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Assessment of possible Roles of Dapagliflozin in Non-Alcoholic Fatty Liver

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Abstract:Background: A broad category including benign liver steatosis, non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and hepatocellular cancer is non-alcoholic fatty liver disease (NAFLD). Recent years have seen NAFLD emerge as a significant health concern. It has become a worldwide priority to find ways to stop or slow the development of NAFLD. Even though there are a number of medicinal therapies for NAFLD that are undergoing clinical trials, changing one's lifestyle continues to be the most important part of treatment. Agents such as dapagliflozin, which are sodium-glucose co-transporter type-2 inhibitors (SGLT-2i), are showing great promise. Apoptosis, low-grade inflammation, autophagy, and processes regulated by SGLT-2i, including the endoplasmic reticulum (ER), are all involved in the pathophysiology of NAFLD. We provide the present state of knowledge on the pathophysiology of NAFLD in this review, with an emphasis on the possible role of SGLT-2i in the onset and progression of NAFLD, supported by the best available evidence from in vitro and animal research. Considering this information, doing additional mechanistic studies would enhance our comprehension of the specific pathways contributing to the development of NAFLD and the possible positive effects of SGLT-2i when used to treat NAFLD.

Keywords: Dapagliflozin,sodium-glucose co-transporter type-2 inhibitors, Non-Alcoholic Fatty

Introduction

The rising prevalence of non-alcoholic fatty liver disease (NAFLD)—which ranges from 13% in Africa to 42% in South-East Asia [1,2]—has emerged as a significant global health concern. Various diseases fall under the umbrella of NAFLD. These include steatosis, which occurs when fat accumulates in more than 5% of hepatocytes, non-alcoholic steatohepatitis, which can lead to necroinflammation and fibrosis, and hepatocellular carcinoma [3,4]. Finding solutions to slow the development of NAFLD has shifted the spotlight worldwide.

Because a high body mass index (BMI) is present in more than 50% of NAFLD patients, it is crucial to treat this condition by making changes to one's lifestyle, such as eating habits, weight control, and exercise routine.

Addressing any co-morbidities, such as obesity, dyslipidemia, or type 2 diabetes mellitus (T2DM), should continue to be the principal focus in treating individuals with NAFLD, even if drugs targeting NAFLD are currently being developed [7,8].

An interesting development is that a group of international experts has recently suggested the term metabolic (dysfunction) associated fatty liver disease (MAFLD) to better reflect the underlying pathogenesis of the NAFLD spectrum [9,10,11]. This is because the cardiometabolic disorders that accompany NAFLD are responsible for the increased morbidity and mortality in these patients. All type 2 diabetic patients with high transaminases during biopsy had NAFLD, according to Masarone et al., who found a prevalence of 94.82% among MS patients [12]. It was shown that 96.82% of type 2 diabetic patients and 58.52% of MS patients in this study had NASH, which is quite surprising. They came to the conclusion that NASH might be one of the first clinical signs of T2DM [12] since insulin resistance (IR) is significantly associated with the pathophysiology of both T2DM and NASH [13]. It is worth mentioning that none of the MS components—age, sex, smoking habit, cholesterolemia, or otherwise—was linked with a lower risk of cardiovascular-related mortality when NAFLD was present, especially steatohepatitis [11]. An increase in preclinical atherosclerotic damage and adverse coronary, cerebral, and peripheral vascular events were found to be related with NAFLD, according to a number of studies and meta-analyses [14]. In addition, there is evidence that systolic and diastolic cardiac dysfunction are correlated with the severity of fibrosis in liver biopsies [15,16].

SGLT-2i, or sodium-glucose co-transporter type 2, are drugs that reduce blood sugar levels; they also aid in weight loss and reduce levels of uric acid in the blood. These medicines have gained permission for use in non-diabetic patients with chronic renal disorders and heart failure because of the significant benefits they have shown even in people without diabetes [17,18]. As a result of their anti-hyperglycemic and weight-reducing properties as well as their apparent strong antioxidant and anti-inflammatory actions, SGLT-2i show great promise as a therapy for NAFLD.

Indeed, imaging techniques and improvements in biological indicators of NAFLD show that SGLT-2i has a favorable effect on fatty liver buildup; however, these benefits have been observed mostly in patients with type 2 diabetes.

With an emphasis on the underlying mechanisms of action, we provide here the most recent findings regarding the impact of SGLT-2i on the progression of NAFLD. One way that SGLT-2i helps NAFLD is by regulating a number of processes, including ER stress, oxidative stress, low-grade inflammation, autophagy, and apoptosis. Thus, we summarize the data from in vitro, animal, investigations that support the idea that SGLT-2i may influence the onset and progression of NAFLD.

