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Animal studies of sodium-glucose co-transporter 2 inhibitors in nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is considered one of the most common chronic liver diseases. Modern lifestyle, characterized by increasing rates of obesity and type 2 diabetes mellitus (T2DM), has led to a "pandemic" of NAFLD that imposes a personal health and socioeconomic burden. Apart from overnutrition and insulin resistance, various metabolic aberrations, gut microbiota and genetic predispositions are involved in the pathogenesis of the disease. The multifactorial nature of NAFLD's pathogenesis makes the development of pharmacological therapies for patients with this disease challenging. Sodium-glucose co-transporter 2 inhibitors (SGLT-2i) are antidiabetic agents that reduce blood glucose mainly by increasing its renal excretion. As T2DM is one of the major contributors to NAFLD, SGLT-2i have emerged as promising agents for the management of NAFLD. In this review, we summarize the main animal studies on SGLT-2i in models of NAFLD.

Keywords Fibrosis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, pathophysiology, sodium-glucose co-transporter 2

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Introduction

The modern lifestyle of the so-called "Western World" has led to novel health concerns. New diseases have emerged, including nonalcoholic fatty liver disease (NAFLD), one of the most common chronic liver diseases [1]. The prevalence of NAFLD has been increasing in recent years and is estimated to be 25-30% worldwide [2]. There appears to be a preference for the elderly and men of younger ages, whereas its rates are similar in men and women after menopause [3]. The rising prevalence is partially explained by the increase in the rates of obesity and type 2 diabetes mellitus (T2DM) [4,5]. In patients with these conditions, the prevalence of NAFLD can be over 90% and 55%, respectively [6]. However, a considerable percentage of non-obese individuals also develop NAFLD [7].

Histologically, NAFLD is characterized by liver fat accumulation in at least 5% of the hepatocytes, after excess alcohol consumption and other hepatic diseases, such as viral and autoimmune hepatitis, and drug-induced liver injury having been ruled out [8]. Thus, the diagnosis of NAFLD is based on the exclusion of certain diseases causing secondary fatty liver: this has motivated many researchers to pursue a new definition and a diagnosis based on definite criteria (positive diagnosis) rather than the exclusion criteria (negative diagnosis) [9]. Two nomenclatures were most prevalent over the years and were published in consensuses: 1) metabolic (dysfunction)-associated fatty liver disease (MAFLD) [10,11]; and 2) metabolic dysfunction-associated steatotic liver disease (MASLD) [12]. However, there is still controversy over the most appropriate definition of the disease. Furthermore, most existing studies have used the term and criteria of NAFLD. Therefore, for the purposes of this review we adopted the term NAFLD.

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Simple hepatic steatosis, known as nonalcoholic fatty liver (NAFL), may progress to nonalcoholic steatohepatitis (NASH), characterized by the addition of inflammation and hepatocellular ballooning, and even to hepatic fibrosis and cirrhosis [8]. NASH patients are reported to be 2-6% worldwide and among them 21-50% have advanced fibrosis, whereas 7% of all NAFLD patients develop advanced fibrosis [6]. The need to efficiently manage NAFLD derives from its higher hepatic (e.g., cirrhosis, hepatocellular carcinoma, hepatic failure) and extra-hepatic (cardiovascular diseases, chronic kidney disease and extra-hepatic malignancies) morbidity and mortality [13-15].

Despite the large number of studies and the variety of agents that have been investigated or are under investigation, until recently there was no approved treatment specifically for NAFLD [16-19]. Ideally, we need a medication that improves both NAFLD (liver function tests and histology) and related metabolic aberrations [20,21]. In this regard, sodium-glucose co-transporter 2 inhibitors (SGLT-2i) are antidiabetic drugs with potentially beneficial effects on liver steatosis [21]. In this review, after a brief summary of the main pathophysiologic contributors to NAFLD, we aim to focus on data derived from animal studies that investigated the potential therapeutic effects of SGLT-2i on NAFLD.

Pathophysiologic mechanisms in NAFLD

Following the previously suggested "2-hit hypothesis", which claimed that one pathogenic contributor leads to the development of hepatic steatosis, while a second pathogenic contributor leads to the progression to hepatic inflammation and fibrosis, the most prevalent model for the pathophysiology of NAFLD is that suggested by the "multiple-hit hypothesis" (Fig. 1) [22,23]. The pathogenesis of NAFLD is multifactorial, with numerous factors acting in parallel, thus leading to the development of hepatic steatosis and the progression to advanced disease [17]. Overnutrition leads to the intrahepatic accumulation of high concentrations of free fatty acids and lipid metabolites that exceed the liver's capacity to appropriately oxidize or store them, thus leading to lipotoxicity, oxidative stress and the production of reactive oxygen species [5]. Consequently, inflammatory and apoptotic pathways are activated, with fibrogenic potential [24]. Not only overnutrition, but also obesity is related with NAFLD development, a process complicated by the production of adipokines, such as adiponectin and leptin, by the adipose tissue [5,25-27]. Insulin resistance (IR) represents another major contributor to the pathogenesis of NAFLD, since it leads to oversupply of free fatty acids to the liver, as a result of the increased lipolysis of adipose tissue triglycerides and hepatic *de novo* lipogenesis [28,29]. In close association with IR, better regulation of glucose levels also leads to an improvement in NAFLD, and delays or even prevents its progression to advanced disease [20]. It is important to note that dysbiosis of gut microbiota stimulates hepatic inflammation via increased absorption of endotoxins and by altering bile-acid metabolism (gut–liver axis) [30].

