosis was demonstrated in histologic NASH patients (n=10) evaluated by liver biopsy.[51] The number of patients included in this study was small, which could limit the study power. Since this study also enrolled both T2DM and impaired glucose tolerance patients, the mean HbA1c at baseline (5.8%) was lower than other studies. Several studies already showed that metformin was ineffective among patients without diabetes,[7,8,12] thus further investigations in diabetes population are required. All these reports are summarized in Table 4.

Similar to findings from preclinical studies, metformin use is associated with decreased liver fat content in diabetic

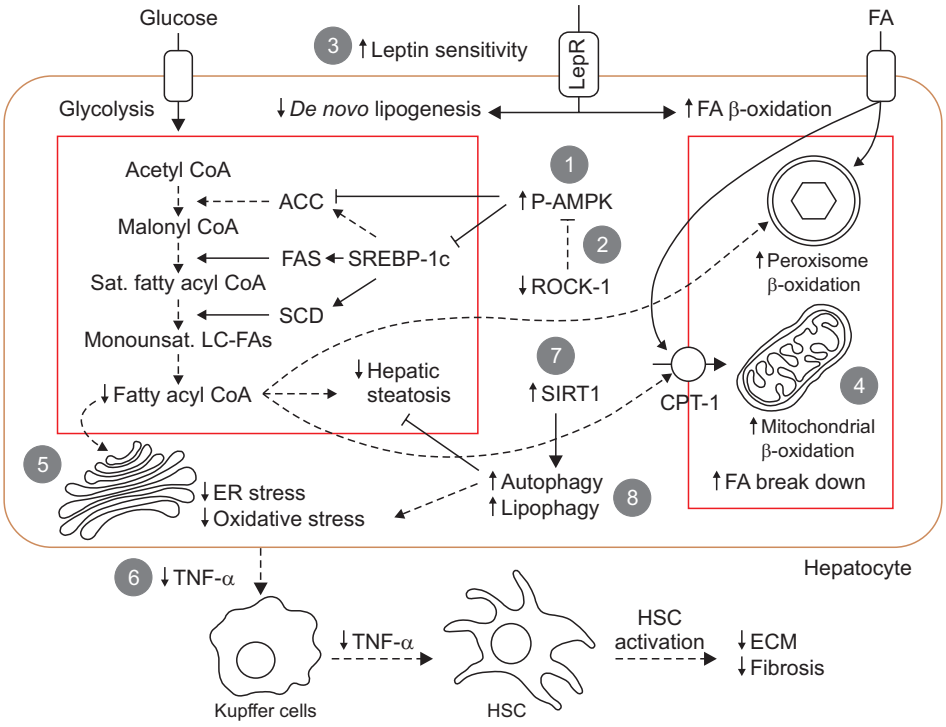


**Fig. 1.** Mechanism of action of metformin in nonalcoholic fatty liver disease. (A) Decrease in *de novo* lipogenesis: (1) AMPK activation and increase inhibitory phosphorylation of ACC; (2) inhibition of ROCK-1 by metformin resulting in inhibitory phosphorylation of ACC; and (3) increase in leptin sensitivity attenuates *de novo* lipogenesis pathway. Decreasing fatty acyl CoA also decreases hepatic steatosis, decreases lipid-induced ER stress and decreases substrate for FA β-oxidation. (B) Increase in FA β-oxidation: (3) increase in leptin sensitivity induces PPARα-dependent FA β-oxidation; (4) up-regulation of proteins involved in mitochondrial lipid oxidation by metformin results in increased FA breakdown and energy combustion. (C) Decrease in inflammation and HSC activation: (5) decreased lipid-induced ER stress and oxidative stress due to decreased *de novo* lipogenesis; (6) TNF-α reduction decreases Kupffer cell and HSC activation resulting in reducing inflammation and fibrosis in the liver. (D) Direct degradation of intracellular lipid: (7, 8) induction of autophagy by restoration of SIRT1 activity causing lipolysis by lysosome (lipophagy).
ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyltransferase; ECM, extracellular matrix proteins; ER, endoplasmic reticulum; FA, fatty acid; FAS, fatty acid synthase; HSC, hepatic stellate cells; LepR, leptin receptor; Monounsat. LC-FAs, monounsaturated long-chain FAs; P-AMPK, phosphorylated AMP-activated protein kinase; PPARα, peroxisome proliferator-activated receptor gamma coactivator-1α; ROCK1, Rho-kinase 1; Sat., saturated; SCD, stearoyl-CoA desaturase; SIRT1, sirtuin 1; SREBP-1c, sterol regulatory element-binding protein 1; TNF-α, tumor necrosis factor.