Advances in our understanding of this multi-factorial illness have led to changes in the main theory underlying the pathogenesis of NAFLD. For a long time, the "two-hit" notion was widely accepted. This theory proposed that NAFLD pathophysiology included two "hits" from different sources (e.g., oxidative stress, ER stress, other injury) that were necessary for the onset and advancement of hepatic inflammation (NASH) and fibrosis, and a first "hit" representing the stage of simple steatosis alone (NAFL) that includes hepatocytic lipid accumulation and hepatic insulin resistance [19]. A more recent idea, the "multiple parallel-hit" model [20], has superseded this earlier one and aims to clarify the intricate pathophysiology and development of NAFLD. This theory proposes that various combinations of various (epi)genetic and environmental factors, or "hits," interact in a dynamic manner and can contribute to the onset and progression of NAFLD. Certain genetic variations and epigenetic alterations [21], metabolic syndrome features [22,23,24,25] (e.g., a Western diet, insufficient physical exercise, central obesity, dysregulation of adipoknes and insulin resistance), lipotoxicity [26,27], gut microbiota dysbiosis [26], autophagy and mitochondrial function dysregulation [28,29,30], endoplasmic reticulum stress [31], hepatocyte homeostasis and death [32,33], inflammatory and fibrotic responses [34,35]. When the liver's ability to process primary metabolic energy sources is overloaded, harmful lipid species build up, causing hepatocyte malfunction, apoptosis, metabolically activated inflammation, and fibrosis [36].

When adipose tissue's ability to store surplus energy from food decreases, the characteristic of NAFLD pathogenesis appears to be an increase in hepatocytes' adipocyte-like (dys)function [37,38,39,40]. Hepatic de novo lipogenesis [41,42,43] is driven by increased metabolic substrates in tissues [27] during energy surplus situations, which in turn enhances IR and creates a vicious cycle. The equilibrium among lipid production, absorption, and lysis controls the buildup of intrahepatic lipid levels [44]. Intracellular buildup of free fatty acids (FFA) results from an imbalance between lipogenesis and lipolysis, which in turn causes hepatocellular damage, IR, impaired liver function, hepatic steatosis, and the development of NASH, cirrhosis, and hepatocellular cancer [44].

Impairment of insulin signaling in adipocytes is caused by metabolic-induced inflammation and dysregulation of adipokines (such as leptin and adiponectin) in the majority of persons with NAFLD [45,46]. This deficit leads to an increase in FA supply to the liver through sped-up lipolysis in subcutaneous adipose tissue and decreased FA absorption [45,46,47]. Overloaded hepatocytes experience an increase in reactive oxygen species (ROS) production [41,48] and the generation of highly reactive aldehyde by-products [49] due to the upregulation of physiologically minor β-oxidation processes in the peroxisomes and the endoplasmic reticulum (ER). Damage to DNA in the nucleus and mitochondria, as well as rupture of phospholipid membranes and cell death, result from this occurrence. In patients with non-alcoholic fatty liver disease (NAFLD), toxic lipid metabolites like diacylglycerols [24], ceramides [52,53], and lysophosphatidyl choline species [53] are generated as a result of mitochondrial dysfunction and impaired β-oxidation. This leads to hepatocyte dysfunction (lipotoxicity) [54,55] and endothelial dysfunction (ER stress). Patients with non-alcoholic fatty liver disease (NAFLD) have problems with the unfolded protein response (UPR), an adaptive homeostatic mechanism of endoplasmic reticulum (ER) stress [56]. As a result, more cell stress sensors are turned on, and inflammatory and apoptotic pathways are elevated [57,58]. Another important factor in the development of NAFL and NASH appears to be the gut microbiota. Inflammasome activation and Toll-like receptor signaling initiate an intrahepatic inflammatory response in response to damage-associated molecular patterns and pathogens originating in the gut [35,59,60]. Neutrophils, monocytes, T-lymphocytes, and macrophages are recruited and progressively penetrate the liver parenchyma after activation of hepatic innate immune cells, including Kupffer cells, dendritic cells, and hepatic stellate cells (HSCs) [34,61]. Then, in an unsuccessful effort at tissue regeneration, the released growth factors and cytokines amplify the inflammatory response and add to the fibrotic process [35]. The complex pathogenetic process of NAFLD involves multiple pathways, making the search for a "wonder drug" an arduous undertaking.

