Impact of Sodium Glucose Cotransporter 2 Inhibitors on Nonalcoholic Fatty Liver Disease Complicated by Diabetes Mellitus

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Sodium glucose cotransporter 2 (SGLT2), a type of membrane protein highly expressed in the kidney, can regulate plasma glucose through the glomerular filtration process by reabsorption from the kidney. SGLT2 inhibitors, which are newly developed oral antidiabetic drugs, can play a role in liver diseases by inhibiting SGLT2-mediated renal glucose reabsorption and inducing glycosuria. Nonalcoholic fatty liver disease (NAFLD) is the most common type of liver disease, resulting in severe liver dysfunction. During the progression of NAFLD, there are some hallmark complications, including lipid metabolism disorders, inflammation induction, and hepatocyte death. Herein, we review several SGLT2 inhibitors that are capable of protecting individuals with NAFLD from severe complications by inhibiting *de novo* lipogenesis, oxidative responses, inflammation induction, and hepatocyte death. (*Hepatology Communications* 2021;5:736-748).

Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by hyperglycemia resulting from insufficient insulin secretion and insulin resistance.[1] T2DM can cause severe complications, including diabetic kidney diseases,[2] cardiovascular diseases,[3] liver diseases (including nonalcoholic fatty liver disease [NAFLD] and liver cancer),[4] and neurosystem syndrome, such as central diabetes insipidus.[5] Over the past several years, the total prevalence of T2DM has greatly increased, and it is now a burden disease globally. The complicated pathogenesis of T2DM means multiple medications may be needed in order to control blood glucose levels (Fig. 1).

Clinically, there are several antidiabetic drugs targeting different organs, including liver, adipocytes, intestine, and pancreas. Generic and branded antidiabetic drugs fall under several types: (1) Biguanides are incredibly common and include metformin, which was reported to lower hepatic glucose production.[6] (2) Sulfonylureas, which were first developed in the 1950s, include glipizide, glyburide, chlorpropamide, and tolbbutamide. Mechanically, this type of drug improved diabetes mainly by increasing the release of insulin from the pancreas.[7] (3) Alpha-glucosidase inhibitors include acarbose and miglitol and mainly target the intestine by competitive and reversible inhibition of alpha-glucosidase in the intestine, which leads to a lower rate of glucose absorption through delayed carbohydrate digestion and extended digestion time.[8] (4) Thiazolidinediones, such as pioglitazone, can bind to a receptor called peroxisome proliferator activated receptor gamma in adipocytes and then, following the sensitivity to insulin, promote fat cell maturation and fat deposition into peripheral tissues.[9] (5) Dipeptidyl

Abbreviations: ACC, acetyl-coenzyme A carboxylase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMPK, adenosine monophosphate–activated protein kinase; AST, aspartate aminotransferase; CD, cluster of differentiation; ChREBP, carbohydrate responsive element binding protein; CoA, coenzyme A; CPT1, carnitine palmitoyl transferase 1; DAG, diacylglycerol; db, diabetic; DGAT, diacylglycerol acyltransferase; DNL, de novo lipogenesis; FA-CoA, fatty-acyl coenzyme A; GGT, gamma-glutamyltransferase; GPAT, glycerol-3-phosphate acyltransferase; HFD, high-fat diet; IFN-γ, interferon-gamma; IL, interleukin; LD, lipid droplet; LPA, lysophosphatidic acid; MAG, monoacylglycerol; MCP-1, monocyte chemoattractant protein 1; MGAT1, monoacylglycerol acyltransferase; miR, microRNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; OxS, oxidative stress; pro, proinflammatory; ROS, reactive oxygen species; SCD1, stearoyl-coenzyme A desaturase 1; SGLT2, sodium-glucose transport protein 2; SOD, superoxide dismutase; SREBP1c, sterol regulatory element binding protein 1c; T2DM, type 2 diabetes mellitus; TAG, triacylglycerol; TCA, tricarboxylic acid; TNF, tumor necrosis factor; VLDL, very low-density lipoprotein.

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peptidase 4 inhibitors, such as sitagliptin, saxagliptin, and vildagliptin, can induce inactivation of incretins, such as glucagon-like peptide 1 and gastric inhibitory polypeptide, following induction of insulin secretion from pancreas and inhibition of glucagon release, which help lower blood glucose.[10]

Excitingly, several types of medicine for T2DM have recently been approved by the U.S. Food and Drug Administration. Sodium-glucose transport protein 2 (SGLT2) inhibitors, such as canagliflozin (Invokana), dapagliflozin (Farxiga), and empagliflozin (Jardiance), can reduce blood glucose levels.[11-13] Other inhibitors are waiting for clinical approval. These include ipragliflozin, tofogliflozin, and luseogliflozin (Fig. 2).

SGLT2, a type of membrane protein highly expressed in the proximal tubules, can regulate plasma glucose in the glomerular filtration process by reabsorption from kidney.[14] This means that inhibiting the level of SGLT2 to control blood glucose level could potentially be used to cure T2DM. Currently, SGLT2 inhibitors are structurally classified into C-glucoside,[15] O-glucoside,[16] N-glucoside,[17] and nonglucoside.[18] Of these, the C-glucoside type, including dapagliflozin, canagliflozin, ipragliflozin, empagliflozin, tofogliflozin, and luseogliflozin, is widely used due to its higher chemical and metabolic stability.[19]

Over the past several years, much research has been undertaken on the role of SGLT2 inhibitors in the treatment of T2DM. SGLT2 inhibitors represent a new class of oral diabetic medication that reduces hyperglycemia by suppressing the reabsorption of glucose in the proximal tubules and improving insulin resistance, glucotoxicity, and lipotoxicity.[20-22] The current review is mainly focused on the potential roles of SGLT2 inhibitors in chronic liver diseases (NAFLD) and related mechanisms.

NAFLD is the most common type of liver disease, affecting 25% of the general population worldwide[23] and 80% of patients with obesity.[24] It is closely associated with a group of disorders that include obesity and T2DM. Although simple steatosis in the initial stage may not cause severe complications, it will develop to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and possibly liver cancer. The number of patients with NASH waiting for a liver transplant has grown dramatically over the last decade, surpassing alcoholic liver diseases except for hepatitis

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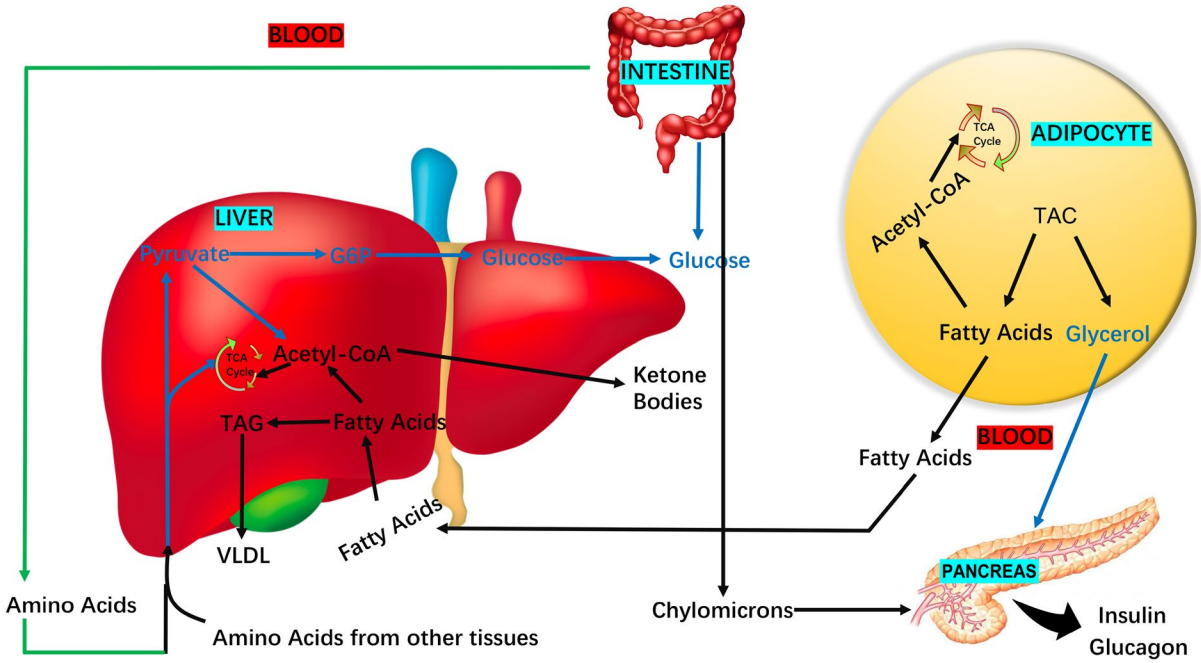

FIG. 1. Intertissue relationships regarding glucose and lipids metobolism. Abbreviation: G6P, glucose 6-phosphate.