patients. However, the effect is not as prominent as the effect shown in the rodent studies as the liver histology improvement was not replicated. It is unclear why there is a difference between animal and human studies. We speculate that this might be due to the uniform pattern of fatty phenotype in the animals studied, and the difference in the pharmacokinetics of metformin between species. Animal studies with an NAFLD model include genetically modified mice or mice fed with a diet promoting the development of a fatty liver. These models generate uniform fatty rodents and the effect of metformin might be seen more clearly than in a human study in which the participants had various degrees of severity of fatty liver and concurrent metabolic derangements. Metformin is taken up in the liver via organic cation transporter-1 (OCT1).[54] Hepatic uptake of various drugs via this transporter has been shown to have differ between species, for example between mice and humans.[55] To our knowledge, no previous research had explored the species difference of metformin uptake. Therefore, it remains unclear whether our speculation would impact the results. Future research should examine this issue as it is important when projecting animal research results to humans. Histologic outcome should be further evaluated in a larger NAFLD population with diabetes. The effect of long-term treatment of metformin on liver-related adverse events are currently unclear, knowledge surrounding this is desirable.

Most of the recent studies conducted in diabetic NAFLD patients enrolled the patients treated with metformin and allowed metformin continuation during the study. Some studies included both metformin users and nonusers. However, despite modest effects being observed, metformin monotherapy decreased liver transaminases and hepatic fat content. These effects were prominent during the 12 to 24 weeks after administration. Therefore, the conduction of clinical studies should consider this possible effect for patient selection to avoid confounding effects caused by metformin treatment.

**Table 4.** Effects of Metformin on the Liver in Diabetic NAFLD Patients

| Author (year) | Populations | Method of NAFLD diagnosis | Design | Metformin, PO (dose/duration) | Major findings (compared to baseline) | | | | Interpretations |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Body anthropometry | Liver fat contents | Biochemistry | Liver fibrosis and histology | |
| Studies showing benefit of metformin use | | | | | | | | | |
| Fan *et al.* (2013)[47] | T2DM with NAFLD (n=68) | US | Randomized study | 1,000-2,000 mg/day/ 12 weeks | ↓ BMI | - | ↓ AST, ALT*<br>↓ HbA1c<br>↓ HOMA-IR | - | Metformin was able to improve hepatic enzymes. |
| Feng *et al.* (2017)[48] | T2DM with NAFLD (n=29) | Quantitative US with IHF ≥10% | Randomized open-label study | 2,000 mg/day/ 24 weeks | ↓ BMI<br>↓ WC | ↓ IHF* | ↓ AST, ALT*<br>↓ HbA1c* | - | Metformin decreased liver aminotransferase level and hepatic fat content. |
| Zhang *et al.* (2017)[46] | T2DM with NAFLD (n=50) | CT (L/S CT ratio <1) | Randomized study | 1,500 mg/day/ 24 weeks | ↓ BMI | ↑ L/S CT ratio | ↓ AST, ALT<br>↓ HbA1c<br>↓ HOMA-IR | ↓ LSM | Metformin was effective in reducing liver enzymes, fat content and hepatic fibrosis. |
| Yabiku *et al.* (2017)[49] | Male T2DM with NAFLD (n=92) | US | Randomized study | 1,000 mg/days/ 24 weeks | ↓ BMI | ↑ L/S CT ratio* | ↓ AST, ALT<br>↓ HOMA-IR | - | Metformin improved liver enzymes and hepatic fat content. |
| Tian *et al.* (2018)[50] | T2DM with NAFLD (n=75) | US | Randomized study | 1,000-1,500 mg/day/ 12 weeks | ↓ BMI | - | ↓ AST, ALT<br>↓ HbA1c<br>↓ HOMA-IR | - | Metformin decreased liver enzymes. |
| Zsóri *et al.* (2019)[53] | New-onset T2DM (n=11) | CT | Prospective study | 1,000 mg/day/ 16 weeks | ↔ BMI | ↑ CT radiation absorption* | ↔ HbA1c<br>↔ HOMA-IR | - | Metformin treatment lowered the amount of fat in the liver of patients with new-onset T2DM. |
| Studies showing uncertain/negative results of metformin use | | | | | | | | | |
| Omer *et al.* (2010)[51] | T2DM or IGT with NASH and elevated ALT (n=22) | Histologic diagnosis of NASH (NAS ≥5) | Open-label randomized study | 1,700 mg/day/ 48 weeks | ↓ BMI<br>↓ WC | - | ↔ AST, ALT*<br>↔ HbA1c<br>↔ HOMA-IR | ↔ NAS*<br>↔ Fibrosis (n=10) | Metformin did not improve transaminase levels and histological NASH score. |
| Shibuya *et al.* (2018)[52] | T2DM with NAFLD (n=16) | US or CT | Randomized open-label study | 1,500 mg/day/ 24 weeks | ↔ BMI | ↓ L/S CT ratio* | ↔ ALT<br>↔ HbA1c | - | Metformin had no beneficial effects on transaminase level and hepatic fat content. |

ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; HbA1c, hemoglobin A1c; HOME-IR, homeostatic model assessment for insulin resistance; IGT, impaired glucose tolerance; IHF, intrahepatic fat; L/S CT ratio, liver/spleen liver/spleen computed tomography (CT) ratio; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PO, per os; T2DM, type 2 diabetes mellitus; US, ultrasonography; WC, waist circumference; ↓, significant decrease; ↑, significant increase; ↔, no significant change.
*An asterisk symbol in the major finding column indicates the primary outcomes in that report.

**Table 5.** Effects of Metformin and Other Antidiabetic Drugs on the Liver in Diabetic NAFLD Patients

| Author (year) | Populations | Method of NAFLD diagnosis | Design | Intervention | Major findings | | | | Interpretations |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Body anthropometry | Liver fat contents | Biochemistry | Liver fibrosis and histology | |
| Fan *et al.* (2013)[47] | T2DM with NAFLD (n=117) | US | Randomized study | Metformin (n=68) | ↓ BMI | - | ↓ AST, ALT*<br>↓ HbA1c<br>↓ HOMA-IR | - | Exenatide was more effective than metformin in improving hepatic enzymes. |
| | | | | Exenatide (n=49) for 12 weeks | ↓↓ BMI | - | ↓↓ AST, ALT*<br>↓ HbA1c<br>↓ HOMA-IR | - | |
| Yabiku *et al.* (2017)[49] | Male T2DM with NAFLD (n=366) | US | Randomized study (compared to no treatment; n=91) | Metformin (n=92) | ↓ BMI | ↑ L/S CT* | ↓↓ AST<br>↓↓ ALT<br>↓↓ HOMA-IR | - | Pioglitazone elicited greatest improvement of liver fat content and hepatic enzymes compared to metformin and Sitagliptin. |
| | | | | Pioglitazone (n=91) | ↑ BMI | ↑ L/S CT* | ↓↓↓ AST<br>↓↓↓ ALT<br>↓↓↓ HOMA-IR | - | |
| | | | | Sitagliptin (n=92) for 6 months | ↔ BMI | ↔ L/S CT* | ↓ AST<br>↓ ALT<br>↓ HOMA-IR | - | |
| Shibuya *et al.* (2018)[52] | T2DM with NAFLD (n=32) | US or CT | Randomized open-label study | Metformin (n=16) | ↔ BMI | ↓ L/S CT ratio* | ↔ ALT<br>↔ HbA1c | - | Luseogliflozin was more effective than metformin in reducing liver fat deposition. |
| | | | | Luseogliflozin (n=16) for 6 months | ↓ BMI | ↑ L/S CT ratio* | ↔ ALT<br>↓ HbA1c | - | |
| Feng *et al.* (2017)[48] | T2DM with NAFLD (n=87) | Quantitative US method with IHF ≥10% | Randomized open-label study | Metformin (n=29) | ↓ BMI<br>↓ WC | ↓↓ IHF* | ↓↓ AST, ALT*<br>↓ HbA1c* | - | Liraglutide provided greater improvement in liver function and IHF contents than metformin. |
| | | | | Liraglutide (n=29) | ↓ BMI<br>↓ WC | ↓↓↓ IHF* | ↓↓ AST, ALT*<br>↓ HbA1c* | - | |
| | | | | Gliclazide (n=29) for 24 weeks | ↔ BMI<br>↔ WC | ↓ IHF* | ↔ AST, ALT*<br>↓ HbA1c* | - | |
| Tian *et al.* (2018)[50] | T2DM with NAFLD (n=127) | US | Randomized study | Metformin (n=75) | ↓ BMI | - | ↓ ALT<br>↓ HbA1c<br>↓ HOMA-IR | - | Liraglutide was more effective than metformin in decreasing ALT levels. |
| | | | | Liraglutide (n=52) for 12 weeks | ↓↓ BMI | - | ↓↓ ALT<br>↓ HbA1c<br>↓ HOMA-IR | - | |
| Omer *et al.* (2010)[51] | T2DM or IGT with NASH and elevated ALT (n=42) | Histologic diagnosis of NASH (NAS ≥5) | Open-label randomized study | Metformin (n=22) | ↓ BMI<br>↓ WC | - | ↔ AST, ALT*<br>↔ HbA1c<br>↔ HOMA-IR | ↔ NAS*<br>↔ Fibrosis (n=10) | Rosiglitazone was more effective than metformin in improving liver enzymes and histology. |
| | | | | Rosiglitazone (n=20) for 48 weeks | ↔ BMI<br>↔ WC | - | ↓ AST, ALT*<br>↔ HbA1c<br>↔ HOMA-IR | ↓ NAS*<br>↔ Fibrosis (n=13) | |

ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; HbA1c, hemoglobin A1c; HOME-IR, homeostatic model assessment for insulin resistance; IGT, impaired glucose tolerance; IHF, intrahepatic fat; L/S CT ratio, liver/spleen liver/spleen computed tomography (CT) ratio; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; PO, per os; SC, subcutaneous injection; T2DM, type 2 diabetes mellitus; US, ultrasonography; WC, waist circumference; ↓, significant decrease; ↑, significant increase; ↔, no significant change.
*An asterisk symbol in the major finding column indicates the primary outcomes in that report.

**Table 6.** Effects of Combined Treatment with Metformin and Other Antidiabetic Drugs on the Liver in Diabetic NAFLD Patients

| Author (year) | Populations | Method of NAFLD diagnosis | Design | Intervention | Major findings | | | | Interpretations |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Body anthropometry | Liver fat contents | Biochemistry | Liver fibrosis and histology | |
| Omer *et al.* (2010)[51] | T2DM or IGT with NASH and elevated ALT (n=22) | Histologic diagnosis of NASH (NAS ≥5) | Open-label randomized study | Metformin+ Rosiglitazone (n=22) | ↓ BMI<br>↓ WC | - | ↓ AST, ALT*<br>↔ HbA1c<br>↔ HOMA-IR | ↓ NAS*<br>↔ Fibrosis (n=12) | Combination of metformin and rosiglitazone improved liver enzymes and liver histology. |
| Choi *et al.* (2018)[58] | T2DM with NAFLD and elevated ALT (n=102) | US | Retrospective study | Metformin+ Dapagliflozin (n=50) | ↓ BW | - | ↓ AST*<br>↓ ↓ ALT* | - | Combination of metformin and dapagliflozin was more effective in improving liver biochemistry than a combination of metformin and DPP4i. |
| | | | | Metformin+ Sitagliptin or Linagliptin (n=52) | ↔ BW | - | ↓ AST*<br>↓ ALT* | - | |
| Cuthbertson *et al.* (2012)[56] | T2DM and NAFLD treated with metformin (n=25) | $^{1}$H MRS with IHL >5.5% | Prospective single arm study | Exenatide (n=19) or Liraglutide (n=6) | ↓ BMI<br>↓ WC | ↓ IHL by $^{1}$H MRS* | ↔ AST<br>↓ ALT<br>↓ HbA1c<br>↑ Adiponectin | - | GLP1-RA added to metformin treatment decreased liver fat content and ALT. |
| Yan *et al.* (2019)[57] | T2DM with NAFLD treated with metformin (n=65) | MRI-PDFF >10% | Randomized study | Liraglutide (n=18) | ↓ BMI<br>↓ WC | ↓ MRI-PDFF* | ↔ AST, ALT<br>↓ HbA1C<br>↓ HOMA-IR<br>↓ Serum IL-6 | ↔ FIB-4<br>↔ NFS | Combination of Metformin with liraglutide and sitagliptin reduced IHL. |
| | | | | Sitagliptin (n=26) | ↓ BMI<br>↓ WC | ↓ MRI-PDFF* | ↓ AST, ALT<br>↓ HbA1C<br>↔ HOMA-IR<br>↔ Serum IL-6 | ↔ FIB-4<br>↔ NFS | |
| | | | | Insulin glargine (n=21) | ↔ BMI<br>↓ WC | ↔ MRI-PDFF* | ↔ AST, ALT<br>↓ HbA1C<br>↔ HOMA-IR<br>↔ Serum IL-6 | ↔ FIB-4<br>↔ NFS | |

ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; DPP4i, dipeptidyl peptidase-4 inhibitors; FIB-4, fibrosis-4 index; GLP1-RA, glucagon-like peptide-1 receptor agonists; $^{1}$H MRS, proton magnetic resonance spectroscopy; HbA1c, hemoglobin A1c; HOME-IR, homeostatic model assessment for insulin resistance; IGT, impaired glucose tolerance; IHL, intrahepatocellular lipid; IL, interleukin; MRI-PDFF, magnetic resonance imaging-estimated proton density fat fraction; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; T2DM, type 2 diabetes mellitus; US, ultrasonography; WC, waist circumference; ↓, significant decrease; ↑, significant increase; ↔, no significant change.
*An asterisk symbol in the major finding column indicates the primary outcomes in that report.

### 2. Effects of metformin compared to other antidiabetic drugs in NAFLD patients with diabetes

In the past decades, new classes of antidiabetic drugs have been approved to be used in T2DM patients. Although metformin as a monotherapy has been shown to reduce hepatic steatosis and improve liver biochemistry in diabetic NAFLD patients, the magnitude of the benefits seems to be more subtle than the newer antidiabetic drugs. These newer agents include thiazolidinediones,[49,51] glucagon-like peptide-1 (GLP-1) receptor agonists,[47,48,50] and sodium-glucose co-transporter-2 (SGLT2) inhibitors.[52]

Sitagliptin, a dipeptidyl peptidase-4 inhibitor (DPP4i), had less potency than metformin in reducing liver enzymes.[49] Gliclazide, a drug in sulfonylurea class, was able to decrease hepatic fat content but to a lower extent than that observed after metformin therapy.[48] A summary of reports comparing the effects of metformin to other antidiabetic drugs on the liver in diabetic NAFLD patients is shown in Table 5.

### 3. Effects of combined treatment with metformin and other antidiabetic drugs in NAFLD patients with diabetes

A combination of metformin with other antidiabetic drugs in NAFLD patients with diabetes had been studied and reported. Since most of diabetic patients had previously received metformin, enrollment of these patients with other antidiabetic medications being added on was common across most of these studies. Addition of thiazolidinediones,[51] GLP-1 receptor agonists,[56,57] DPP4i[57] and SGLT2i[58] all provided additional benefit to metformin as a monotherapy. However, it should be noted that the synergistic effect of metformin added on to other antidiabetic medications has not yet been reported. Insulin glargine treatment did not improve NAFLD parameters further, as insulin treatment did not affect the insulin resistance nor body weight reduction.[57] A summary of the reports regarding the effects of a combined treatment with metformin and other antidiabetic drugs on the liver in NAFLD patients with diabetes is shown in Table 6.

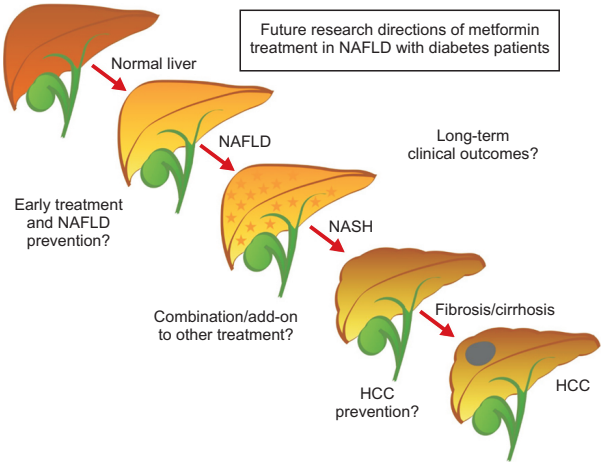


**Fig. 2.** Future directions.
NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma.

## EFFECTS OF METFORMIN ON HCC DEVELOPMENT

The use of metformin was associated with a reduced risk of hepatocellular carcinoma (HCC).[59] Several epidemiological studies suggested that metformin had potential antitumor effect with potential effects in cancer prevention.[60,61] A large matched-paired cohort conducted in Taiwan found that metformin was associated with HCC incidence reduction in patients with T2DM with a hazard ratio of 0.76 (0.67 to 0.85).[62] In a mouse model of NASH and liver tumor, metformin decreased the proportion of tumor-carrying mice.[63] However, this effect was not observed in the liver in mice that had already developed NAFLD.[63] Another study of a HFD-fed, HCC model of transgenic zebrafish demonstrated the HFD enhanced malignancy-related histologic and morphologic features.[64] Metformin treatment reduced liver size and reversed the diet-induced increase in steatosis, vessel formation, and inflammation and restored T cell infiltration.[64] These results suggested potential benefits of metformin in the prevention of HFD-induced liver tumorigenesis and progression, especially if administered early prior to the onset of NAFLD. Further studies are needed to warrant this benefit of metformin as regards liver cancer.