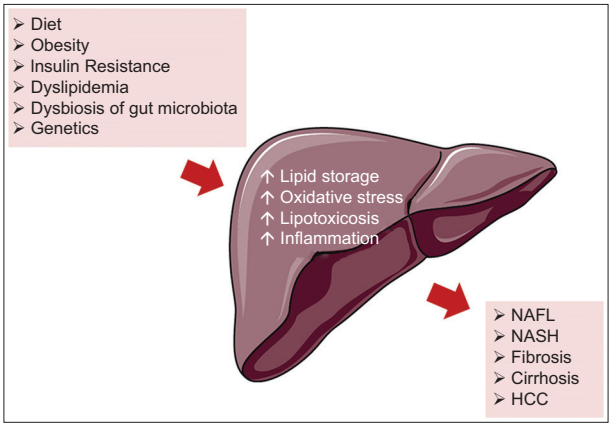

Figure 1 Brief pathophysiology of NAFLD. The pathophysiology of NAFLD starts from lipid accumulation within the hepatocytes. Insulin resistance, obesity and dyslipidemia are some of the major contributors related to the pathogenesis of NAFLD. A high-calorie diet rich in carbohydrates (especially fructose) and saturated fats may promote hepatic steatosis, which may subsequently progress to hepatic inflammation, fibrosis or even cirrhosis and HCC in some patients. Long-standing hepatic steatosis predisposes for a mild but chronic intra-hepatic inflammation, which enhances liver injury and may lead to structural disorganization and fibrosis. NAFLD is also influenced by alterations in the composition of the gut microbiota (dysbiosis) or genetic factors, as well as other factors. Though the precise mechanisms leading to NAFLD development and progression are not fully understood, its pathophysiology is seemingly affected by complex interactions among metabolic, environmental and genetic factors
HCC, hepatocellular carcinoma; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis

Pathophysiologic association of SGLT-2 inhibitors with NAFLD

Sodium-glucose co-transporters (SGLTs) are responsible for glucose transportation from the renal tubule to the adjacent epithelial cells [31]. SGLT-2 is a protein expressed mainly in the kidney, and more precisely in the luminal membrane of the proximal renal tubules, that leads to the reabsorption of the major proportion of glucose [31]. SGLT-2i are antidiabetic medications that block SGLT-2, thus increasing the urinary excretion of glucose, diminishing circulating glucose levels independently of insulin secretion and, consequently, without inducing hypoglycemia (Fig. 2) [32]. Better glycemic control, reduction in insulin levels and a possible increase in glucagon release render SGLT-2i potentially beneficial medications for NAFLD [33]. Moreover, metabolic changes include decreased triglycerides, enhanced lipolysis and elevated fat oxidation supported by ketogenesis [34]. Taking these mechanisms into consideration, it seems that SGLT-2i shift substrate utilization from carbohydrate to lipid, and as we already know, the alteration of lipid metabolism represents a crucial step in the development and progression of NAFLD [34,35].

In addition to the previous actions, the elevated glucose urinary excretion promotes osmotic diuresis, and eventually natriuresis, uricosuria and calorie loss. The body weight reduction could be considered a potentially useful contributor