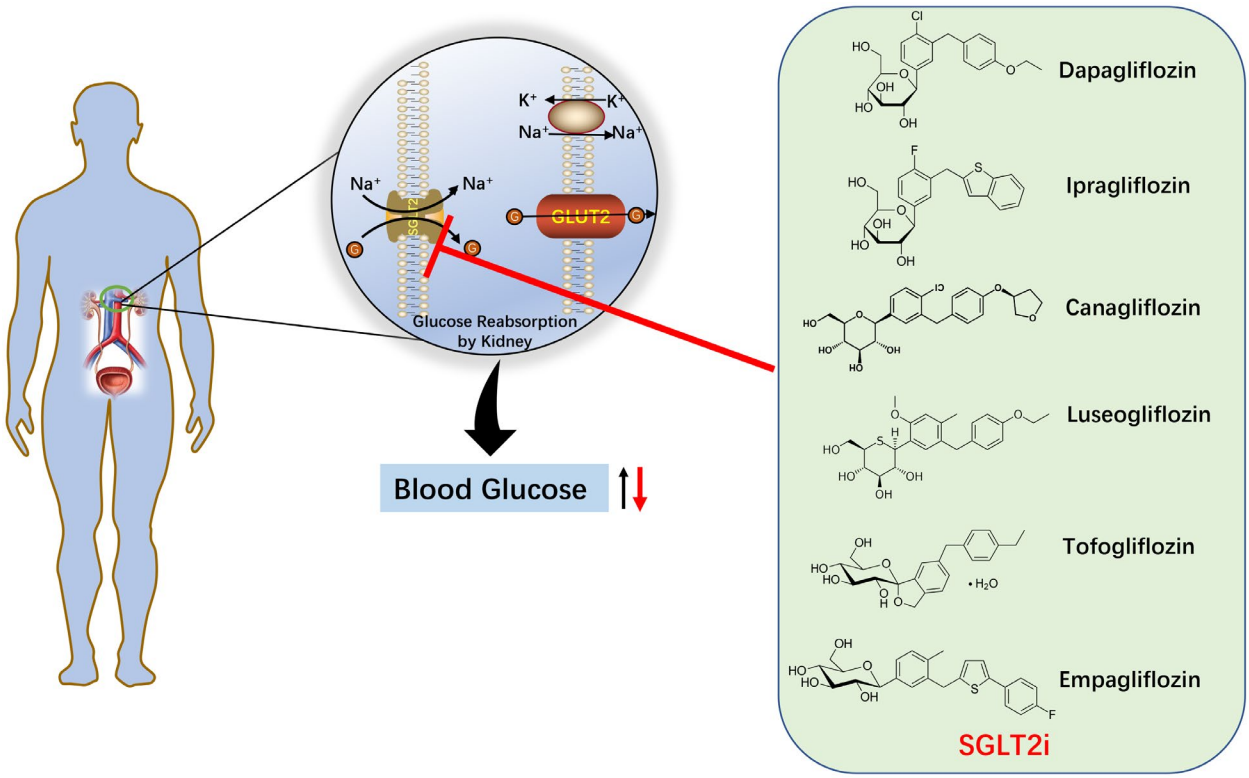

FIG. 2. Mechanims of SGLT2 inhibitors and its structure. Abbreviation: GLUT2, glucose transporter 2.

C.[25] During the progression of NASH, abnormal lipid accumulation, one of the disease's key features, combined with inflammatory response, oxidative response, and fibrosis, make it a risk factor for hepatic cirrhosis and hepatocellular carcinoma (HCC). Based on the mechanisms of NASH, some results have indicated that patients with NAFLD could benefit from body weight loss; a moderate caloric restriction of around 1,200 kcal/day would be sufficient to decrease lipid droplet (LD) accumulation following normalized insulin level.[26] However, there are as yet no established treatments for NASH even though several novel drugs are under clinical trials, including an inhibitor of apoptosis signal regulating kinase-1, a farnesoid X receptor agonist, and a fibroblast growth factor 19 agonist. Therefore, searching for novel drugs for NASH is an urgent task.[27]

SGLT2 inhibitors, which are newly developed, oral, antidiabetic drugs, in liver diseases could inhibit SGLT2-mediated renal glucose reabsorption and induce glycosuria.[28] SGLT2, one type of membrane protein highly expressed in the kidney, could regulate plasma glucose in the glomerular filtration process by reabsorption from the kidney. SGLT2 inhibitors have been shown to have roles in reducing the renal threshold for glucose, thus allowing excretion of glucose into urine,[29] which is beneficial to the patient with obesity and T2DM. In terms of roles in improving obesity, SGLT2 inhibitors could be useful for the treatment of NAFLD. Recently, SGLT2 inhibitors have been demonstrated to benefit the liver in mouse models of NASH by reducing body weight[30]; reducing some liver enzyme levels in serum, including alanine aminotransferase [ALT] and aspartate aminotransferase [AST][31,32]; and lowering LD accumulation,[33] oxidative response,[34] and inflammation induction.[35] In addition, some clinical cases have shown that patients suffering from NASH are benefiting from some SGLT2 inhibitors.[36-40]

Histopathologic Improvement of NAFLD With SGLT2 Inhibitors

Nonalcoholic liver diseases cover a wide spectrum of liver pathologies ranging from nonalcoholic fatty liver, liver cirrhosis, HCC, and liver failure, even without excessive alcohol intake. One study[41] found that histopathologic features in 5 patients showed a decrease in steatosis score, lobular inflammation, ballooning and fibrosis stage after 24 weeks of treatment. During their research, 6 patients showed decreases in serum exosome of microRNA-122 (miR-122) ratios along with histologic improvement after 1 day of SGLT2 inhibitors. They also found that miR-122 in serum is associated with the histologic severity of liver disease and the risk of metabolic syndrome development and evidenced the usefulness of serum exosome miR-122 as an early predictor of histologic improvement. However, long-term perspective research should be applied to confirm the impacts of SGLT2 inhibitors on histologic improvement, including glucose metabolism.[42] Additionally, Lai et al.[43] reported that a 6-month treatment of empagliflozin could significantly improve steatosis, hepatocyte ballooning, and fibrosis in a small cohort of patients with biopsy-proven NASH with T2DM. In summary, SGLT2 inhibitors show significant potential in the improvement of NAFLD/NASH and T2DM.

Key Regulatory Mechanisms of SGLT2 Inhibitors Involved in the Prevention of NAFLD/NASH

TARGETING LIVER FUNCTION AND LIVER ENZYMES

Liver is one of the main organs in our body metabolizing carbohydrate, protein, and fats[44]; therefore, maintaining normal liver function is important to ensure quality of life. Patients with NASH suffer from liver dysfunction, so targeting liver function would seem to be a suitable method for therapy for these patients. Because of the metabolic role of liver, a number of enzymes in the liver and some products of the metabolic pathway that are very sensitive to abnormalities are considered to be biochemical markers of liver dysfunction; these include ALT, AST, ratio of aminotransferases (ALT/AST), alkaline phosphatase (ALP), and

gamma-glutamyltransferase (GGT). Patients with NAFLD/NASH and related liver dysfunction have a high level of ALT, AST, ALP, and GGT compared to the normal condition.[45] Excitingly, treatment with SGLT2 inhibitors could correct the abnormal levels of these liver enzymes.