## CONCLUSION AND FUTURE PERSPECTIVES

Metformin treatment was shown to be effective in alleviating hepatic lipogenesis in animal models of NAFLD through various mechanisms. However, in clinical studies, metformin could modestly reduce the BMI, liver fat content, and liver enzymes in NAFLD patients with diabetes. Despite these reports on benefits of metformin, some contradicting reports still exist. Combination treatments with other antidiabetic drugs, especially the drugs in the thiazolidinediones, GLP-1 receptor agonists and SGLT2 inhibitors groups demonstrated increased efficacy. Among diabetic patients with biopsy-proven NASH, currently available data from a small enrolled study suggested that

metformin was not associated with histologic or liver fibrosis improvement. Further research with a larger sample size is warranted to confirm these findings. A long-term clinical study to evaluate liver-related complications, and a study to elucidate the role of metformin in HCC prevention are necessary. Summaries of the future directions are shown in Fig. 2. Nevertheless, there is a potential benefit in the continued use of metformin in NAFLD patients with diabetes, either alone or in combination with other antidiabetic drugs.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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## REFERENCES

1. Allen AM, Therneau TM, Larson JJ, Coward A, Somers VK, Kamath PS. Nonalcoholic fatty liver disease incidence and impact on metabolic burden and death: a 20 year-community study. Hepatology 2018;67:1726-1736.
2. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183-1192.
3. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011;140:124-131.
4. Lomonaco R, Bril F, Portillo-Sanchez P, et al. Metabolic impact of nonalcoholic steatohepatitis in obese patients with type 2 diabetes. Diabetes Care 2016;39:632-638.
5. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017;60:1577-1585.
6. Li Y, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Biomed Rep 2013;1:57-64.
7. Haukeland JW, Konopski Z, Eggesbø HB, et al. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. Scand J Gastroenterol 2009;44:853-860.
8. Uygun A, Kadayifci A, Isik AT, et al. Metformin in the treatment of patients with non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2004;19:537-544.
9. Bugianesi E, Gentilcore E, Manini R, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol 2005;100:1082-1090.
10. Duseja A, Das A, Dhiman RK, et al. Metformin is effective in achieving biochemical response in patients with nonalcoholic fatty liver disease (NAFLD) not responding to lifestyle interventions. Ann Hepatol 2007;6:222-226.
11. Nair S, Diehl AM, Wiseman M, Farr GH Jr, Perrillo RP. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. Aliment Pharmacol Ther 2004;20:23-28.
12. Shields WW, Thompson KE, Grice GA, Harrison SA, Coyle WJ. The effect of metformin and standard therapy versus standard therapy alone in nondiabetic patients with insulin resistance and nonalcoholic ateatohepatitis (NASH): a pilot trial. Therap Adv Gastroenterol 2009;2:157-163.
13. Song YM, Lee YH, Kim JW, et al. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. Autophagy 2015;11:46-59.
14. Fullerton MD, Galic S, Marcinko K, et al. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. Nat Med 2013;19:1649-1654.
15. Ford RJ, Fullerton MD, Pinkosky SL, et al. Metformin and salicylate synergistically activate liver AMPK, inhibit lipogenesis and improve insulin sensitivity. Biochem J 2015;468:125-132.
16. Huang H, Lee SH, Sousa-Lima I, et al. Rho-kinase/AMPK axis regulates hepatic lipogenesis during overnutrition. J Clin Invest 2018;128:5335-5350.
17. Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biol Rev Camb Philos Soc 2016;91:452-468.
18. Brownsey RW, Boone AN, Elliott JE, Kulpa JE, Lee WM.