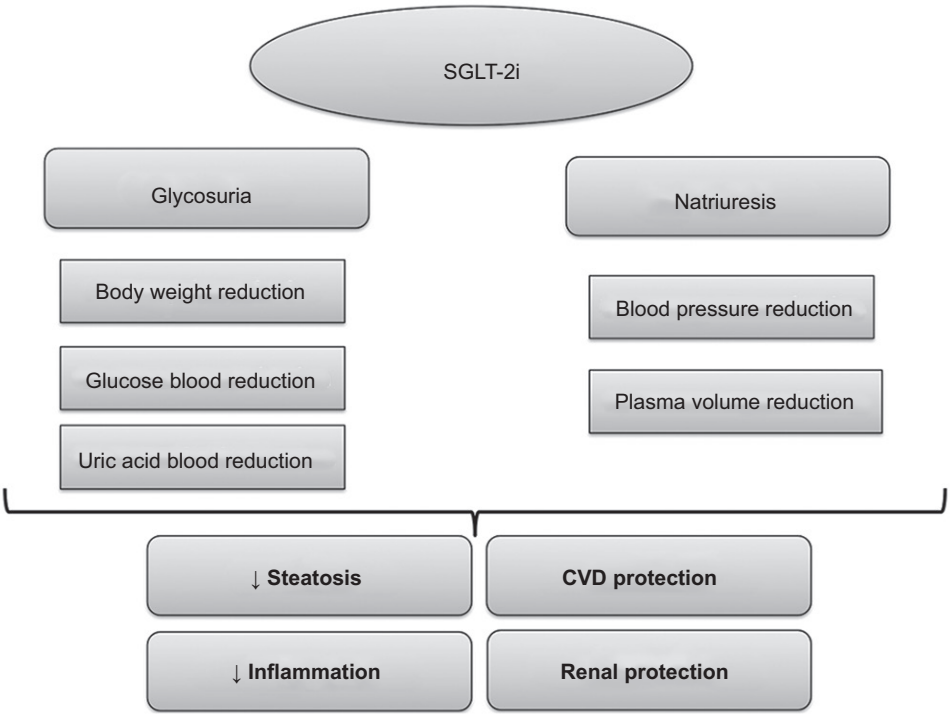

Figure 2 Potential mechanisms of actions of SGLT-2i in NAFLD. By glucose excretion in the urine, SGLT-2i lead to reduced blood glucose levels and body weight loss, while the induced uricosuria diminishes circulating uric acid. Moreover, natriuresis, which occurs in parallel with glycosuria, contributes to the reduction of blood pressure and plasma volume. These effects were shown to favor cardiorenal protection, but they may also be beneficial against the development of steatosis and its progression to hepatic inflammation
NAFLD, nonalcoholic fatty liver disease; SGLT-2i, sodium-glucose co-transporter 2 inhibitors; CVD, cardiovascular disease

towards the improvement of NAFLD, as data indicated that a weight loss of 7% or more decreases hepatic fat, and even inflammation, while a 10% reduction may reverse hepatic fibrosis [36]. Notably, natriuresis leads to decreases in blood pressure and plasma volume, whereas uricosuria may protect against chronic kidney disease and possibly atherosclerosis [37].

As we have already mentioned, SGLT-2 are responsible for the glucose reabsorption in the kidney. Another type of SGLT, SGLT-1, are also expressed in the kidney, but more predominantly in the small intestine and are largely responsible for glucose reabsorption from the small intestine [38]. The administration of an SGLT-1i showed a protective effect against the pathogenesis of NAFLD in a rodent model [39]. Moreover, SGLTs are also reported to be expressed in other organs. SGLT-1 are also expressed in the heart and the skeletal muscle, whereas SGLT-2 activity was detected in rat brain and, in a more recent study, the hepatic expression of SGLT-2 was found to be greater in NAFLD and NASH compared to normal livers [38,40,41].

Collectively, SGLT-2i seem to have properties that may be advantageous in the management of NAFLD. Furthermore, initial data may indicate some favorable effects of SGLT-1i. However, a great deal more data are needed to fully elucidate the potentiality of SGLT-2i against NAFLD, and in this regard animal studies are of high importance.

Data from animal studies

Animal studies, conducted primarily on mice and rats, have provided valuable insights into the effects of SGLT-2i on NAFLD. Their main results are summarized here and in Table 1.

Search strategy

We searched in the PubMed database using the search string: "(("Non-alcoholic Fatty Liver Disease"[Mesh]) OR ("Fatty Liver"[Mesh]) OR (non-alcoholic fatty liver disease) OR (nonalcoholic fatty liver disease) OR (fatty liver) OR (hepatic steatosis) OR (nonalcoholic steatohepatitits) OR (non-alcoholic steatohepatitis) OR NAFLD OR NASH OR (liver fibrosis) OR (liver cirrhosis)) AND (("Sodium-Glucose Transporter 2 Inhibitors" [Mesh]) OR (sodium glucose co-transporter 2 inhibitors) OR (SGLT2 inhibitors) OR SGLT2i OR dapagliflozin OR empagliflozin OR canagliflozin OR ipragliflozin OR luseogliflozin OR tofogliflozin)", restricted with the filter "Other Animals". Using this string, 142 articles were retrieved (last update March 20, 2024). The search was extended to the reference lists of some of the selected articles. Since this is a narrative review, the selection of the included articles was based on the personal judgment of the authors,

Table 1 Effect of various SGLT-2i on main histological lesions in animal models of NAFLD