Based on results from clinical cases, doses of 50 mg/day of ipragliflozin for 3 months,[36] 6 months,[46] and 12 months[47]; 5 mg/day[48] and 10 mg/day[37] of dapagliflozin for 6 months; 100 mg/day of canagliflozin for 6 months[40] and 12 months,[49]; 10 mg/day of empagliflozin for 6 months[39]; and 2.5 mg/day of luseogliflozin for 6 months[50] could normalize the level of ALT, AST, ALP, and GGT and induce a loss of body weight. In addition, treatment with an SGLT2 inhibitor could decrease hepatic triacylglycerol (TAG) level, resulting in a reduced liver to body ratio.[51] In line with clinical case findings, Tahara et al.[34] reported that ipragliflozin has a therapeutic effect in an amylin liver NASH model by decreasing levels of ALT and AST, hepatic lipid content, number of apoptotic cells, and areas of fibrosis.

In addition, the ratio of liver to spleen on computed tomography is used as an indicator of liver function clinically; this ratio has a negative correlation with the degree of chronic liver disease.[52,53] A liver to spleen ratio <1.0 is defined as liver dysfunction.[54] As Bando et al. reported,[55] a 12-week ipragliflozin treatment given to Japanese patients with T2DM resulted in an increased liver to spleen ratio from 0.92 ± 0.26 to 0.98 ± 0.25 ($P < 0.001$). Consistently, two other types of SGLT2 inhibitors, dapagliflozin and luseogliflozin, showed the same positive outcomes as ipragliflozin by increasing the liver to spleen ratio of patients with NASH clinically.[50,56] Although almost all these clinical studies involved Japanese patients, these studies did suggest significant therapeutic effects of SGLT2 inhibitors in patients with NASH with T2DM through liver function modification.

TARGETING LIPID METABOLISM

The hallmark of NAFLD is hepatic lipid accumulation, which results from an imbalance when fatty acid uptake and *de novo* synthesis exceed oxidation and secretion. Regarding lipid metabolism, patients with NAFLD show decreased oxidation and elevated lipogenesis, resulting from insulin resistance.[57] Elevated *de novo* lipogenesis (DNL) is activated by two major pathways, sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate responsive element binding protein (ChREBP), which can be activated by increased insulin signaling and increased glucose concentrations, respectively. SREBP1c can be activated by liver X receptor alpha. Once activated, alpha will heterodimerize with retinoid X receptor following the increase of SREBP1c messenger RNA (mRNA). The activated SREBP1c can in turn activate DNL through transcriptional up-regulation of several genes involved in fatty acid synthesis, including Fas cell surface death receptor (*FAS*) and acetyl-coenzyme A (CoA) carboxylase (*ACC*). In addition, SREBP1c can be activated by the phosphoinositide 3-kinase and protein kinase B pathways, resulting in forkhead box protein O1 (FOXO1) phosphorylation. Phosphorylation of FOXO1 can prevent its translocation to the nuclear periphery, resulting in the inhibition of glycogenesis. In contrast to SREBP1c, ChREBP can be activated by the postprandial rise in glucose delivery to hepatocytes following the increase in glycolysis and pyruvate. Pyruvate, in turn, will form acetyl-CoA as a lipogenic substrate through pyruvate dehydrogenase. ChREBP loss can induce inhibition of acetyl-CoA, resulting in decreased secretion of very low-density lipoproteins (VLDLs), followed by the accumulation of fatty acids in cells and potentially forming TAG,[58-61] which is presumed to be removed from liver by VLDLs. However, in NAFLD, the excretion of VLDLs is inhibited. Thus, a decrease in DNL and an increase in fatty acid oxidation and VLDL excretion will contribute to improved hepatic steatosis. Herein, we review the effects of some SGLT2 inhibitors on lipid metabolism (Fig. 3).

According to Paglialunga and Dehn,[62] hepatic DNL is only elevated in healthy people postprandially but patients with NAFLD gain an increase in DNL in a fasting condition and no further increase postprandially. Considering the sustained elevation of DNL in NAFLD, some research with SGLT2 inhibitors have shown SGLT2 inhibitor treatment exerts an inhibitory role in DNL. Hepatic lipogenesis involves a complex network of nuclear receptors that coordinates the regulation of enzymes involved in different steps of hepatic lipid metabolism from DNL to fatty acid oxidation and uptake and to TAG secretion (Fig. 3).

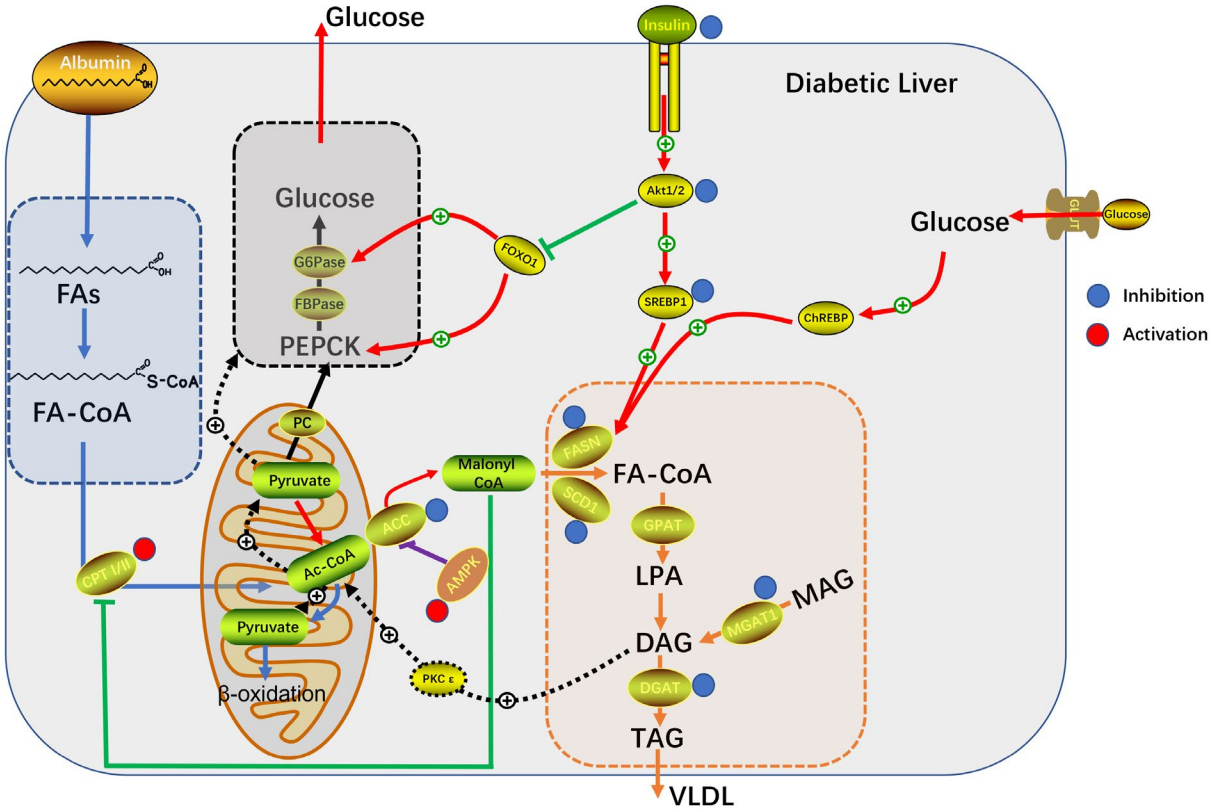

FIG. 3. Metabolic pathways in diabetic liver. Abbreviations: Ac-CoA, acyl-coenzyme A; Akt1/2, protein kinase B 1 and 2; FASN, fatty acid synthase; FBPase, G6Pase, GLUT, PC, PEPCK, PKCε, protein kinase C epsilon.

