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Review

Glucagon-like Peptide-1 Receptor-based Therapeutics for Metabolic Liver Disease

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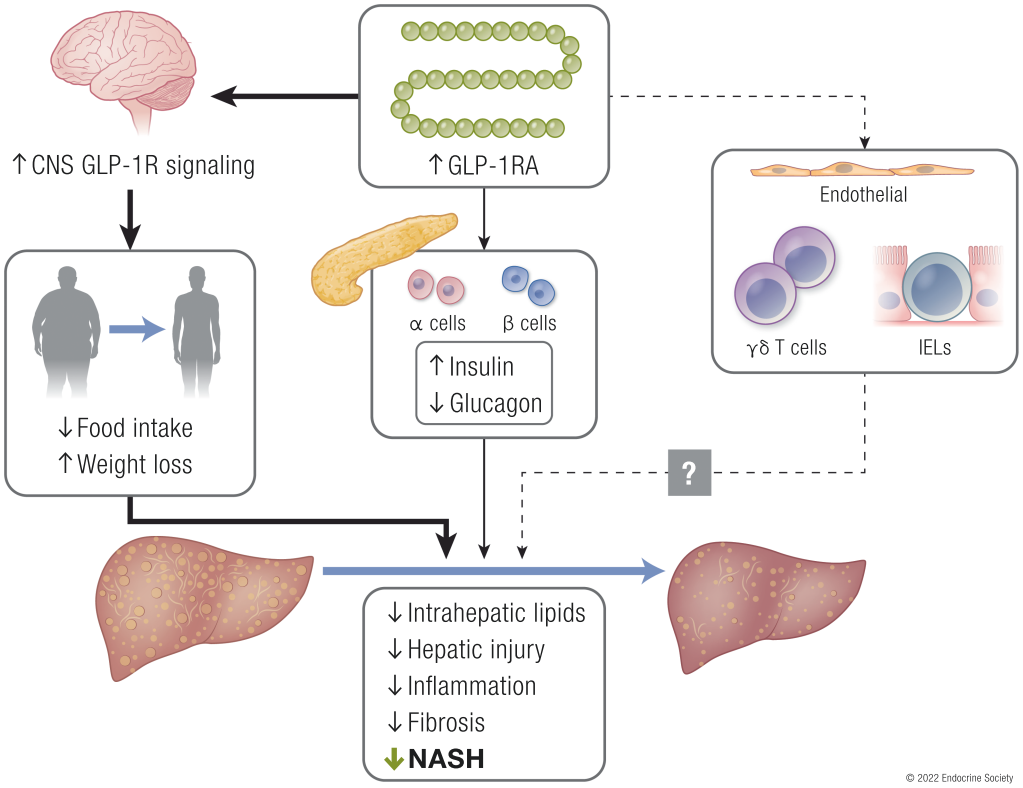
Abstract

Glucagon-like peptide-1 (GLP-1) controls islet hormone secretion, gut motility, and body weight, supporting development of GLP-1 receptor agonists (GLP-1RA) for the treatment of type 2 diabetes (T2D) and obesity. GLP-1RA exhibit a favorable safety profile and reduce the incidence of major adverse cardiovascular events in people with T2D. Considerable preclinical data, supported by the results of clinical trials, link therapy with GLP-RA to reduction of hepatic inflammation, steatosis, and fibrosis. Mechanistically, the actions of GLP-1 on the liver are primarily indirect, as hepatocytes, Kupffer cells, and stellate cells do not express the canonical GLP-1R. GLP-1RA reduce appetite and body weight, decrease postprandial lipoprotein secretion, and attenuate systemic and tissue inflammation, actions that may contribute to attenuation of metabolic-associated fatty liver disease (MAFLD). Here we discuss evolving concepts of GLP-1 action that improve liver health and highlight evidence that links sustained GLP-1R activation in distinct cell types to control of hepatic glucose and lipid metabolism, and reduction of experimental and clinical nonalcoholic steatohepatitis (NASH). The therapeutic potential of GLP-1RA alone, or in combination with peptide agonists, or new small molecule therapeutics is discussed in the context of potential efficacy and safety. Ongoing trials in people with obesity will further clarify the safety of GLP-1RA, and pivotal studies underway in people with NASH will define whether GLP-1–based medicines represent effective and safe therapies for people with MAFLD.