Study [ref.], year	SGLT-2i	Animal model	Improvement in steatosis	Improvement in lobular inflammation	Improvement in ballooning	Improvement in fibrosis	Improvement in NAS	Duration
Omori *et al* [42], 2019	Dapagliflozin	db/db mice	No	NA	NA	NA	NA	6 wo dapagliflozin (1 mg/kg) for 8 w
El Mahdy *et al* [43], 2020	Dapagliflozin	Wistar rats on HFD	Yes	Yes (generally as inflammation)		NA	NA	HFD for 12 w then dapagliflozin for 6 w
Hazem *et al* [44], 2022	Dapagliflozin	Albino STZ rats on HFD	Yes	Yes (generally as inflammation)		NA	NA	HFD for 8 w then STZ then dapagliflozin (0.75, 1.5 or 3 mg/kg) for 6 w
Ji *et al* [47], 2017	Canagliflozin	C57BL/6J mice on HFD	NA	NA	NA	NA	NA	4 wo HFD for 4 w then canagliflozin (15 or 60 mg/kg) for 4 w
Kabil *et al* [45], 2018	Canagliflozin	Wistar nicotinamide and STZ diabetic rats on HFD	Yes	Yes	Yes	NA	Yes	HFD+canagliflozin (10 or 20 mg/kg) for 8 w
Shiba *et al* [46], 2018	Canagliflozin	MC4R-KO mice on WD	No	No	Yes	NA	Yes	8 wo WD+canagliflozin for 8, 20 and 52 w
Yoshino *et al* [48], 2021	Canagliflozin	KK-Ay diabetic mice	NA	NA	NA	NA	NA	7 wo canagliflozin for 4 w
Jojima *et al* [49], 2016	Empagliflozin	C57BL/6J STZ diabetic mice on HFD	NA	NA	NA	NA	Yes	HFD from week 4. Empagliflozin from week 6 for 3 w
Xu *et al* [55], 2017	Empagliflozin	C57BL/6J mice on HFD	Yes	NA	NA	NA	NA	8 wo HFD or HFD+empagliflozin (3 or 10 mg/kg) for 16 w
Petito-da-Silva *et al* [51], 2019	Empagliflozin	C57BL/6J mice on HFD	Yes	NA	NA	NA	NA	3 mo of age start HFD for 10 w then empagliflozin for 5 w
Hupa-Breier *et al* [57], 2021	Empagliflozin	C57BL/6J mice on HFD	No	No	No	No	No	6-8 wo HFD for 12 w then empagliflozin (10 mg/kg) for 4 w
Hüttl *et al*[58], 2021	Empagliflozin	Wistar and HHTg rats	No differences in histological evaluation					6 mo empagliflozin (10 mg/kg) for 8 w
Meng *et al* [50], 2021	Empagliflozin	C57BL/6J STZ diabetic mice on HFD	Yes	NA	NA	NA	Yes	6-8 wo start HFD for 12 w then empagliflozin for 8 w
Nasiri-Ansari *et al* [53], 2021	Empagliflozin	C57BL/6J ApoE(-/-) mice on HFD	Yes	Yes	No	No liver fibrosis was detected neither on the control nor on empagliflozin group	Yes	5 wo HFD for 5 w then empagliflozin for 5 w
Perakakis *et al* [60], 2021	Empagliflozin	C57BL/6JRj mice with biopsy-confirmed steatosis and fibrosis on AMLN diet	No	No	No	No	Yes	5 wo AMLN for 36 w then empagliflozin (10 mg/kg) for 12 w

(Contd...)

Table 1 *(Continued)*

Study [ref.], year	SGLT-2i	Animal model	Improvement in steatosis	Improvement in lobular inflammation	Improvement in ballooning	Improvement in fibrosis	Improvement in NAS	Duration
Kurtz *et al* [59], 2022	Empagliflozin	TallyHo, SWR/J and C57BL/6J mice on HFD	No	NA	NA	NA	NA	12-14 wo HFD or HFD+empagliflozin (10 mg/kg) for 12 w
Lee *et al* [56], 2022	Empagliflozin	Choline-deficient, L-amino acid-defined, HFD C57BL/6J mice	No	Yes	Yes	Yes	Yes	7 wo HFD for 2 w then empagliflozin (10 μg/g) for 5 w
Chun *et al* [41], 2023	Empagliflozin	C57BL/6J mice on AMLN diet	severe fat accumulation and collagen deposition with the AMLN diet and these NASH characteristics were ameliorated with empagliflozin treatment					7 wo AMLN for 10 w then empagliflozin (10 mg/kg) for 10 w
Kim *et al* [61], 2023	Empagliflozin	C57BL/6N choline-deficient on HFD	No	No (generally as inflammation)		No	NA	Experiment 1: 6 wo HFD for 8 w then empagliflozin (10 mg/kg) for 8 w/Experiment 2:6 wo HFD for 30 w then empagliflozin (10 mg/kg) for 12 w
Radlinger *et al* [52], 2023	Empagliflozin	C57BL/6 mice on WD	Yes	NA	NA	NA	NA	6 wo WD or WD+empagliflozin (30 mg/kg) for 10 w
Hayashizaki-Someya *et al* [63], 2015	Ipragliflozin	Wistar rats on CDAA diet	Yes	No (generally as inflammation)		Yes	NA	9 wo CDAA or CDAA+ipragliflozin (0.3 or 3 mg/kg) for 5 w
Honda *et al* [64], 2016	Ipragliflozin	C57BL/6J mice on AMLN diet	Yes	Yes	No	Yes	Yes	8 wo AMLN for 12 w then ipragliflozin (40 mg) for 8 w
Komiya *et al* [66], 2016	Ipragliflozin	C57BL/6J ob/ob mice on HFD	Yes	NA	NA	NA	NA	8 wo HFD for 12 w then ipragliflozin (10 mg/kg) for 4 w
Tahara *et al* [65], 2019	Ipragliflozin	KK-A^y diabetic mice on HFD	Yes	Yes (generally as inflammation)		Yes	NA	7 wo HFD+ipragliflozin (0.1-3 mg/kg) for 4 w
Qiang *et al* [67], 2015	Luseogliflozin	C57BL/6J nicotinamide and STZ-induced diabetic mice on HFD	Yes	NA	NA	Yes	NA	HFD+luseogliflozin for 8 w
Yoshioka *et al* [68], 2021	Tofogliflozin	MC4R-KO mice on WD, with diethyl nitrosamine	No	Yes	Yes	Yes	Yes	2 wo diethyl nitrosamine then 6 wo WD+tofogliflozin for 14 w