Inhibition of Hepatic DNL

Targeting the process of DNL, ACC2 catalyzes acetyl-CoA to form malonyl-CoA, which is catalyzed by fatty acid synthase and stearoyl-CoA desaturase 1 (SCD1) to fatty-acyl (FA)-CoA. Accumulated FA-CoA becomes lysophosphatidic acid (LPA) by glycerol-3-phosphate acyltransferase (GPAT). Then, LPA stimulates diacylglycerol (DAG) generation, which also comes from monoacylglycerol (MAG) by monoacylglycerol acyltransferase (MGAT1). Finally, all the DAG is catalyzed by diacylglycerol acyltransferase (DGAT) to TAG.

ACC INHIBITION

ACC can catalyze carboxylation of acetyl-CoA into malonyl-CoA, and its activity can be regulated by phosphorylation and allosteric and protein–protein interaction.[63] Phosphorylation of adenosine monophosphate–activated protein kinase (AMPK) will inhibit ACC activity, leading to the inhibition of DNL and activation of oxidation. In addition, Srebp1c can activate ACC to enhance lipogenesis. Therefore, inhibition of ACC activity will negatively regulate DNL, which is beneficial for controlling NAFLD. SGLT2 inhibitors have been shown to negatively regulate the activity of ACC. As demonstrated by Komiya et al.,[64] ipragliflozin treatment in a high-fat diet (HFD) mouse model could attenuate the expression of DNL, including Srebp1c and Acc1 in the liver. Additionally, empagliflozin treatment in an Otsuka Long Evans Tokushima Fatty Rat model could induce a high level of phosphorylation of AMPK, which can inhibit the activity of ACC.[65] In summary, a therapeutic role of SGLT2 inhibitors is promising in chronic liver diseases by inhibiting ACC activity.

SCD1

SCD1 is responsible for converting stearoyl-CoA to oleoyl-CoA; thus, chemical or genetic inhibition of SCD1 is capable of inhibiting DNL and plays a significant role in NAFLD therapy.[66,67] Ntambi

et al.[66] have reported that SCD1-knockout mice are protected from diet-induced obesity, and SCD1 antisense oligodeoxynucleotide (ASO) therapy was shown to protect mice from NAFLD development in an HFD model.[56] Taken together, SCD1 is now a therapy target for NAFLD. As reported by Omori et al,[68] after 8 weeks of dapagliflozin treatment, diabetic (db)/db mice showed lower SCD1 expression, which helped to protect mice from liver steatosis. Ipragliflozin treatment given to obese (ob)/ob mice also consistently showed significant hepatic protective outcomes by inhibiting SCD1.[64] Considering the research of SGLT2 inhibitor involvement in the inhibition of SCD1, we cannot deny the protective therapeutic role of SGLT2 inhibitors in NAFLD.

MGAT

MGAT enzymes comprise a family of three enzymes (MGAT1-3) that catalyze the acylation of both MAG and DAG to form DAG and TAG, respectively.[69] MGAT1 and MGAT2 are mostly active in rodents and humans, while MGAT3 is active in humans.[70] Mice fed an HFD gained high levels of MGAT1,[71] and MGAT1 knockout reduced the TAG content in the liver of mice fed a 40% high-fat/fructose and cholesterol diet.[72] In addition, the inhibitor of MGAT2 has been reported to have the capacity of preventing hepatic steatosis.[73] Research on MGAT in NAFLD has shown that dapagliflozin treatment in db/db mice (NAFLD mouse model) reduced the expression of MGAT1,[68] which led to a healthier liver.

Acyl-CoA:DGAT

DGAT is capable of acylating DAGs into triglycerides. There are two isoforms, DGAT1 and DGAT2. Hepatic steatosis and insulin resistance were reversed by either DGAT1 knockout in mice or knocking down by ASO.[74,75] Compared with the application of DGAT1 inhibitors, DGAT2 inhibitors are better tolerated in many patients with NAFLD.[76] Ji et al.[77] found that the hepatic DGAT2 mRNA level of HFD mice was elevated whereas canagliflozin reversed this effect, which is indicative of its role in suppressing TAG synthesis and ultimately LD accumulation by DGAT2 down-regulation. However, DGAT2 had a paradoxical effect on hepatic DAG levels; its inhibition decreased DAG, and its activation increased DAG. DAG-induced activation of protein kinase C epsilon impaired the insulin signal[78] but increased the hepatic content of acetyl-CoA, which potentially goes to mitochondria for β-oxidation following an increase in the mitochondrial tricarboxylic acid (TCA) cycle, pyruvate carboxylase, and hepatic glucogenesis.[76] Finally, all those can be attributed to the most severe pathologic disorders, such as inflammation and cell toxicity.[79,80] It is possibly the transient increases in early lipid intermediates following DAG inhibition might reduce DNL.[76] More clinical trials should be conducted to measure the function of DGAT in patients with NAFLD.

ACTIVATION OF β-OXIDATION

Free fatty acids (FFAs) absorbed into cells will move to mitochondria from acyl-CoA to CO_2 and H_2O through the TCA cycle. During this process, the carnitine shuttle plays a key role. FA-CoA is transported from outside to inside the mitochondria by carnitine palmitoyl transferase 1 (CPT1); however, hepatic CPT1 activation was inhibited by an overwhelming level of malonyl-CoA in patients with NAFLD.[81] This suggests that CPT1 is a promising therapeutic target for NAFLD.

As mentioned previously, SGLT2 inhibitors can inhibit the activation of ACC, which has a role in the formation of malonyl-CoA. Honda et al.[32] reported ipragliflozin treatment in amylin liver-NASH diet NAFLD mice could partially reverse the expression of CPT1 compared with the vehicle group, following decreasing FFA levels, which induce inflammation and lipotoxicity.[82-84] Consistently, plasma nonesterified fatty acids and 3-hydroxybutyrate increases were detected in fasted ipragliflozin-treated animals and urinary 3-hydroxybutyrate excretion,[53] suggesting possible enhanced fatty acid oxidation. In summary, SGLT2 inhibitors can increase fatty acid β-oxidation.

Targeting Hepatocyte Death and Fibrosis

The pathogenesis of NASH involves lipotoxicity, gut/nutrient-derived signals, adipocytokines, and genetic factors.[85-87] Although the mechanisms for NAFLD pathogenesis are still enigmatic, the two-hit

hypothesis is the most widespread theory for the development of NAFLD. Abnormal triglyceride accumulation in the liver induced by hepatic DNL increases and impaired fatty acid oxidation results from insulin resistance following reactive oxygen species (ROS) production (first hit). Hepatocytes overwhelmed by ROS due to oxidative stress (OxS) increase following endoplasmic reticulum stress and inflammation (second hit), which then leads to NASH, which may, in turn, progress to hepatic fibrosis and cirrhosis due to marked cell death, ballooning, apoptosis, and necrosis (third hit).[88] Therefore, maintaining oxidative balance is essential to control NAFLD, even for normal subjects. This next section will focus on the effects of SGLT2 inhibitors on OxS reduction and anti-inflammatory effects (Fig. 4).