Graphical Abstract



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Key Words: diabetes, obesity, liver disease, non-alcoholic steatosis, glucagon-like-peptide-1, cardiovascular disease

Abbreviations: [^{1}H]-MRS, proton magnetic resonance spectroscopy; Akt, protein kinase B; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAT, brown adipose tissue; BMI, body mass index; CNS, central nervous system; CVOTs, cardiovascular outcome trials; DPP-4, dipeptidyl peptidase-4; FGF21, fibroblast growth factor 21; GCG, glucagon; GCGR, glucagon receptor; GIP, glucose-dependent insulinotropic polypeptide; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; GLP-1RA, glucagon-like peptide-1 receptor agonists; HbA1c, glycated hemoglobin; HFD, high fat diet; HFFC, high fat, high fructose and cholesterol; icv, intracerebroventricular; iNKT, invariant natural killer T cell; IL, interleukin; IEL, intraepithelial lymphocytes; LDL, low-density lipoprotein; MAFLD, metabolic-associated fatty liver disease; mRNA, messenger RNA; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; SGLT-2, sodium-glucose cotransporter-2; T2D, type 2 diabetes; TAG, triacylglycerol; td, tandem dimer; TG, triglyceride; TRL, triglyceride-rich lipoprotein; TRL-ApoB, triglyceride-rich lipoprotein-apolipoprotein B; VLDL, very low density lipoprotein.

Gut peptides such as glucagon-like peptide-1 (GLP-1) exhibit pleiotropic actions to control metabolism. Originally characterized as a nutrient-stimulated incretin hormone that potentiates meal-stimulated insulin secretion, GLP-1 also reduces glucagon (GCG) secretion, gastric emptying, gut lipoprotein secretion, food intake, and body weight, leading to improved metabolic health in animals and humans (1, 2). Data from animal studies and randomized trials in humans demonstrate reduction of hepatic fat, inflammation, and fibrosis following sustained GLP-1 receptor (GLP-1R) agonism, through incompletely understood mechanisms. Here we review mechanisms potentially underlying the actions of GLP-1-based therapeutics, mediated via the canonical GLP-1R, in the setting of metabolic-associated fatty liver disease (MAFLD). We update current metabolic concepts of GLP-1 action, identifying mechanisms, opportunities, and uncertainties surrounding the use of GLP-1R agonists (GLP-1RA) for the treatment of MAFLD and nonalcoholic steatohepatitis (NASH).

Glucagon-like Peptide-1

Nutrient intake initiates the release of intestinal-derived incretin hormones, secreted at low levels in the basal state, that reduce postprandial hyperglycemia by augmenting meal-stimulated insulin secretion (3). Glucose-dependent insulinotropic polypeptide (GIP), the first incretin to be identified, was isolated using biochemical purification followed by functional characterization in the 1970s (4). The sequence of mammalian GLP-1 was discovered in the 1980s, deduced from cloning and sequencing of proglucagon (*GCG*) complimentary DNAs and genes (2). N-terminally truncated GLP-1(7-37) and GLP-1(7-36)NH_2 are the bioactive forms secreted from gut enteroendocrine L cells, with the majority of GLP-1 content localized to the gut. GLP-1 is continuously secreted at low basal levels in the fasting or interprandial state, and circulating levels of GLP-1 increase 2- to 3-fold after meal ingestion (1). GLP-1 is also synthesized in the brain stem, transported along axonal pathways to different regions of the central nervous system (CNS). Although the injured pancreas and islets ex vivo are capable of synthesizing GLP-1, the pancreatic levels of intact bioactive GLP-1 are extremely low in the normal mouse and human pancreas (5-7). Bioactive GLP-1 is rapidly cleaved by dipeptidyl peptidase-4 (DPP-4), a widely expressed serine protease (8) responsible, together with renal clearance, for the short half-life of biologically active GLP-1 in the circulation ($t_{1/2}$ = <2 minutes). DPP-4 activity generates GLP-1(9-37) and GLP-1(9-36)NH_2, GLP-1 metabolites that do not activate the canonical GLP-1R at physiological concentrations.