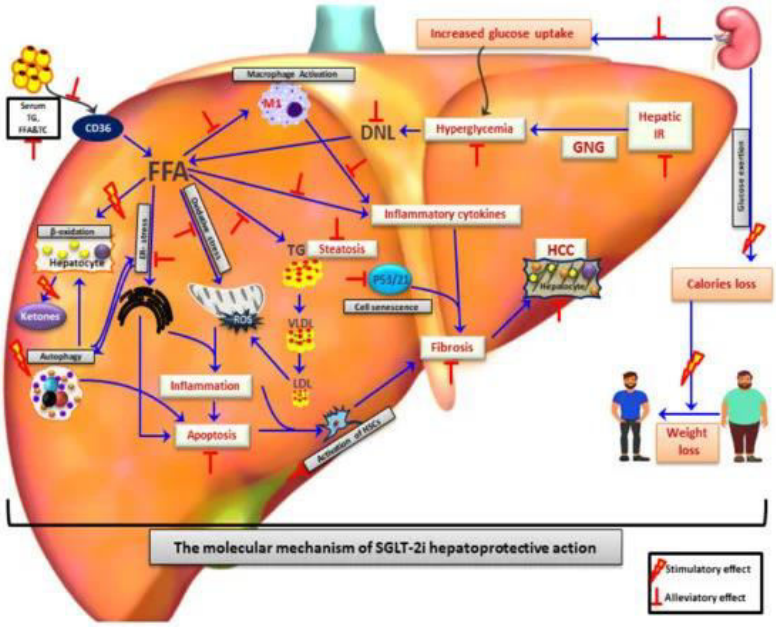

Figure 1. SGLT-2 inhibitors as a promising therapeutic agents for treatment of NAFLD/NASH patients. SGLT-2i treatment contributes to alleviation of NAFLD by reduction of hyperglycaemia, improvement of systematic insulin resistance, elevation of caloric loss and reduction of body weight mostly due to glycosuria. Apart from that, SGLT-2i play a hepatoprotective effect through reduction of hepatic de novo lipogenesis, hepatic inflammation, apoptosis, ER-stress, oxidative stress, and increase of hepatic beta-oxidation. Reduced activation of hepatic satellite cells and p53/p21 pathways by SGLT-2i leads to amelioration of hepatic fibrosis and HCC development. FFA: Free fatty acids; DNL: De novo lipogenesis; HCC: Hepatocellular carcinoma; TC: Total cholesterol; TG: Triglycerides; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; GNG: Gluconeogenesis; HSC: Hepatic stellate cells; IR: Insulin resistance; ROS: Reactive oxygen species; ER-stress: Endoplasmic reticulum stress.

SGLT-2i Overview

In 1835, the first naturally occurring non-selective SGLT-2i was phlorizin, a b-Dglucoside that had been extracted from apple tree bark. It was originally utilized to treat infectious illnesses and fevers [62,63]. Glucose and an aglycone, which are two aromatic carbocycles linked by an alkyl spacer, are the components of phlorizin. Scientists found about fifty years later that, unrelated to insulin secretion, strong doses of phlorizin reduced plasma glucose levels via glucosuria [62,63,64]. Nevertheless, it wasn't until the early 1970s that the process by which phlorizin exerts its effects was uncovered [62,65]. This process involves the proximal tubule's active glucose transport system.

The precise action mechanism of phlorizin and its possible use in hyperglycemia treatment were subsequently uncovered by researchers. Oral administration of phlorizin causes intestinal glucosidases to hydrolyze the O-glycosidic bond, resulting in the production of phloretin. Phloretin inhibits the GLUT transporter and uncouples oxidative phosphorylation. The antidiabetic drug Phlorizin was never developed because of its low SGLT-2 selectivity, rapid degradation, inhibition of the ubiquitous glucose transporter 1 (GLUT1), poor intestinal absorption, and gastrointestinal side effects (e.g., diarrhea and dehydration) [62,66,67,68].

However, a researcher created a devphlorizin-based analog that is more selective for SGLT-2 than SGLT-1, has higher bioavailability and stability, and overcomes the aforementioned negative effects of phlorizin. T-1095, sergliflozin, and remogliflozin were among the O-glucoside analogs that were initially their focus [69,70]. Pharmaceutical companies instead began using C-glucosides, another phlorizin derivative, because of its superior stability and enhanced SGLT-2 selectivity [62].