OxS INHIBITION (OXIDATIVE RESPONSE) BY SGLT2 INHIBITION

OxS plays a key role in the progression of NAFLD from simple steatosis to NASH[88] and occurs through ROS production initiating lipid peroxidation by targeting the double bonds of polyunsaturated fatty acid. This is followed by the production of 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA),[88] which can be inhibited by some antioxidant compounds, such as catalase, glutathione (GSH), GSH S-transferase, superoxide dismutase (SOD), coenzyme Q, and Cu-Zn SOD.[89-91] Recently, Tahara et al.[34] reported that one of the SGLT2 selective inhibitors (ipragliflozin) has a great capacity to control hepatic steatosis by OxS inhibition in type 2 diabetic mice. Consistently, canagliflozin, another type of SGLT2 inhibitor, has also reversed the diabetic high-fat fed induced hepatic diabetic high fat fed group.[34] According to their research, hepatic MDA levels in the HFD group treated with canagliflozin (20 mg/kg) were significantly decreased compared with the HFD group without treatment. In contrast, hepatic activity of SOD and glutathione peroxidase increased significantly in the HFD group treated with canagliflozin. Taken together, SGLT2 inhibitors display promising therapeutic effects on NAFLD/NASH by the ablation of ROS production.

ANTI-INFLAMMATORY EFFECTS

Hepatic inflammation is associated with the majority of acute and chronic liver diseases.[92] Lipid

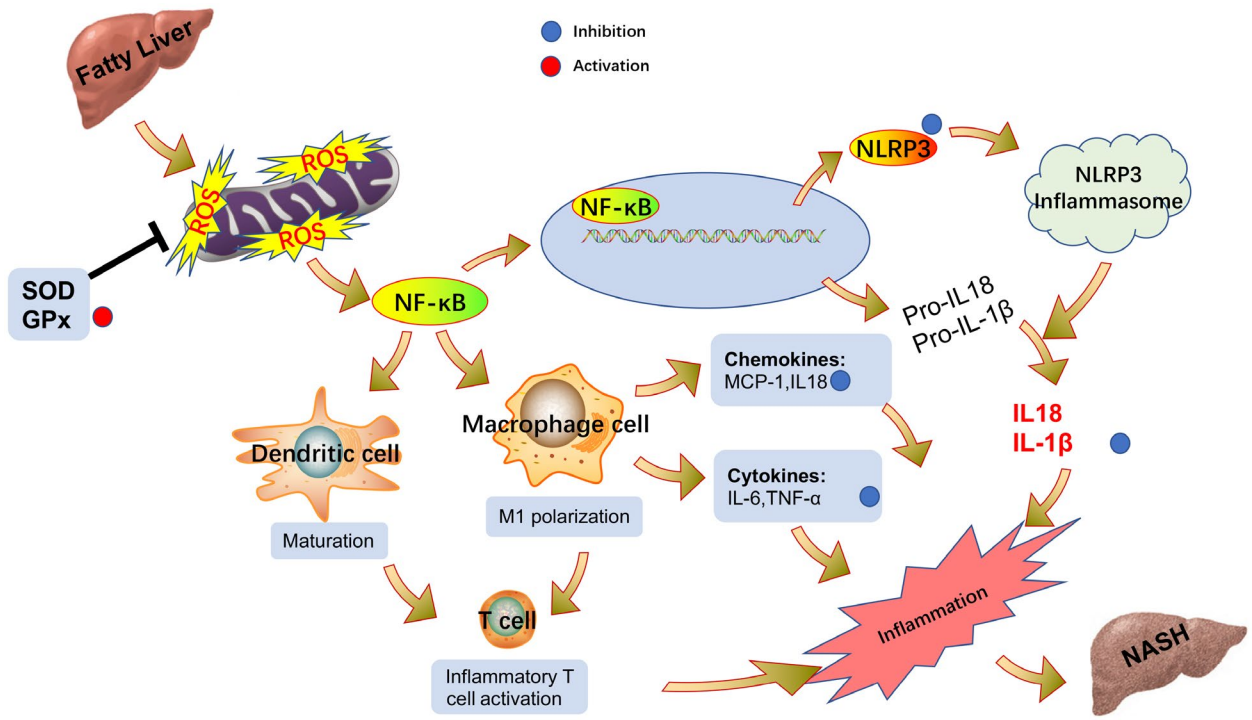

FIG. 4. Inflammatory responses induction in NAFLD/NASH. Abbreviation: GPx, glutathione peroxidase.

accumulation induced by an HFD in mouse liver leads to subacute hepatic inflammation through nuclear factor kappa B (NF-κB) activation, with increased downstream inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β,[93] and chemokines, including monocyte chemoattractant protein 1 (MCP-1) and IL-18.[94] Several types of cells are involved during cytokine secretion following inflammatory effects; these include dendritic cells, macrophages, and T cells. Dendritic cells are antigen-presenting cells in our body that act mainly as messengers between the innate and the adaptive immune system. For example, stimulated dendritic cells can induce rapid production of IL-12, which can help send naive cluster of differentiation (CD)4 T cells toward a Th1 phenotype.[95] Regarding macrophages, there are two main types of activated macrophages, M1 and M2. M1 is the classic macrophage; it can be activated by lipopolysaccharide and interferon-gamma (IFN-γ), following the induction of IL-12 secretion.[96] In contrast, M2 is an alternatively activated macrophage. Its main role is in wound repair and regeneration by secretion of anti-inflammatory cytokines, such as IL-10. The T cell is a type of lymphocyte that develops in the thymus gland and plays a central role in the immune response. T cells are grouped into a series of subsets based on their function, with the two main groups being conventionally adaptive T cells and innate-like T cells.[97] Among conventionally adaptive T cells, helper $CD4^+$ T cells can produce several cytokines, such as IL-4, IL-17, and IFN-γ. Another subset of conventionally adaptive T cells consists of cytotoxic cells, which are $CD8^+$ T cells. These cells can also produce the key cytokines IL-2 and IFN-γ, which influence the functions of other cells, in particular macrophages and natural killer cells.[98] The increased inflammatory signaling is both a hallmark and driver of more advanced liver diseases, including NASH. Treatment with ipragliflozin in HFD mice can inhibit TNF-α and IL-6 levels.[34] Consistently, empagliflozin treatment in HFD mice is also capable of reducing the levels of TNF-α, IL-6, and MCP-1.[99]

Considering the mechanisms of NF-κB signaling in inflammation, NF-κB targets inflammation directly not only by increasing the production of inflammatory cytokines and chemokines but also by being a central mediator of NLR family pyrin domain containing 3 (NLRP3) inflammasome activation; NF-κB acts by inducing the transcriptional expression of NLRP3