ESSENTIAL POINTS

- GLP-1R agonists improve inflammation and reduce fibrosis in preclinical models of experimental liver disease.
- GLP-1R agonists reduce systemic and hepatic inflammation through incompletely understood mechanisms.
- GLP-1R agonists reduce hepatosteatosis in association with weight loss.
- GLP-1R agonists appear to reduce NASH and halt fibrosis progression in humans.
- The development of unimolecular co-agonists and combination therapy may improve upon the actions of GLP-1R agonists alone for the treatment of NASH.
- The cardiovascular safety of GLP-1RA may provide additional benefit for people living with NASH.

The Glucagon-like Peptide-1 Receptor

The GLP-1R is a heterotrimeric G-protein coupled receptor that transduces the insulinotropic effects of GLP-1 in pancreatic β cells and mediates GLP-1R–dependent actions in extrapancreatic tissues (1, 9). Within the pancreas, GLP-1Rs are expressed in β cells and in some α and δ cells, collectively regulating the stimulation of insulin and somatostatin and the inhibition of GCG secretion (9). The GLP-1R is also expressed in extrapancreatic tissues including the enteric and central nervous systems, kidney, gut, lung, intestinal intraepithelial lymphocytes (IEL), endothelial cells and vascular smooth muscle cells, and the heart (9). The localization of extrapancreatic GLP-1R expression is consistent with roles for GLP-1R activation in the inhibition of food intake and gastric emptying, cardioprotection, and reduction of inflammation, activities conserved in both preclinical and clinical studies (10). In this review, we prioritize studies enabling mechanistic attribution of pathways linking canonical GLP-1R expression and activity to control of hepatosteatosis, inflammation, and fibrosis.

Glucagon-like Peptide-1 Receptor Agonists

Multiple structurally distinct GLP-1RA are utilized for the treatment of type 2 diabetes (T2D). These include short-acting peptides such as exendin-4 or lixisenatide and long-acting molecules such as dulaglutide, liraglutide, and semaglutide. Liraglutide and semaglutide are also approved for the treatment of people with overweight and obesity

(11). In addition to improving glycemic control and reducing body weight, GLP-1RA reduce levels of circulating biomarkers of hepatic injury and decrease liver fat in animals and humans. Nevertheless, the mechanisms linking GLP-1R activation to reduced hepatosteatosis remain uncertain and somewhat controversial, as the canonical GLP-1R is not expressed within most major liver cell types, including hepatocytes (9). Herein, we review the data supporting the use of GLP-1-based medicines for the treatment of metabolic liver disease, highlighting key mechanistic concepts and areas of uncertainty.

GLP-1 Action on the Liver

Hepatic Glucose Production

GLP-1RA inhibit hepatic glucose production, likely through indirect actions via control of insulin and GCG secretion (12) and possibly through CNS-dependent mechanisms. Conversely, loss of function studies using *Glp1r*$^{-/-}$ mice subjected to a hyperinsulinemic-euglycemic clamp reveal elevated endogenous glucose production associated with reduced hepatic phosphorylated protein kinase B (Akt) and phosphorylated GSK3β expression (13). In healthy humans undergoing a 2-hour pancreatic clamp, infusion of glucose with GLP-1 (0.4 pmol/kg/min) through the antecubital vein inhibits hepatic glucose production, independent of insulin and GCG action (14). Pharmacological studies using intracerebroventricular (icv) GLP-1 infusion (0.01 μg/min) through the lateral cerebral ventricle during a 2-hour hyperinsulinemic-euglycemic clamp in mice reduced hepatic glucose production, an effect that was attenuated with infusion (0.01 μg/min) of the GLP-1R antagonist exendin(9-39) (15). Similarly, injection of GLP-1 (0.01 μg/min) into the arcuate nucleus, but not the third cerebral ventricle or paraventricular nucleus, of healthy rats reduced endogenous glucose production during a hyperinsulinemic-euglycemic clamp (16). A role for the gut-brain-GLP-1 axis in the suppression of acute hepatic glucose production was inferred from studies of rats receiving intraduodenal GLP-1, with or without hepatic vagotomy (17). Whether CNS GLP-1R circuits suppress hepatic glucose production in humans is difficult to ascertain.