Data are sorted according to the specific SGLT-2i (primarily) and to year of publication (secondarily)
Yes: refers to statistically significant improvement in the animal group on SGLT-2i compared with the control group
No: refers to statistically non-significant improvement between the animal group on SGLT-2i compared with the control group
AMLN, amylin liver NASH; ApoE(-/-), apolipoprotein E knockout mice; CDAA, choline deficient L-amino acid-defined diet; HFD, high fat diet; HHTg, hereditary hypertriglyceridemic; KK-Ay, diabetic KK and lethal yellow (Ay) mice; MC4R-KO, melanocortin 4 receptor-knockout; NA, not available; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OLETF, Otsuka Long-Evans Tokushima fatty; SGLT-2i, sodium-glucose transporter-2 inhibitors; STZ, streptozotocin; w, weeks; WD, western diet; wo, weeks old

mainly according to their originality and their potential impact on the field. Moreover, some more articles beyond this search were added at the discretion of the authors, when this was considered necessary for the flow of this review.

Dapagliflozin

Dapagliflozin was used in a few experimental models to evaluate its effect on NAFLD. In *db/db* mice, steatosis was not different from the control group, but dapagliflozin retained the beta cell mass of the pancreas through the reduction of glucotoxicity [42]. In contrast, in rat models dapagliflozin histologically improved steatosis and inflammation, as well as liver function tests, reportedly via inhibiting oxidative stress [43,44].

Canagliflozin

Kabil *et al* reported a rat model, treated with nicotinamide and streptozotocin to induce diabetes, that received a high-fat diet (HFD) for 8 weeks [45]. The animals were then categorized into 3 groups fed HFD and 3 groups on chow diet (CD); 2 of the groups received the drug at a dose of 10 or 20 mg/kg [45]. Canagliflozin ameliorated steatosis, hepatic weight, lipid storage in the liver and alanine aminotransferase (ALT) in a dose-dependent manner. Canagliflozin also reduced inflammatory cytokines and oxidative stress, as well as inflammation and hepatocellular ballooning, compared to the untreated group. In another study that used a melanocortin 4 receptor knock-out (MC4R-KO) mouse model fed a Western diet, which is an IR and obesity model, canagliflozin was reported to improve hepatocellular ballooning, NAFLD activity score (NAS) and fibrosis (described by Sirius red stained area percentage), but not hepatic steatosis and inflammation after 20 weeks of treatment [46]. After the initial 8 weeks, treated mice increased calorie intake and body weight and decreased ALT, but this was not accompanied by higher glucose and liver weight compared to the untreated group. In this study, SGLT-2 protein was found to be expressed in the central vein and biliary tract and, importantly, a 52-week treatment was shown to attenuate the occurrence of hepatocellular carcinoma. In another study, after an initial period of 4 weeks on HFD, obese mice were treated with placebo or orlistat (15 mg/kg) or canagliflozin (60 mg/kg) [47]. In the canagliflozin group, body weight was reduced after a 4-week interval; however, the reduction in liver weight was not different compared to the orlistat group. In contrast, the diabetic KK and lethal yellow (Ay) mice (KK-Ay) of Yoshino *et al* demonstrated no reduction in body weight, cholesterol or free fatty acids, but lower glucose and triglyceride levels and liver weight compared to controls over 4 weeks [48]. Lipid droplet accumulation and hepatic triglycerides were improved in the canagliflozin group, while a lipidomics analysis revealed that prostaglandin E2 and resolvin E3, considered as lipid mediators, were elevated in the treated group, which is suggested to improve fat deposition in the liver.