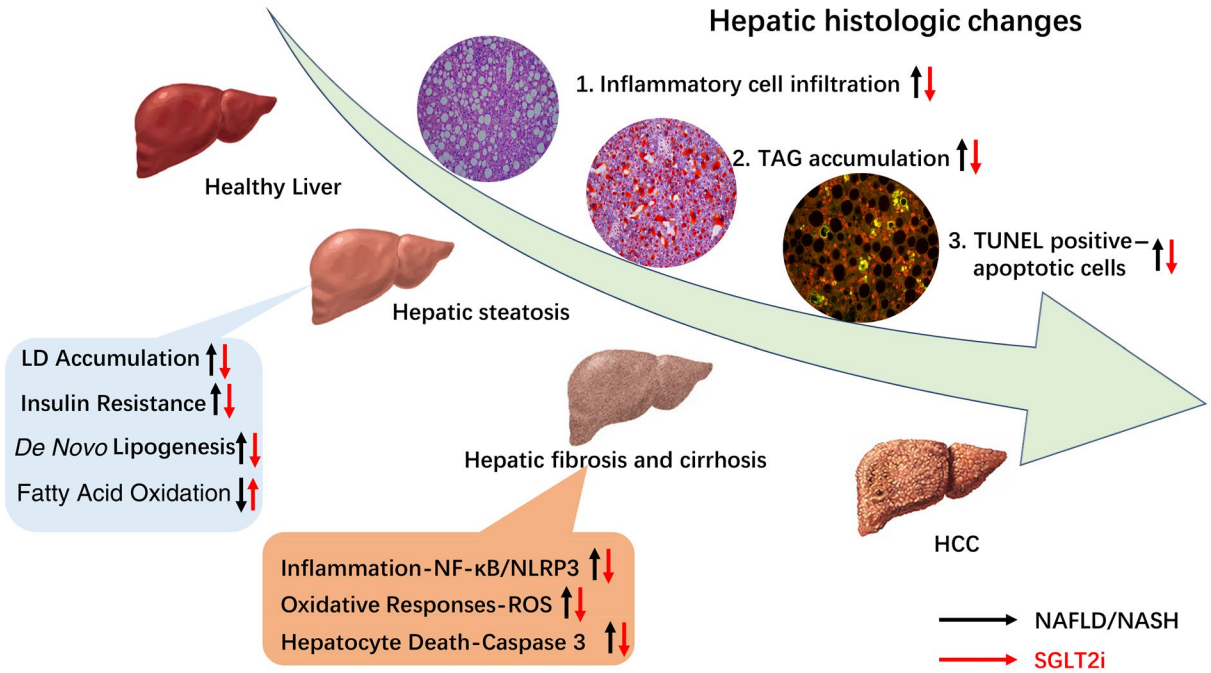

FIG. 5. SGLT2 inhibitors are capable of attenuating the abnormal oxidative response and inflammatory responses following the protective roles in inhibiting hepatocytes death in the development of NAFLD. Abbreviations: SGLT2i, sodium-glucose transport protein 2 inhibitor; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling.

and proinflammatory (pro)IL-1β.[100,101] The NLRP3 inflammasome can promote autocatalytic activation of the cysteine protease caspase-1 and mediates the proteolytic activation of proinflammatory cytokines, including proIL-1β. In particular, a causal role for IL-1β and its contribution to the impairment of insulin signaling is well recognized.[102] As reported by Benetti et al.,[103] empagliflozin reduced activation of the NLRP-3 inflammasome pathway in the liver, which is related to triglyceride decrease. NLRP-3 activation can lead to proIL-1β and proIL-18.[104] In addition, an SGLT2 inhibitor significantly suppresses NLRP3 inflammasome activation and subsequent secretion of IL-1β and IL-18 in human macrophages by decreasing serum levels of insulin among patients with T2DM and cardiovascular disease.[105] Taken together, SGLT2 inhibitors can exert a protective role in steatosis by ablation inflammatory responses following inhibition of caspase-dependent apoptosis (caspase 3 activation) induction.[106]

SGLT2 inhibitors can directly reduce body weight and blood glucose level by reversing the effects of insulin resistance in T2DM. Mechanically, it can inhibit overwhelmed DNL in NAFLD and reactivate inhibited fatty acid oxidation to help lipids move out of the liver. Finally, SGLT2 inhibitors are capable of attenuating the abnormal oxidative response and inflammatory responses through their protective roles in inhibiting hepatocyte death (Fig. 5).

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REFERENCES

1) American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009;32(Suppl. 1):S62-S67.
2) Lim AK. Diabetic nephropathy—complications and treatment. Int J Nephrol Renovasc Dis 2014;7:361-381.
3) Leon BM, Maddox TM. Diabetes and cardiovascular disease: epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes 2015;6:1246-1258.
4) El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 2004;126:460-468.
5) Masri-Iraqi H, Hirsch D, Herzberg D,Lifshitz A, Tsvetov G, Benbassat C, et al. Central diabetes insipidus: clinical characteristics and long-term course in a large cohort of adults. Endocr Pract 2017;23:600-604.
6) Saraiva AM. Biguanides in the treatment of diabetes mellitus. [in Portuguese] Acta Med Port 1989;2(Suppl. 1):15S-18S.
7) Lv W, Wang X, Xu Q, Lu W. Mechanisms and characteristics of sulfonylureas and glinides. Curr Top Med Chem 2020;20:37-56.
8) Chiba S. Molecular mechanism in alpha-glucosidase and glucoamylase. Biosci Biotechnol Biochem 1997;61:1233-1239.
9) Nanjan MJ, Mohammed M, Prashantha Kumar BR, Chandrasekar MJN. Thiazolidinediones as antidiabetic agents: a critical review. Bioorg Chem 2018;77:548-567.
10) Gallwitz B. Clinical use of DPP-4 inhibitors. Front Endocrinol (Lausanne) 2019;10:389.
11) Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al.; CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 2017;377:644-657.
12) Saleem F. Dapagliflozin: cardiovascular safety and benefits in type 2 diabetes mellitus. Cureus 2017;9:e1751.
13) Frampton JE. Empagliflozin: a review in type 2 diabetes. Drugs 2018;78:1037-1048.
14) Lytvyn Y, Bjornstad P, Udell JA, Lovshin JA, Cherney DZI. Sodium glucose cotransporter-2 inhibition in heart failure: potential mechanisms, clinical applications, and summary of clinical trials. Circulation 2017;136:1643-1658.
15) Han S, Hagan DL, Taylor JR, Xin L, Meng W, Biller SA, et al. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. Diabetes 2018;57:1723-1729.
16) Washburn WN. Development of the renal glucose reabsorption inhibitors: a new mechanism for the pharmacotherapy of diabetes mellitus type 2. J Med Chem 2009;52:1785-1794.
17) Gao Y, Zhao G, Liu W, Wang Y, Xu W, Wang J. Thiadiazole-based thioglycosides as sodium-glucose co-transporter 2 (SGLT2) inhibitors. Chin J Chem 2010;28:605-612.
18) Li AR, Zhang J, Greenberg J, Lee T, Liu J. Discovery of non-glucoside SGLT2 inhibitors. Bioorg Med Chem Lett 2011;21:2472-2475.
19) Chen LH, Leung PS. Inhibition of the sodium glucose co-transporter-2: its beneficial action and potential combination therapy for type 2 diabetes mellitus. Diabetes Obes Metab 2013;15:392-402.
20) Sa-Nguanmoo P, Tanajak P, Kerdphoo S, Jaiwongkam T, Pratchayasakul W, Chattipakorn N, et al. SGLT2-inhibitor and DPP-4 inhibitor improve brain function via attenuating mitochondrial dysfunction, insulin resistance, inflammation, and apoptosis in HFD-induced obese rats. Toxicol Appl Pharmacol 2017;333:43-50.
21) Bertinat R, Nualart F, Yáñez AJ. SGLT2 inhibitors: glucotoxicity and tumorigenesis downstream the renal proximal tubule? J Cell Physiol 2016;231:1635-1637.
22) Shimo N, Matsuoka TA, Miyatsuka T, Takebe S, Tochino Y, Takahara M, et al. Short-term selective alleviation of glucotoxicity and lipotoxicity ameliorates the suppressed expression of key β-cell factors under diabetic conditions. Biochem Biophys Res Commun 2015;467:948-954.
23) Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73-84.
24) Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol 2006;45:600-606.
25) Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 2015;148:547-555.