Regulation of acetyl-CoA carboxylase. Biochem Soc Trans 2006;34(Pt 2):223-227.
19. Viollet B, Foretz M, Guigas B, et al. Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. J Physiol 2006;574(Pt 1):41-53.
20. Li YL, Li XQ, Wang YD, Shen C, Zhao CY. Metformin alleviates inflammatory response in non-alcoholic steatohepatitis by restraining signal transducer and activator of transcription 3-mediated autophagy inhibition in vitro and in vivo. Biochem Biophys Res Commun 2019;513:64-72.
21. Geng Y, Hernández Villanueva A, Oun A, et al. Protective effect of metformin against palmitate-induced hepatic cell death. Biochim Biophys Acta Mol Basis Dis 2020;1866:165621.
22. Guo J, Zhou Y, Cheng Y, et al. Metformin-induced changes of the coding transcriptome and non-coding RNAs in the livers of non-alcoholic fatty liver disease mice. Cell Physiol Biochem 2018;45:1487-1505.
23. Tang X, Li J, Xiang W, et al. Metformin increases hepatic leptin receptor and decreases steatosis in mice. J Endocrinol 2016;230:227-237.
24. Karavia EA, Hatziri A, Kalogeropoulou C, et al. Deficiency in apolipoprotein A-I ablates the pharmacological effects of metformin on plasma glucose homeostasis and hepatic lipid deposition. Eur J Pharmacol 2015;766:76-85.
25. Khalaf HM, Ibrahim MA, Amin EF, Abdel-Tawab Ibrahim S, Abdel-Wahab S, Fouad YM. Allopurinol potentiates the hepatoprotective effect of metformin and vitamin E in fructose-induced fatty liver in rats. Clin Exp Hepatol 2019;5:65-74.
26. Brandt A, Hernández-Arriaga A, Kehm R, et al. Metformin attenuates the onset of non-alcoholic fatty liver disease and affects intestinal microbiota and barrier in small intestine. Sci Rep 2019;9:6668.
27. Shin NR, Bose S, Wang JH, et al. Flos Lonicera combined with metformin ameliorates hepatosteatosis and glucose intolerance in association with gut microbiota modulation. Front Microbiol 2017;8:2271.
28. de Jesús Acosta-Cota S, Aguilar-Medina EM, Ramos-Payán R, et al. Therapeutic effect of treatment with metformin and/or 4-hydroxychalcone in male Wistar rats with nonalcoholic fatty liver disease. Eur J Pharmacol 2019;863:172699.
29. Mahzari A, Li S, Zhou X, et al. Matrine protects against MCD-induced development of NASH via upregulating HSP72 and downregulating mTOR in a manner distinctive from metformin. Front Pharmacol 2019;10:405.
30. Sui Y, Kong X, Fan R, et al. Long-term treatment with metformin in the prevention of fatty liver in Zucker diabetic fatty rats. Diabetol Metab Syndr 2019;11:94.
31. Matafome P, Louro T, Rodrigues L, et al. Metformin and atorvastatin combination further protect the liver in type 2 diabetes with hyperlipidaemia. Diabetes Metab Res Rev 2011;27:54-62.
32. Hawley SA, Gadalla AE, Olsen GS, Hardie DG. The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. Diabetes 2002;51:2420-2425.
33. Li Y, Xu S, Mihaylova MM, et al. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab 2011;13:376-388.
34. Polyzos SA, Kountouras J, Mantzoros CS. Leptin in nonalcoholic fatty liver disease: a narrative review. Metabolism 2015;64:60-78.
35. Polyzos SA, Aronis KN, Kountouras J, Raptis DD, Vasiloglou MF, Mantzoros CS. Circulating leptin in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Diabetologia 2016;59:30-43.
36. Fishman S, Muzumdar RH, Atzmon G, et al. Resistance to leptin action is the major determinant of hepatic triglyceride accumulation in vivo. FASEB J 2007;21:53-60.
37. Yokoi N, Hoshino M, Hidaka S, et al. A novel rat model of type 2 diabetes: the Zucker Fatty Diabetes Mellitus ZFDM rat. J Diabetes Res 2013;2013:103731.
38. Stachowicz A, Suski M, Olszanecki R, Madej J, Okoń K, Korbut R. Proteomic analysis of liver mitochondria of apolipoprotein E knockout mice treated with metformin. J Proteomics 2012;77:167-175.
39. Bradham CA, Plümpe J, Manns MP, Brenner DA, Trautwein C. Mechanisms of hepatic toxicity. I. TNF-induced liver injury. Am J Physiol 1998;275:G387-G392.
40. Kakino S, Ohki T, Nakayama H, et al. Pivotal role of TNF-α in the development and progression of nonalcoholic fatty liver disease in a murine model. Horm Metab Res 2018;50:80-87.
41. Singh R. Autophagy and regulation of lipid metabolism. Results Probl Cell Differ 2010;52:35-46.
42. You L, Wang Z, Li H, et al. The role of STAT3 in autophagy. Autophagy 2015;11:729-739.
43. Karavia EA, Papachristou DJ, Liopeta K, Triantaphyllidou IE, Dimitrakopoulos O, Kypreos KE. Apolipoprotein A-I modulates processes associated with diet-induced nonalcoholic fatty liver disease in mice. Mol Med 2012;18:901-912.
44. Martínez-Chantar ML, Vázquez-Chantada M, Ariz U, et al. Loss of the glycine N-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. Hepatology 2008;47:1191-1199.
45. Bäckhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718-15723.
46. Zhang R, Cheng K, Xu S, et al. Metformin and diammonium glycyrrhizinate enteric-coated capsule versus metformin