Empagliflozin

Empagliflozin seems to be the most studied SGLT-2i in animal studies of NAFLD; it thus seems reasonable that most studies selected in this review (Table 1) referred to empagliflozin. Jojima *et al* used diabetic mice fed on an HFD and treated with empagliflozin for 3 weeks to show that NAS was lower in the treated group compared to the untreated one [49]. However, there was no difference in body weight or the expression of type 3 collagen mRNA of mice treated with empagliflozin, although collagen deposition was lower compared to the untreated control group. In line with the aforementioned study, Meng *et al* reported lower NAS in HFD fed mice on triglycerides for 8 weeks compared to controls [50]. They also showed improvement in lipid droplet accumulation, blood tests and expression of genes involved in lipogenesis and oxidation in the same group. Petito-da-Silva *et al* administered empagliflozin to HFD mice for 5 weeks, showing improvement in steatosis, metabolic parameters, and lipogenic and endoplasmic reticulum stress genes [51]. Other studies also showed steatosis improvement by empagliflozin [52,53]. Interestingly, metabolomic analysis of the impact of empagliflozin on mice fed an HFD reported that empagliflozin affects lipid oxidation and lipid metabolism [54]. As for hepatic inflammation, Xu *et al* fed C57BL/6J mice with CD, HFD and empagliflozin at a low dose of 3 and a high dose of 10 mg/kg body weight. They reported upregulated mRNA levels of anti-inflammatory and M2 macrophage markers in the group treated with empagliflozin [55], implying that empagliflozin favor anti-inflammatory activity. Notably, the high dose of empagliflozin ameliorated several morphologic and biochemical parameters more than the low dose, thus indicating a dose-dependent relationship with metabolic alterations. Interestingly, in apolipoprotein E knock-out [ApoE(-/-)] mice on HFD, a 5-week treatment with empagliflozin reduced steatosis, lobular inflammation and NAS compared to controls [53]. Notably, neither group developed fibrosis. The histological amelioration in steatosis and NASH was linked with the lower expression of genes involved in lipogenesis and inflammation. In line with this study, choline-deficient, L-amino acid-defined, HFD C57BL/6J mice treated with empagliflozin for 5 weeks confirmed the alterations in inflammation, NAS, but also fibrosis compared to the control group; surprisingly, no significant difference between groups was observed in steatosis [56]. In contrast, a 16-week study of C57BL/6 mice on an HFD, with added fructose and sucrose in the drinking water, revealed no improvement in body weight, aspartate aminotransferase, ALT, histological parameters or NAS in the empagliflozin group compared to the HFD control group [57]. Other studies involving rodents confirmed that empagliflozin did not show superiority and did not improve histological outcomes compared to controls [58,59]. All in all, it has been shown that empagliflozin exerts its favorable effects when diabetes exists [57,60]. Notably, Kim *et al* used an experimental model to show that choline-deficient C57BL/6N mice on HFD developed NAFL after 8 weeks and NASH after 30 weeks [61]. Empagliflozin improved body and liver weight and ALT, compared to the HFD control group, only when it was co-administered with ezetimibe, but not as monotherapy [61]. In this study, steatosis decreased at the stage of NAFL only when empagliflozin was combined with ezetimibe, whereas no histological differences were

shown in steatosis, inflammation or fibrosis in the empagliflozin-treated group compared to controls, when the disease progressed to NASH [61]. An important piece of research provided evidence that SGLT-2 was not only present in the liver, but was also increased in NAFLD, while its inhibition ameliorated NASH through autophagy activation [41]. Furthermore, empagliflozin was shown to ameliorate hepatic lipid accumulation and collagen deposition [41]. Another pathway highlighting the anti-inflammatory and antifibrotic effects of empagliflozin in NAFLD was demonstrated in a rat model with downregulation of the nuclear factor-kappa B (NF-κB)/sex determining region Y box 9 (SOX9)/osteopontin axis, which reduced collagen accumulation, and in parallel to the upregulation of hepatic osteocalcin through the inhibition of NF-κB [62].

Ipragliflozin

Ipragliflozin was shown to improve hepatic steatosis, not only in a rat model [63], but also in mouse models [64,65], even independently of alterations of body weight [66]. Additionally, improvement in fibrosis stage by ipragliflozin was also demonstrated, without significant simultaneous changes in inflammation [63]. Interestingly, histological improvements in NAS parameters (hepatic steatosis, lobular inflammation) and fibrosis, but not in hepatocellular ballooning, were observed in a diabetic NASH-induced mouse model [64], while in another mouse model histological improvement in steatosis, inflammation and fibrosis were observed only in the groups receiving high-dose ipragliflozin (3 mg/kg) compared to the control group [65]. Finally, in the model reported by Honda *et al*, ipragliflozin improved lipotoxicity and IR [64].

Other SGLT-2i

In accordance with the aforementioned findings, other SGLT-2i, such as luseogliflozin and tofogliflozin also revealed promising results. Luseogliflozin improved hepatic steatosis and collagen deposition, while tofogliflozin also improved hepatic inflammation, NAS and liver fibrosis, without significant differences in steatosis grade [67,68]. Interestingly, it has been suggested that tofogliflozin may prevent the development of NASH-associated hepatic tumors [68]; however, this requires validation by other studies.

Combination therapies

Although treating NAFLD is an appealing topic for researchers and the pharmaceutical industry, most medications failed to meet their endpoints in clinical trials [69]. This may indicate that there is no "magic bullet" to effectively treat all NAFLD patients, partly because of the considerable heterogeneity of the disease's pathogenesis. Thus, it appears rational to target multiple pathogenic factors simultaneously, which may prove to be more fruitful approach to management [17,70,71]. Several researchers have investigated the potential benefits of combining an SGLT-2i with other medications (Table 2). Regarding dapagliflozin, the combination with metformin, a first-line antidiabetic medication, provided the greatest histological benefits in rats on an atherogenic diet [72].

Regarding canagliflozin, its monotherapy lowered hepatic triglyceride content, but no further decrease was observed in the group on a combination of canagliflozin and teneligliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, in mice on an HFD [73]. In another study, the combination of canagliflozin and teneligliptin did not show additional histological improvement compared to canagliflozin monotherapy in Fisher rats on a choline deficient L-amino acid-defined diet; however, the collagen content, as an index of fibrosis, was decreased in the combination group compared to either canagliflozin or teneligliptin monotherapy [74].