26) Eslamparast T, Tandon P, Raman M. Dietary composition independent of weight loss in the management of non-alcoholic fatty liver disease. Nutrients 2017;9:800.
27) Bansal R, Nagórniewicz B, Prakash J. Clinical advancements in the targeted therapies against liver fibrosis. Mediators Inflamm 2016;2016:7629724.
28) Chao EC. SGLT-2 inhibitors: a new mechanism for glycemic control. Clin Diabetes 2014;32:4-11.
29) Vallon V, Thomson SC. Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia 2017;60:215-225.
30) Yamane M, Matono T, Okano JI, Nagahara R, Matsuki Y, Okamoto T, et al. Protective effects of ipragliflozin, a sodium-glucose cotransporter 2 inhibitor, on a non-alcoholic steatohepatitis mouse model. Yonago Acta Med 2019;62:30-35.
31) Shimizu M, Suzuki K, Kato K, Jojima T, Iijima T, Murohisa T, et al. Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. Diabetes Obes Metab 2019;21:285-292.
32) Honda Y, Imajo K, Kato T, Kessoku T, Ogawa Y, Tomeno W, et al. The selective SGLT2 inhibitor ipragliflozin has a therapeutic effect on nonalcoholic steatohepatitis in mice. PLoS One 2016;11:e0146337.
33) Goto R, Kamimura K, Shinagawa-Kobayashi Y, Sakai N, Nagoya T, Niwa Y, et al. Inhibition of sodium glucose cotransporter 2 (SGLT2) delays liver fibrosis in a medaka model of nonalcoholic steatohepatitis (NASH). FEBS Open Bio 2019;9:643-652.
34) Tahara A, Kurosaki E, Yokono M, Yamajuku D, Kihara R, Hayashizaki Y, et al. Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice. Eur J Pharmacol 2013;715:246-255.
35) Han JH, Oh TJ, Lee G, Maeng HJ, Lee DH, Kim KM, et al. The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE-/- mice fed a western diet. Diabetologia 2017;60:364-376.
36) Tabuchi H, Maegawa H, Tobe K, Nakamura I, Uno S. Effect of ipragliflozin on liver function in Japanese type 2 diabetes mellitus patients: a subgroup analysis of the STELLA-LONG TERM study (3-month interim results). Endocr J 2019;66:31-41.
37) Eriksson JW, Lundkvist P, Jansson PA, Johansson L, Kvarnström M, Moris L, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. Diabetologia 2018;61:1923-1934.
38) Kusunoki M, Natsume Y, Sato D, Tsutsui H, Miyata T, Tsutsumi K, et al. Luseogliflozin, a sodium glucose co-transporter 2 inhibitor, alleviates hepatic impairment in Japanese patients with type 2 diabetes. Drug Res (Stuttg) 2016;66:603-606.
39) Kuchay MS, Krishan S, Mishra SK, Farooqui KJ, Singh MK, Wasir JS, et al. Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: a randomized controlled trial (E-LIFT Trial). Diabetes Care 2018;41:1801-1808.
40) Inoue M, Hayashi A, Taguchi T, Arai R, Sasaki S, Takano K, et al. Effects of canagliflozin on body composition and hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease. J Diabetes Investig 2019;10:1004-1011.
41) Akuta N, Watanabe C, Kawamura Y, Arase Y, Saitoh S, Fujiyama S, et al. Effects of a sodium-glucose cotransporter 2 inhibitor in nonalcoholic fatty liver disease complicated by diabetes mellitus: preliminary prospective study based on serial liver biopsies. Hepatol Commun 2017;1:46-52.
42) Akuta N, Kawamura Y, Watanabe C, Nishimura A, Okubo M, Mori Y, et al. Impact of sodium glucose cotransporter 2 inhibitor on histological features and glucose metabolism of non-alcoholic fatty liver disease complicated by diabetes mellitus. Hepatol Res 2019;49:531-539.
43) Lai LL, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK. Empagliflozin for the treatment of nonalcoholic steatohepatitis in patients with type 2 diabetes mellitus. Dig Dis Sci 2020;65:623-631.
44) Rui L. Energy metabolism in the liver. Compr Physiol 2014;4:177-197.
45) Sattar N, Forrest E, Preiss D. Non-alcoholic fatty liver disease. BMJ 2014;349:g4596.
46) Ohta A, Kato H, Ishii S, Sasaki Y, Nakamura Y, Nakagawa T, et al. Ipragliflozin, a sodium glucose co-transporter 2 inhibitor, reduces intrahepatic lipid content and abdominal visceral fat volume in patients with type 2 diabetes. Expert Opin Pharmacother 2017;18:1433-1438.
47) Nakamura I, Maegawa H, Tobe K, Uno S. Safety and effectiveness of ipragliflozin for type 2 diabetes in Japan: 12-month interim results of the STELLA-LONG TERM post-marketing surveillance study. Adv Ther 2019;36:923-949.
48) Arase Y, Shiraishi K, Anzai K, Sato H, Teramura E, Tsuruya K, et al. Effect of sodium glucose co-transporter 2 inhibitors on liver fat mass and body composition in patients with nonalcoholic fatty liver disease and type 2 diabetes mellitus. Clin Drug Investig 2019;39:631-641.
49) Itani T, Ishihara T. Efficacy of canagliflozin against nonalcoholic fatty liver disease: a prospective cohort study. Obes Sci Pract 2018;4:477-482.
50) Shibuya T, Fushimi N, Kawai M, Yoshida Y, Hachiya H, Ito S, 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.
51) Hayashizaki-Someya Y, Kurosaki E, Takasu T, Mitori H, Yamazaki S, Koide K, et al. Ipragliflozin, an SGLT2 inhibitor, exhibits a prophylactic effect on hepatic steatosis and fibrosis induced by choline-deficient l-amino acid-defined diet in rats. Eur J Pharmacol 2015;754:19-24.
52) Zeb I, Li D, Nasir K, Katz R, Larijani VN, Budoff MJ. Computed tomography scans in the evaluation of fatty liver disease in a population-based study: the multi-ethnic study of atherosclerosis. Acad Radiol 2012;19:811-818.
53) Wilson GA, Keyes JW Jr. The significance of the liver-spleen uptake ration in liver scannning. J Nucl Med 1974;15:593-597.
54) Piekarski J, Goldberg HI, Royal SA, Axel L, Moss AA. Difference between liver and spleen CT numbers in the normal adult: its usefulness in predicting the presence of diffuse liver disease. Radiology 1980;137:727-729.
55) Bando Y, Ogawa A, Ishikura K, Kanehara H, Hisada A, Notumata K, et al. The effects of ipragliflozin on the liver-to-spleen attenuation ratio as assessed by computed tomography and on alanine transaminase levels in Japanese patients with type 2 diabetes mellitus. Diabetol Int 2017;8:218-227.
56) Kurinami N, Sugiyama S, Yoshida A, Hieshima K, Miyamoto F, Kajiwara K, et al. Dapagliflozin significantly reduced liver fat accumulation associated with a decrease in abdominal subcutaneous fat in patients with inadequately controlled type 2 diabetes mellitus. Diabetes Res Clin Pract 2018;142:254-263.
57) Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cell Mol Life Sci 2018;75:3313-3327.
58) Hall AM, Kou K, Chen Z, Pietka TA, Kumar M, Korenblat KM, et al. Evidence for regulated monoacylglycerol acyltransferase expression and activity in human liver. J Lipid Res 2012;53:990-999.