In the time after, several efforts were made to create phlorizin C-glucoside analogs that could inhibit SGLT-2 effectively and selectively. As a result of these efforts, dapagliflozin was developed in 2008 by Meng et al. [71]. The effectiveness of dapagliflozin against human SGLT-2 was about 1,200 times greater than that against SGLT-1. The United States Food and Drug Administration (FDA) authorized dapagliflozin and multiple other C-glucoside inhibitors in the subsequent years. The inhibitory actions of canagliflozin, a thiophene derivative of C-glucoside, were found to be more than 400 times different between SGLT-2 and SGLT-1 in humans [72]. The third agent to be approved by both the EMA and the FDA was empagliflozin, which has a selectivity for SGLT-2 over SGLT-1 that is approximately 2700 times higher than that of luseogliflozin and topogliflozin. Up to this point, only Japan and Russia have authorized the use of ipragliflozin [73,74,75,76,77].

Here are the chemical formulas and brand names of the SGLT-2 inhibitors that are currently approved by the FDA in the US: ertugliflozin, dapagliflozin, canagliflozin, and empagliflozin, as well as the structure of phlorizin [78]. Many patents and articles have already revealed the different structures of other SGLT-2 inhibitors [79,80].

SGLT-2 Inhibitors in NAFLD

In Vitro Data

Human hepatocellular carcinoma cells (HepG2) have been found to express both the SGLT-1 and SGLT-2 co-transporters [81,82,83], whereas SGLT-2 has also been found in immortalized human primary hepatocyte cells (HuS-E/2), and in immortalized normal human hepatocyte-derived liver cells (L02) [84,85].

Research has shown that the majority of SGLT-2i have an anti-proliferative effect in several hepatic cell lines. This effect is achieved, among other things, by reducing glucose absorption [86]. Canagliflozin significantly reduced cell proliferation by increasing the number of cells in the G0/G1 phase and decreasing the number of cells in the G2/M phase when incubated with L02 and HepG2 cells for 24 hours at different doses [86]. Furthermore, activation of caspase 3 in HepG2 cells demonstrated an apoptotic effect [87]. Theoretically, SGLT-2i regulates cell proliferation and survival by targeting cyclins and cyclin-dependent kinases (CDKs), which are cell growth regulators. Consequently, HepG2 cells that were treated with canagliflozin showed an upregulation of the cell growth regulator hepatocyte nuclear factor 4α (HNF4α) [82]. In addition, canagliflozin therapy has been associated with cell cycle arrest in a hepatocellular carcinoma (HCC) cell line due to decreased expression of cyclin D1, cyclin D2, and cdk4 [82,87]. Another study found that by incubating HepG2 cells with canagliflozin, the anti-carcinogenic capability of γ-irradiation was enhanced by modulation of ER stress-mediated autophagy and cell apoptosis, and the survival of clonogenic cells was decreased [88]. Contrarily, at doses of 10, 50, and 100 μM, the HepG2 cell proliferation rate was boosted by trilobatin, a new SGLT-1/2 inhibitor, while human HCC cell proliferation rate was unaffected by incubation with tofogliflozin at different dosages [89]. There was no effect on cancer cell survival [81,86] or adhesion capacity [81] when canagliflozin, empagliflozin, or dapagliflozin were incubated with HepG2 cells. However, an increase in the number of floating HepG2 cells [81] indicated that the cells were sensitive to dapagliflozin after UDP Glucuronosyltransferase Family 1 Member A9 (UGT1A9) was silenced.

The treatment with dapagliflozin significantly reduced the buildup of lipids and total lipids in L02 cells caused by oleic acid (OA) by increasing FA β-oxidation. This was demonstrated by increased levels of proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) and activation of the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway [84]. Multiple in vitro investigations have demonstrated that AMPK plays a critical role in mediating the numerous positive effects of SGLT-2i on glucose and cholesterol metabolism in liver cells. In OA-stimulated HuS-E/2 cells, dapagliflozin inhibited glucose absorption by activating AMPK and decreasing SGLT-2 expression [90]. After subjecting cells to compound C, a strong AMPK inhibitor, this effect was reversed [84]. In addition, canagliflozin treatment of HepG2 cells activated AMPK, which permitted hepatic cholesterol efflux. Then, the reverse transport of cholesterol was stimulated because AMPK activation elevated the expression of the liver X receptor (LXR) and the proteins that interact with it [91]. In addition, activation of LXR regulates the expression of the ATP-binding cassette (ABC) transporters ABCG5 and ABCG8, which speeds up the elimination of cholesterol in the

feces [92]. The expression of ABCG5, ABCG8, and LXR were all decreased after canagliflozin treatment, but this effect was blocked when cells were treated with compound C [91]. The activation of AMP-kinase is triggered by an elevated AMP/ADP ratio. Indirectly activating the AMPK signaling pathway, SGLT-2i lowers cellular ATP levels is well-documented [83,93]. The control of glucose metabolism is the mechanism by which dapagliflozin reduces intracellular ATP levels [84].