Regarding empagliflozin, its combination with linagliptin, another DPP-4 inhibitor, reduced hepatic lipid content compared to monotherapy with empagliflozin in *db/db* mice [75]. The combination of empagliflozin with metformin in mice with CCl_4-induced hepatic fibrosis reduced necroinflammation compared to CCl_4 mice on no treatment (active control group), and resulted in a smaller fibrosis area compared to monotherapy with either metformin or empagliflozin [76]. As mentioned above, the combination of empagliflozin with ezetimibe (a hypolipidemic medication) reduced steatosis, but not inflammation or fibrosis, compared to a control group in choline-deficient mice on HFD, whereas monotherapy with either drug was not effective [61].

Regarding ipragliflozin, its combination with pioglitazone, a peroxisome proliferator-activated receptor-γ agonist, resulted in a bigger decrease in steatosis, inflammation and fibrosis compared to control and monotherapy with ipragliflozin in diabetic mice with NASH [65]. When ipragliflozin was combined with liraglutide, a glucagon-like peptide-1 receptor agonist, in two mouse models, the combination reduced NAS compared to controls (Table 2), but did not provide extra histological benefits compared to monotherapy with either ipragliflozin or pioglitazone [77]. The combination of ipragliflozin with metformin reduced hepatic steatosis, inflammation and fibrosis compared to a control group and metformin monotherapy in diabetic mice with NASH [78].

Regarding tofogliflozin, its combination with pemafibrate decreased hepatocellular ballooning, but not steatosis or fibrosis, compared to the control group, but showed no additional histological benefit compared to monotherapy with tofogliflozin in streptozotocin mice on HFD [79].

Discussion

NAFLD is a global public health problem with increasing prevalence, making the need for pharmacological therapy imperative. Since T2DM is considered a major contributor to the pathophysiology of NAFLD, antidiabetic medications hold promise for the management of NAFLD. SGLT-2i have been approved for T2DM treatment and their mechanisms of action render them potentially therapeutic agents for NAFLD.

Table 2 Animal studies having evaluated combination therapies in rodents with NAFLD.

Study [ref.], year	Animal model	Groups	Improvement in steatosis	Improvement in inflammation	Improvement in fibrosis	Improvement in additional parameters
Shaaban *et al* [72], 2022	Wistar rats on atherogenic diet supplemented with high fat	(1) Controls on CD; (2) controls on atherogenic diet; (3) metformin; (4) pioglitazone; (5) metformin+pioglitazone; (6) dapagliflozin; (7) metformin+dapagliflozin; (8) pioglitazone+dapagliflozin; (9) silymarin	Group 7 vs. 1 and group 8 vs. 2 (histological)	Group 8 vs. 2 (histological)	Group 7 and 8 vs. 2 (molecular markers)	NAS: group 7 and 8 vs. 2 (histological)
Kawarasaki *et al* [73], 2023	C57BL/6N mice on HFD	(1) Controls on CD; (2) controls on HFD; (3) teneligliptin; (4) canagliflozin; (5) teneligliptin+canagliflozin	Group 4 vs. 2 (hepatic TGs content)	NA	NA	-
Ozutsumi *et al* [74], 2020	Fisher 344 rats on CDAA	(1) Controls on CSAA; (2) controls on CDAA; (3) canagliflozin; (4) teneligliptin; (5) canagliflozin+teneligliptin	Group 3 and 5 vs. 2 (histological)	Group 3 and 5 vs. 2 (histological)	Group 5 vs. 3 and 4 (histological)	-
Kern *et al* [75], 2016	*db/db* mice	(1) Controls; (2) empagliflozin; (3) linagliptin; (4) empagliflozin+linagliptin	Group 4 vs. 1 and 2 (hepatic lipid content %)	NA	NA	-
Abdelhamid *et al* [76], 2021	BALB/c mice on olive oil	(1) Controls on olive oil; (2) metformin; (3) empagliflozin; (4) CCl_4; (5) CCl_4+metformin; (6) CCl_4+empagliflozin; (7) CCl_4+metformin+empagliflozin	NA	Group 7 vs. 4 (histological)	Group 7 vs. 4, 5 and 6 (histological: fibrosis area%)	-
Kim *et al* [61], 2023	C57BL/6N choline-deficient mice on HFD: for 8 weeks (NAFL model 1) or for 30 weeks (NASH model 2)	(1) Controls on HFD; (2) empagliflozin; (3) ezetimibe; (4) empagliflozin+ezetimibe	Group 4 vs. 1 (model 1)	No differences between groups (histological)	No differences between groups (histological)	-
Tahara *et al* [65], 2019	KK-Ay mice, on HFD	(1) Controls on HFD; (2) ipragliflozin; (3) pioglitazone; (4) ipragliflozin+pioglitazone	Group 4 vs. 1 and 3 (histological)	Group 4 vs. 1 and 3 (histological)	Group 4 vs. 1 and 3 (histological)	-
Koike *et al* [77], 2021	DIO mice (model 1), C57BL/6 *db/db* mice (model 2)	(1) Controls; (2) liraglutide; (3) ipragliflozin; (4) liraglutide+ipragliflozin. Both models were divided into these groups.	NA	NA	NA	NAS: group 2, 3 and 4 vs. 1 (model 1), group 3 and 4 vs. 1 (model 2) (histological)
Tahara *et al* [78], 2020	KK-Ay mice, on HFD	(1) Control on HFD; (2) ipragliflozin; (3) metformin; (4) ipragliflozin+metformin	Group 4 vs. 1 and 3 (histological)	Group 4 vs. 1 and 3 (histological)	Group 4 vs. 1 and 3 (histological)	-
Murakami *et al* [79], 2022	STZ mice on HFD	(1) Control on HFD; (2) pemafibrate; (3) tofogliflozin; (4) pemafibrate+tofogliflozin	No differences between groups (histological)	Group 3 and 4 vs. 1 (histological: hepatocellular ballooning)	No differences between groups (histological: sirius red area %)	NAS: No differences between groups (histological)