59) Shi Y, Cheng D. Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. Am J Physiol Endocrinol Metab 2009;297:E10-E18.
60) Gao M, Ma Y, Liu D. High-fat diet-induced adiposity, adipose inflammation, hepatic steatosis and hyperinsulinemia in outbred CD-1 mice. PLoS One 2015;10:e0119784.
61) Cnop M, Hannaert JC, Hoorens A, Eizirik DL, Pipeleers DG. Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. Diabetes 2001;50:1771-1777.
62) Paglialunga S, Dehn CA. Clinical assessment of hepatic de novo lipogenesis in non-alcoholic fatty liver disease. Lipids Health Dis 2016;15:159.
63) Hunkeler M, Stuttfeld E, Hagmann A, Imseng S, Maier T. The dynamic organization of fungal acetyl-CoA carboxylase. Nat Commun 2016;7:11196.
64) Komiya C, Tsuchiya K, Shiba K, Miyachi Y, Furuke S, Shimazu N, et al. Ipragliflozin improves hepatic steatosis in obese mice and liver dysfunction in type 2 diabetic patients irrespective of body weight reduction. PLoS One 2016;11:e0151511.
65) Kim JW, Lee YJ, You YH, Moon MK, Yoon KH, Ahn YB, et al. Effect of sodium-glucose cotransporter 2 inhibitor, empagliflozin, and α-glucosidase inhibitor, voglibose, on hepatic steatosis in an animal model of type 2 diabetes. J Cell Biochem 2019;120:8534-8546.
66) Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci U S A 2002;99:11482-11486.
67) Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, et al. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. J Clin Invest 2005;115:1030-1038.
68) Omori K, Nakamura A, Miyoshi H,Takahashi K, Kitao N, Nomoto H, et al. Effects of dapagliflozin and/or insulin glargine on beta cell mass and hepatic steatosis in db/db mice. Metabolism 2019;98:27-36.
69) 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.
70) Hu Q, Mao Y, Liu M, Luo R, Jiang R, Guo F. The active nuclear form of SREBP1 amplifies ER stress and autophagy via regulation of PERK. FEBS J 2020;287:2348-2366.
71) Bengoechea-Alonso MT, Ericsson J. The phosphorylation-dependent regulation of nuclear SREBP1 during mitosis links lipid metabolism and cell growth. Cell Cycle 2016;15:2753-2765.
72) Hall AM, Soufi N, Chambers KT, Chen Z, Schweitzer GG, McCommis KS, et al. Abrogating monoacylglycerol acyltransferase activity in liver improves glucose tolerance and hepatic insulin signaling in obese mice. Diabetes 2014;63:2284-2296.
73) Take K, Mochida T, Maki T, Satomi Y, Hirayama M, Nakakariya M, et al. Pharmacological inhibition of monoacylglycerol O-acyltransferase 2 improves hyperlipidemia, obesity, and diabetes by change in intestinal fat utilization. PLoS One 2016;11:e0150976.
74) Choi CS, Savage DB, Kulkarni A, Yu XX, Liu ZX, Morino K, et al. Suppression of diacylglycerol acyltransferase-2 (DGAT2), but not DGAT1, with antisense oligonucleotides reverses diet-induced hepatic steatosis and insulin resistance. J Biol Chem 2007;282:22678-22688.
75) Jornayvaz FR, Birkenfeld AL, Jurczak MJ, Kanda S, Guigni BA, Jiang DC, et al. Hepatic insulin resistance in mice with hepatic overexpression of diacylglycerol acyltransferase 2. Proc Natl Acad Sci USA 2011;108:5748-5752.
76) Samuel VT, Shulman GI. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. Cell Metab 2018;27:22-41.
77) Ji W, Zhao M, Wang M, Yan W, Liu Y, Ren S, et al. Effects of canagliflozin on weight loss in high-fat diet-induced obese mice. PLoS One 2017;12:e0179960.
78) Dasgupta S, Bhattacharya S, Maitra S, Pal D, Majumdar SS, Datta A, et al. Mechanism of lipid induced insulin resistance: activated PKCε is a key regulator. Biochim Biophys Acta 2011;1812:495-506.
79) Weber LW, Lebofsky M, Stahl BU, Gorski JR, Muzi G, Rozman K. Reduced activities of key enzymes of gluconeogenesis as possible cause of acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-diox in (TCDD) in rats. Toxicology 1991;66:133-144.
80) Sunny NE, Parks EJ, Browning JD, Burgess SC. Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab 2011;14:804-810.
81) Goedeke L, Bates J, Vatner DF, Perry RJ, Wang T, Ramirez R, et al. Acetyl-CoA carboxylase inhibition reverses NAFLD and hepatic insulin resistance but promotes hypertriglyceridemia in rodents. Hepatology 2018;68:2197-2211.
82) Boden G. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. Curr Diab Rep 2006;6:177-181.
83) Engin AB. What is lipotoxicity? Adv Exp Med Biol 2017;960: 197-220.
84) Yazıcı D, Sezer H. Insulin resistance, obesity and lipotoxicity. Adv Exp Med Biol 2017;960:277-304.
85) Marra F, Lotersztajn S. Pathophysiology of NASH: perspectives for a targeted treatment. Curr Pharm Des 2013;19:5250-5269.
86) Caligiuri A, Gentilini A, Marra F. Molecular pathogenesis of NASH. Int J Mol Sci 2016;17:1575.
87) Ertunc ME, Hotamisligil GS. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. J Lipid Res 2016;57:2099-2114.
88) Spahis S, Delvin E, Borys JM, Levy E. Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis. Antioxid Redox Signal 2017;26:519-541.
89) Erhardt A, Stahl W, Sies H, Lirussi F, Donner A, Häussinger D. Plasma levels of vitamin E and carotenoids are decreased in patients with nonalcoholic steatohepatitis (NASH). Eur J Med Res 2011;16:76-78.
90) Sugimoto H, Okada K, Shoda J, Warabi E, Ishige K, Ueda T, et al. Deletion of nuclear factor-E2-related factor-2 leads to rapid onset and progression of nutritional steatohepatitis in mice. Am J Physiol Gastrointest Liver Physiol 2010;298:G283-G294.
91) Kabil SL, Mahmoud NM. Canagliflozin protects against non-alcoholic steatohepatitis in type-2 diabetic rats through zinc alpha-2 glycoprotein up-regulation. Eur J Pharmacol 2018;828:135-145.
92) Blasco-Algora S, Masegosa-Ataz J, Gutiérrez-García ML, Alonso-López S, Fernández-Rodríguez CM. Acute-on-chronic liver failure: pathogenesis, prognostic factors and management. World J Gastroenterol 2015;21:12125-12140.
93) Del Campo JA, Gallego P, Grande L. Role of inflammatory response in liver diseases: therapeutic strategies. World J Hepatol 2018;10:1-7.
94) Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023.
95) Gaudino SJ, Kumar P. Cross-talk between antigen presenting cells and T cells impacts intestinal homeostasis, bacterial infections, and tumorigenesis. Front Immunol 2019;10:360.
96) Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage polarization: different gene signatures in M1(LPS+) vs. classically and M2(LPS-) vs. alternatively activated macrophages. Front Immunol 2019;10:1084.

97) Chou C, Li MO. Tissue-resident lymphocytes across innate and adaptive lineages. Front Immunol 2018;9:2104.
98) Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 2015;74:5-17.
99) Jojima T, Tomotsune T, Iijima T, Akimoto K, Suzuki K, Aso Y. Empagliflozin (an SGLT2 inhibitor), alone or in combination with linagliptin (a DPP-4 inhibitor), prevents steatohepatitis in a novel mouse model of non-alcoholic steatohepatitis and diabetes. Diabetol Metab Syndr 2016;8:45.
100) Jo EK, Kim JK, Shin DM, Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 2016;13:148-159.
101) Wree A, McGeough MD, Peña CA, Schlattjan M, Li H, Inzaugarat ME, et al. NLRP3 inflammasome activation is required for fibrosis development in NAFLD. J Mol Med (Berl) 2014;92:1069-1082.
102) Ballak DB, Stienstra R, Tack CJ, Dinarello CA, van Diepen JA. IL-1 family members in the pathogenesis and treatment of metabolic disease: focus on adipose tissue inflammation and insulin resistance. Cytokine 2015;75:280-290.
103) Benetti E, Mastrocola R, Vitarelli G, Cutrin JC, Nigro D, Chiazza F, et al. Empagliflozin protects against diet-Induced NLRP-3 inflammasome activation and lipid accumulation. J Pharmacol Exp Ther 2016;359:45-53.
104) Leng W, Ouyang X, Lei X, Wu M, Chen L, Wu Q, et al. The SGLT-2 inhibitor dapagliflozin has a therapeutic effect on atherosclerosis in diabetic ApoE-/- mice. Mediators Inflamm 2016;2016:6305735.
105) Kim SR, Lee SG, Kim SH, Kim JH, Choi E, Cho W, et al. SGLT2 inhibition modulates NLRP3 inflammasome activity via ketones and insulin in diabetes with cardiovascular disease. Nat Commun 2020;11:2127.
106) Jojima T, Wakamatsu S, Kase M, Iijima T, Maejima Y, Shimomura K, et al. The SGLT2 inhibitor canagliflozin prevents carcinogenesis in a mouse model of diabetes and non-alcoholic steatohepatitis-related hepatocarcinogenesis: association with SGLT2 expression in hepatocellular carcinoma. Int J Mol Sci 2019;20:5237.