To simulate the hepatocyte condition in NASH, palmitic acid (PA) is used to stimulate hepatic cell lines [94,95]. Dapagliflozin significantly reduced intracellular lipid accumulation when incubated with PA-stimulated L02 and HepG2 cell lines. The decrease in lipid synthesis-related proteins, an increase in genes involved in fatty acid oxidation (such as PPARα and CPT1a), modulation of the AMPK/mTOR pathway, and autophagy were the factors that were thought to be responsible for this result. Intriguingly, the positive effect of dapagliflozin on reducing intracellular lipid accumulation was reversed when cells were incubated with compound C [85]. The results indicate that activation of AMPK signaling is directly related to the SGLT-2i-induced improvement of NAFLD.

Animal Studies

There are primarily two types of animal models used to study NAFLD and NAASH. One group includes rats with specific genetic modifications, such as those with leptin deficiency (ob/ob), resistance to leptin (db/db), Agouti mutation (KK-Ay), and apolipoprotein E knockout (ApoE−/−) mutations. Another group includes rats with specific genetic modifications, such as the Zucker diabetic fatty (ZDF) rats, the Prague hereditary hypertriglyceridemic (HHTg) rats, and the Otsuka Long-Evans Tokushima Fried (OLETF) rat. Animal models of non-alcoholic steatohepatitis and non-alcoholic fatty liver disease (NAFLD) that have been induced by dietary or pharmaceutical manipulation fall into a different category. These models have been fed different diets, such as the MCD diet, the AMLN diet (amylin liver non-alcoholic steatohepatitis, NASH), the CDAA diet, a high-fat diet (HFD), an HFD + cholesterol diet, or a high-fat, high-calorie (HFHC) diet [30,96,97,98,99,100]. Hepatic tissues from rats and mice express the SGLT-1 and SGLT-2 genes, according to the majority of recent evidence [30,85,87,101]. Hepatic macrophages and T lymphocytes also show elevated SGLT-2 protein levels [101]. Our understanding of the possible hepato-protective mechanisms of SGLT-2i actions is primarily derived from animal research, while the precise molecular processes by which SGLT-2i induces its favorable effects on NAFLD remain unclear.

Animal studies indicate that other pathways are more likely to be responsible for the documented SGLT-2i-mediated hepato-protection, even if SGLT-2i-induced weight loss appears to play a significant role in hepato-protective effects on NAFLD in humans [102,103,104].

In addition to decreasing the accumulation of white adipose tissue, administration of SGLT-2i causes a net loss of calories, which in turn reduces body weight growth [99,105,106,107]. Research has shown that SGLT-2i can enhance weight loss by regulating the AMP/ATP ratio, which in turn increases basal metabolism, increases body temperature, and improves systemic IR [19]. In a study conducted by Petito da Silva et al., it was found that male C57Bl/6 mice that were fed a high-fat diet (HFD) for five weeks at a dose of 10 mg/kg/day of empagliflozin (Table 1) considerably reduced their body weight and mass, even if their appetites were somewhat increased [107]. Canagliflozin improved liver function, TG content, and NAS score in male mice that were fed a high-fat diet for four weeks (Table 2), just as it did in humans [108]. Also, in both the HFD and HFD+MCD diet-fed C57BL/6J mice, dapagliflozin lowered body weight and ALT levels in the short-term (Table 3) [109]. Although SGLT-2i may have a positive impact on weight loss, not all research have found this to be the case. No significant effect on body weight growth, epididymal fat weight, or food consumption was seen in studies that administered SGLT-2i [30,105,110,111]. Supplementing an AMLN diet with 40 mg ipragliflozin/kg for 8 weeks did not significantly change the body weight of AMLN diet fed mice. However, it did improve liver function, hepatic fibrosis, and NA score [114], which is in contrast to the strong effects of low dose ipragliflozin on weight loss reported in several studies (Table 4). While 10 mg/kg/day of

empagliflozin improved liver function and NAFLD status, it had no discernible impact on body weight in ApoE knockout mice [30], ZDF, HHTg, and Wistar rats [115,116].

While some research suggests that taking SGLT-2i won't make you put on any pounds [30,100,110,111,112,113,114], the bulk of the evidence suggests that it will help you lose weight.