+: Indicates combination of medications

BALB, Bagg Albino; CD, chow diet; CDAA, choline-deficient, L-amino acid-defined; CSAA, choline-sufficient, L-amino acid-defined; DIO, diet induced obese; HFD, high fat diet; KK-Ay, diabetic KK and lethal yellow (Ay) mice; NA, not available; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; STZ, streptozotocin; TGs, triglycerides

Preclinical studies have demonstrated promising results regarding specific histological features of NAFLD, such as hepatic steatosis and inflammation, whereas there is less and more conflicting evidence for liver fibrosis (Table 1)—though this is considered a histological predictor of advanced disease and a difficult target to treat [70]. Moreover, the metabolic effects of SGLT-2i influence the pathophysiologic mechanisms that lead to NAFLD, including but not limited to weight loss and glycemic control (Fig. 2). SGLT-2i have also shown pleiotropic effects, thus leading to their approval, apart from T2DM, for heart failure and chronic kidney disease [80], diseases that are closely associated with NAFLD [81]. More specifically, SGLT-2i were shown to decrease cardiovascular risk and the rate of chronic kidney disease, which are particularly important for individuals with NAFLD, and even more important when we consider that cardiovascular diseases are the first cause of death among patients with NAFLD [82,83]. Regarding the effects of SGLT-2i in a clinical setting, a recent meta-analysis of 16 randomized controlled trials evaluating the effectiveness of SGLT-2i on hepatic steatosis and fibrosis in patients with NAFLD showed favorable effects of SGLT-2i on imaging parameters of hepatic steatosis (e.g., controlled attenuation parameter, liver-to-spleen attenuation ratio and magnetic resonance imaging–proton density fat fraction) [84]. On the other hand, SGLT-2i showed a potentially limited effect on noninvasive indices of hepatic fibrosis, whereas studies with paired liver biopsies are scarce [84]. These effects resemble those observed in the relevant animal studies (Table 1), generally showing a favorable effect of SGLT-2i on hepatic steatosis, but a so far questionable effect on fibrosis. Taking the above into consideration, more data on the effects of SGLT-2i are required in patients with NAFLD from studies designed with paired liver biopsies; in this regard, animal studies are highly constructive, since they generate the hypotheses on which human studies are based. However, an important limitation of different animal studies is the heterogeneity in their design, including but not limited to animal models, doses of medications, duration and combinations of medications (Tables 1 and 2). This heterogeneity may generate conflicting data that are sometimes difficult to explain, and impedes the applicability of animal studies to clinical studies [85].

Recently, resmetirom, an oral thyroid hormone receptor beta-selective agonist, being selective for the liver, was approved in the USA for patients with NASH and fibrosis stage 2 or 3 [86]. However, a "one pill fits all" approach for NAFLD patients is challenging and unlikely, given the complex and multifactorial pathogenesis of the disease. In this scenario, combination therapies targeting multiple pathogenic contributors simultaneously appear to be a potentially promising strategy [17]. Combination therapies may also have additive or synergistic effects on the same target or may help reduce the dose of another medication, thus diminishing the possibility of the latter having adverse effects. Most importantly, a personalized approach, targeting different pathogenic contributors based on the individual situation, should be considered [17]. Although data are currently limited on the effects of combinations of SGLT-2 with other medications (Table 2), this approach may be promising; for example, the combination of resmetirom with SGLT-2i may be investigated, possibly starting from animal models of diabetes and fibrotic NASH, and then moving on to patients with diabetes and fibrotic NASH.

Regarding their adverse effects, SGLT-2i are well-tolerated in patients with T2DM, with the main side-effects being urinary and genital tract infections, which are generally mild and do not lead to discontinuation; however, there are cases of the more alarming euglycemic diabetic ketoacidosis, which seems to occur rarely, but needs vigilance on the part of physicians [87].

In conclusion, the current findings indicate that SGLT-2i may be beneficial for NAFLD through multiple mechanisms, which may lie beyond their weight loss and glucose-regulating properties. Given their pleiotropic effects, SGLT-2i, as monotherapy or in combination with other medications, may prove to be valuable allies in the management of NAFLD in some selected individuals, thus providing hepatic benefits in addition to antidiabetic, cardiovascular and renal benefits. Of course, further research is needed in the field to better elucidate the pharmacological mechanisms underlying the observed benefits with regard to NAFLD. This may lead to a more efficacious translation of the findings of animal studies into tailored clinical studies in patients with NAFLD.

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