alone versus diammonium glycyrrhizinate enteric-coated capsule alone in patients with nonalcoholic fatty liver disease and type 2 diabetes mellitus. Gastroenterol Res Pract 2017;2017:8491742.
47. Fan H, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. Arq Bras Endocrinol Metabol 2013;57:702-708.
48. Feng W, Gao C, Bi Y, et al. Randomized trial comparing the effects of gliclazide, liraglutide, and metformin on diabetes with non-alcoholic fatty liver disease. J Diabetes 2017;9:800-809.
49. Yabiku K, Mutoh A, Miyagi K, Takasu N. Effects of oral antidiabetic drugs on changes in the liver-to-spleen ratio on computed tomography and inflammatory biomarkers in patients with type 2 diabetes and nonalcoholic fatty liver disease. Clin Ther 2017;39:558-566.
50. Tian F, Zheng Z, Zhang D, He S, Shen J. Efficacy of liraglutide in treating type 2 diabetes mellitus complicated with non-alcoholic fatty liver disease. Biosci Rep 2018;38:BSR20181304.
51. Omer Z, Cetinkalp S, Akyildiz M, et al. Efficacy of insulin-sensitizing agents in nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2010;22:18-23.
52. Shibuya T, Fushimi N, Kawai M, et al. Luseogliflozin improves liver fat deposition compared to metformin in type 2 diabetes patients with non-alcoholic fatty liver disease: a prospective randomized controlled pilot study. Diabetes Obes Metab 2018;20:438-442.
53. Zsóri G, Illés D, Iványi E, et al. In new-onset diabetes mellitus, metformin reduces fat accumulation in the liver, but not in the pancreas or pericardium. Metab Syndr Relat Disord 2019;17:289-295.
54. Wang DS, Jonker JW, Kato Y, Kusuhara H, Schinkel AH, Sugiyama Y. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. J Pharmacol Exp Ther 2002;302:510-515.
55. Morse BL, Kolur A, Hudson LR, et al. Pharmacokinetics of Organic Cation Transporter 1 (OCT1) substrates in Oct1/2 knockout mice and species difference in hepatic OCT1-mediated uptake. Drug Metab Dispos 2020;48:93-105.
56. Cuthbertson DJ, Irwin A, Gardner CJ, et al. Improved glycaemia correlates with liver fat reduction in obese, type 2 diabetes, patients given glucagon-like peptide-1 (GLP-1) receptor agonists. PLoS One 2012;7:e50117.
57. Yan J, Yao B, Kuang H, et al. Liraglutide, sitagliptin, and insulin glargine added to metformin: the effect on body weight and intrahepatic lipid in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. Hepatology 2019;69:2414-2426.
58. Choi DH, Jung CH, Mok JO, Kim CH, Kang SK, Kim BY. Effect of dapagliflozin on alanine aminotransferase improvement in type 2 diabetes mellitus with non-alcoholic fatty liver disease. Endocrinol Metab (Seoul) 2018;33:387-394.
59. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Antidiabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. Am J Gastroenterol 2013;108:881-891.
60. Noto H, Goto A, Tsujimoto T, Noda M. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. PLoS One 2012;7:e33411.
61. Kim HJ, Lee S, Chun KH, et al. Metformin reduces the risk of cancer in patients with type 2 diabetes: an analysis based on the Korean National Diabetes Program Cohort. Medicine (Baltimore) 2018;97:e0036.
62. Tseng CH. Metformin and risk of hepatocellular carcinoma in patients with type 2 diabetes. Liver Int 2018;38:2018-2027.
63. Tajima K, Nakamura A, Shirakawa J, et al. Metformin prevents liver tumorigenesis induced by high-fat diet in C57Bl/6 mice. Am J Physiol Endocrinol Metab 2013;305:E987-E998.
64. de Oliveira S, Houseright RA, Graves AL, et al. Metformin modulates innate immune-mediated inflammation and early progression of NAFLD-associated hepatocellular carcinoma in zebrafish. J Hepatol 2019;70:710-721.