Nevertheless, it appears that SGLT-2i's protective benefits against NAFLD progression are mediated by mechanisms other than weight. Reducing the expression of genes involved in de novo lipogenesis, FA uptake, and hepatic TG secretion, while promoting the expression of key regulatory genes of fatty acid β-oxidation, SGLT-2i treatment improves IR and ameliorates intracellular FFA, total cholesterol (TC), and TG accumulation [30,84,100,107,109,112,117]. At higher doses, lipotoxic intermediates like diacylglycerols (DAG) contribute to insulin resistance (IR) by activating the protein kinase C (PKC)ε pathway [118], but taking empagliflozin lowers hepatic TAG levels and improves IR [115]. Importantly, Hüttl et al. recently showed that an increase in Nrf2, fibroblast growth factor 21, and altered gene expression of the cytochrome P450 (CYP) enzyme superfamily of cytochrome [115] mediates the decrease in hepatic lipid accumulation after empagliflozin treatment. Curiously, Nrf2 controls lipid metabolism by reducing insulin sensitivity whereas FGF21 controls lipogenesis [119,120].

Some of the most important genes involved in lipogenesis, such as fatty acid synthase (Fasn), acetyl-CoAcarboxylase 1 (Acc1), and stearoyl-CoA desaturase1 (Scd-1), are regulated by sterol regulatory element-binding transcription factor 1 (SREBP1). There is evidence that suggests that elevated SREBP1 expression can worsen hepatic steatosis [121]. Several studies have shown that canagliflozin and empagliflozin both suppress SREBP1 and Scd-1 expression [30,107,108,115]. However, Kern et al. [122] showed that eight weeks of treatment with empagliflozin had no significant effect on Scd-1 levels in db/db mice. In ZDF rats, Scd-1 gene expression was down-regulated and ACC1 phosphorylation was lowered after nine weeks of therapy with dapagliflozin (1 mg/kg/day). Due to the decreased mTOR expression/activity found following SGLT-2i treatment [84,123], which could explain the decreased expression of SREBP1 and its downstream lipogenic targets, as SREBP1 activity is directly regulated by mTOR signaling [123].

The overexpression of the peroxisome proliferator-activated receptor gamma (PPAR-γ) causes the production of new lipoproteins and the subsequent accumulation of these lipid droplets within the liver cells (hepatocytes) [124]. When compared to insulin glargine-treated rats, those given dapagliflozin for eight weeks had a lower liver weight and no liver steatosis progression [125]. Without influencing insulin sensitivity, liver TC, or oleate content, this effect was discovered to be mediated through reduced expression of PPAR-γ targeted genes involved in fatty acid synthesis, including Scd-1, Mogat1, Cell death inducing DFFA like effector A (Cidea), and cell death inducing DFFA like effector C (Cidec) [125].

SGLT-2i has also been demonstrated to undo the down regulation of genes involved in FA β-oxidation and lipolysis that has been caused by a high-fat diet in the livers of different NAFLD mice models [30,84,107,108]. The fact that SGLT-2i induces PPARα expression has been demonstrated [84,105,107,114]. The majority of genes involved in the FA β-oxidation pathway in mitochondria, including ACOX1, CPT1, and cytochrome-mediated (CYP4A1 and CYP4A3), are regulated by PPARα activation [44]. Research has demonstrated that AMLN-fed C57BL/6J mice with elevated levels of PPARα, CPT1A, and Microsomal Triglyceride Transfer Protein (MTTP) gene expression experienced an acceleration of β-oxidation and the export of very-low-density lipoprotein (VLDL) after receiving ipragliflozin therapy. The export of hepatic LDL is greatly impacted by MTTP [114]. In a similar vein, dapagliflozin reduced the buildup of hepatic lipids in rats with ZDF by increasing levels of the FA β-oxidation enzyme ACOX1 [85]. While tofogliflozin did not affect the expression of genes associated to FA β-oxidation in tumorous liver tissues, it did so in non-tumorous liver lesions [126]. On the other hand, not every study finds that SGLT-2i promotes the expression of transcription factors and enzymes that are involved in regulating FA β-oxidation [107,114,126]. Importantly, after eight weeks of empagliflozin therapy, the expression of PPARα did not change in either Wistar or HHTg rats [107,115]. Zinc alpha 2 glycoprotein (ZAG) is an essential regulator of hepatic lipolysis; its down-regulation was ameliorated by canagliflozin administration at doses of 10 and 20 mg/kg/day in response to the high-fat diet (HFD).

Similar SGLT-2i treatment of KK-Ay animals increased levels of the prostaglandin E2 (PGE2) protein in the liver, which in turn decreased lipid droplet accumulation [111]. In addition, when canagliflozin and exercise were administered together, they increased hepatic PPARγ Coactivator 1 Beta (PGC1b) levels and decreased the expression of genes that cause lipogenesis in the liver, like Scd1 [108]. Notably, PGC1 is involved in regulating hepatic gluconeogenesis, and studies have linked its overexpression to an increase in hepatic FA oxidation [127].

Reduced oxidative stress, inflammation in the liver, and cell death are the results of SGLT-2-i's ability to limit de novo lipogenesis while simultaneously increasing lipolysis and β-oxidation [128]. Reduced levels of oxidative stress, inflammation, and apoptosis were observed in the livers of diabetic Wistar adult male rats given canagliflozin. This was supported by measures such as decreased levels of plasma malondialdehyde (MDA), serum tumor necrosis factor (TNF), and caspase-3, and increased expression of interleukin-6 (IL-6) in the livers of these rats [128,129]. Treatment with dapagliflozin also alleviated hepatic steatosis and decreased expression of hepatic inflammatory cytokines (TNF-α, IL-1β, and IL-18) in male Wistar rats fed HCHF [130]. Ipragliflozin has anti-inflammatory and anti-oxidative properties, according to research by Tahara et al. [131]. This was shown in mice that were either streptozotocin-nicotinamide created type 2 diabetics or were fed a high-fat diet. Similarly, decreasing hepatic ROS generation of myeloperoxidase (MPO) and F4/80 with dapagliflozin administration at a dose of 1 mg/kg/day ameliorated blood ALT levels and hepatic fibrosis [132]. Enzyme MPO controls the start of the acute inflammatory response and encourages the progression of chronic inflammation by producing oxidants [133]. Additionally, levels of hepatic ROS, MDA, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were decreased after 16 weeks of canagliflozin administration in F344 rats [110]. Lipidomic acid dehydrogenase (MDA) and oxidative DNA damage (8-OHdG) are two well-established indicators, correspondingly. Reducing hepatic oxidative stress and ameliorating hepatic inflammation were both improved by combination therapy with canagliflozin and teneligliptin, which was noteworthy [110]. Thiobarbituric acid reactive substances (TBARS) levels were decreased as an indicator of reduced oxidative stress in patients treated with remogliflozin and ipragliflozin [100,134]. Alternatively, neither the hepatic TBARS nor the plasma ALT levels were significantly affected by four weeks of dapagliflozin administration at a dose of 1 mg/kg/twice daily [135]. Empadiozin treatment reduced oxidative stress, which improved hepatic inflammation [30,107,116] and steatosis [30,107,115], as measured by down-regulation of inflammatory markers. Intriguingly, SGLT-2-anti-inflammatory i's effects in the liver have been associated with reduced macrophage infiltration expression [107,117,134] and increased autophagy markers [30,117]. In particular, empagliflozin therapy decreased oxidative stress by increasing antioxidant enzyme activity (SOD) and decreasing hepatic inflammation through upregulating Nrf2 [115]. In diet-induced obese mice and NASH mouse models, empagliflozin reduced inflammation by suppressing hepatic nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α) expression, and by inhibiting the IL17/IL23 axis [115,117,136]. Mice and rats treated with low doses of dapagliflozin and ipragliflozin (1 mg/kg/day) showed reduced hepatic inflammation. By reducing expression of pro-inflammatory markers Emr1 and Itgax in non-tumorous lesions, ipragliflozin treatment for 12 weeks did relieve hepatic fibrosis [126]. Hupa-Breier et al. recently found that empagliflozin alone, at a dose of 10 mg/kg/day, had no beneficial effect on diet-induced NASH after four weeks of treatment, but it did increase expression of pro-inflammatory and pro-fibrotic genes. By regulating the pro-inflammatory immune response and microbiome dysbiosis, the combination of dulaglutide and empagliflozin had a hepato-protective impact on diabetic background mice in the same study [137].

The positive effects of empagliflozin on liver inflammation and ALT levels were shown by Meng et al. to be mediated mostly through the stimulation of AMPK/mTOR activity [117]. Liver kinase B1 (LKB1) is a master serine/threonine kinase that activates downstream AMPK pathway kinases [105,138]. Dapagliflozin therapy also activates the AMPK/mTOR pathway by phosphorylation of LKB1. Prior research has shown that autophagy-dependent lipid catabolism caused by SGLT-2i requires AMPK activation [30,117]. By increasing

AMPK phosphorylation and BECLIN gene expression and decreasing P62 levels and mTOR levels and activity, both dapagliflozin and empagliflozin induce hepatic autophagy [30,85].

Steatosis and the advancement of NAFLD/NASH are both impacted by ER stress, which in turn impairs autophagy [30,107]. In line with the idea that endoplasmic reticulum stress plays a regulatory role in autophagy, Nasiri-Ansari et al. showed that empagliflozin can reduce hepatic cell death and improve endoplasmic reticulum stress by stimulating autophagy [30]. Specifically, there are three branches of ER stress that empaglifilozin administration inhibits: inositol-requiring enzyme 1 (IRE1a), X-box binding protein 1 (Xbp1), activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), and activating transcription factor 6 (ATF6) [30,107]. In a recent study, Chen et al. found that reducing ATF6 signaling reduced inflammation and hepatic cell death caused by endoplasmic reticulum stress [139]. In HFD-fed ApoE−/− animals with NASH, the hepatic cell apoptosis was controlled by the combined effects of the ER stress response and autophagy process, which led to a decrease in cleaved caspase 3 levels and an increase in the B-cell lymphoma-2 (Bcl-2)/BCL2 Associated X (Bax) ratio [30]. Notably, canagliflozin has been shown to have an anti-apoptotic effect on mice that are fed a high-fat diet, as indicated by strong expression of Bcl-2 in their livers [129].

The conversion of hepatic stellate cells to myofibroblasts, a byproduct of chronic inflammation, is a factor in liver fibrosis and the development of NASH from NAFLD [140]. The most powerful inducer of liver fibrosis is Transforming growth factor beta (TGF-β). The expression of hepatic TGF-β was decreased in both F344 rats and MC4R-KO mice after 16 and 20 weeks of canagliflozin administration, respectively [101,110]. It is worth noting that the activation of TGF-β results in a rise in the production of fibronectin and collagen types I, II, and IV [141]. Mice treated with both empagliflozin and canagliflozin had their collagen expression levels decreased, suggesting that the two drugs worked together [87,137]. Reduced expression of collagen1a1, collagen1a2, TGF-β, and smooth muscle actin (SMA) is another mechanism by which luteogliflozin produces anti-fibrotic effects [142]. There is a wealth of information demonstrating that tissue inhibitors of metalloproteinases (TIMPs) play a pivotal role in the development of fibrosis in the liver. Both luseogliflozin and canagliflozin have been found to alleviate hepatic fibrosis and decrease TIMP expression [101,126,142]. Treating cells with empagliflozin had no effect on the expression of markers for stellate cell functioning, including galectin-3, a-SMA, and collagen1a1 [99].

The development of cirrhosis and HCC is more likely in individuals with NASH. Several variables, including inflammation, abnormal metabolism, and hepatic lipotoxicity, lead to DNA instability, which in turn causes hepatic tumorogenesis [141,143]. Since most studies have demonstrated that SGLT-2i protects against the progression of NAFLD/NASH, researchers set out to determine whether it has any influence on the formation or progression of HCC [87,101,142]. Liver cancer and induced fibrosis are both favorably associated with P53/P21 signaling pathway activation. Effective reduction of P21 expression and amelioration of NASH progression through, at least in part, modulating genes relevant to the hepatocyte cellular senescence-associated secretory phenotype was seen after a 14-week therapy with tofogliflozin [89]. Canagliflozin, when administered daily, significantly reduced the incidence of hepatic tumorous lesions, preventing hepatocellular carcinoma (HCC) in both the STAM (a NASH model) mice (11 weeks) and the MC4R-KO (HFD-fed) mice (52 weeks) [87,101]. Canagliflozin also had an anti-carcinogenic impact in F344 rats that were fed CDAA for 66 weeks; this was shown by a decrease in the number of cells positive for placental glutathione S-transferase, a major indicator of hepatocarcinogenesis [110]. Finally, by suppressing hepatic neovascularization indicators, such as cluster of differentiation 31 (CD31) and vascular endothelial growth factor (VEGF) [110], canagliflozin treatment decreased the development of hepatic preneoplastic lesions in rats given CDAA.

Conclusion

No matter how common type 2 diabetes is, there is growing interest in SGLT-2i as a potential treatment for non-alcoholic fatty liver disease (NAFLD). According to in vitro, animal, and clinical studies, SGLT-2i has a number of beneficial effects on NAFLD development and progression, including weight loss and the

regulation of multiple processes, such as oxidative stress, low-grade inflammation, autophagy, and apoptosis. Also, different SGLT-2i medications have different impacts on NAFLD, which may indicate that there are characteristics unique to each drug in this class in terms of its action mechanism(s) and how it affects NAFLD

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