Hepatic Glucose Disposal

Several studies support a role for GLP-1 in hepatic and nonhepatic glucose disposal. Infusion of GLP-1 (7.5 pmol/kg/min) via the jejunal and splenic vein in healthy mongrel dogs increased hepatic glucose uptake 3-fold compared to saline controls, effects that were independent of insulin or GCG secretion and not due to selective activation of GLP-1R signaling in the portal vein (18, 19). Similarly, a single 20-μg injection of exenatide in mongrel dogs elevated net hepatic glucose uptake during an intraportal glucose infusion clamp under conditions of postprandial hyperinsulinemia and hyperglycemia, findings abolished in the absence of hyperinsulinemia (20). Conversely, loss-of-function studies in chow-fed *Glp1r*$^{-/-}$ mice reveal lower levels of hepatic glycogen and phosphorylated Akt, consistent with impaired stimulation of hepatic glucose uptake (21). Collectively, these studies suggest that GLP-1 indirectly inhibits hepatic glucose production and stimulates liver glucose uptake.

GLP-1 Action on Hepatic Lipid Metabolism

GLP-1RA reduce liver fat and inflammation in rodent and human studies, supporting their investigational use in people with T2D and nonalcoholic fatty liver disease (NAFLD) at risk for developing NASH (10). The effects of GLP-1 on de novo lipogenesis, β-oxidation, chylomicron import, and very low-density lipoprotein (VLDL) export collectively contribute to the lipid-lowering effects of GLP-1RA (Fig. 1).

De Novo Lipogenesis

GLP-1 inhibits hepatic de novo lipogenesis, likely through indirect mechanisms. Administration of exendin-4 or liraglutide reduced levels of hepatic triglyceride (TG) and cholesterol in fructose-fed hamsters or high-fat diet (HFD)-fed mice, respectively, findings associated with a reduction in hepatic expression of *Srebpf1* and A*cc1* (22). Administration of the GLP-1RA CNTO3649 or exendin-4 for 4 weeks reduced hepatic TG and cholesterol and decreased levels of VLDL-TG and VLDL-apoB production in HFD-fed ApoE*3 Leiden transgenic mice (23). These findings were accompanied by reduced expression of *Fasn*, *Dgat1*, and *Srebf1*, genes controlling hepatic lipogenesis.

In a subset of subjects studied in the Liraglutide Safety and Efficacy in Patients with Non-alcoholic Steatohepatitis trial, adults with obesity treated with liraglutide for 12 weeks [age 18-70 years, body mass index (BMI > 30)] exhibited reduced BMI and glycated hemoglobin (HbA1c), improved insulin-mediated suppression of hepatic glucose production and adipose tissue lipolysis, and reduced hepatic de novo lipogenesis (24). In contrast, administration of liraglutide (1.8 mg daily) for 16 weeks to people with T2D reduced body weight, HbA1c, and liver fat, without any change in de novo lipogenesis. Moreover, no differences were detected in levels of fasting or postprandial β-hydroxybutyrate, a marker of hepatic fat oxidation (25).

Delivery of Lipid Substrate to the Liver via the Gut

Small-bowel lipid absorption and subsequent chylomicron formation are an important source of lipids transported directly to the liver. GLP-1R activation robustly reduces enterocyte chylomicron production and secretion in animals and humans, with or without diabetes. Physiologically, GLP-1R blockade with exendin(9-39) (50 nmol/kg) increased levels of TG-rich apolipoprotein B-48 (TRL-ApoB-48; a marker of intestinally-derived chylomicron secretion) in chow-fed hamsters (26). Similarly, genetic disruption of the murine *Glp1r* increased the excursion of TG-rich lipoproteins after olive oil loading. How GLP-1 inhibits chylomicron secretion from the gut in vivo or isolated intestinal preparations ex vivo (26) in the absence of the canonical GLP-1R in enterocytes remains uncertain. The most abundant cellular site of GLP-1R expression within the gut is localized to the IEL (27); however, there are few data indicating a role for the IEL GLP-1R in the control of lipid metabolism. icv injection of exendin-4 (250 ng) reduces TRL-apoB48 levels in fat-loaded hamsters at 2, 4, and 6 hours post injection, findings attenuated by adrenergic and melanocortin-4 receptor blockade (28). Interestingly, melanocortin-4 receptor blockade did not abrogate the exendin-4–mediated reductions in plasma TGs. Furthermore, the actions of intraperitoneal exendin-4 to reduce levels of TRL-apoB-48 in hamsters were not blocked by icv injection

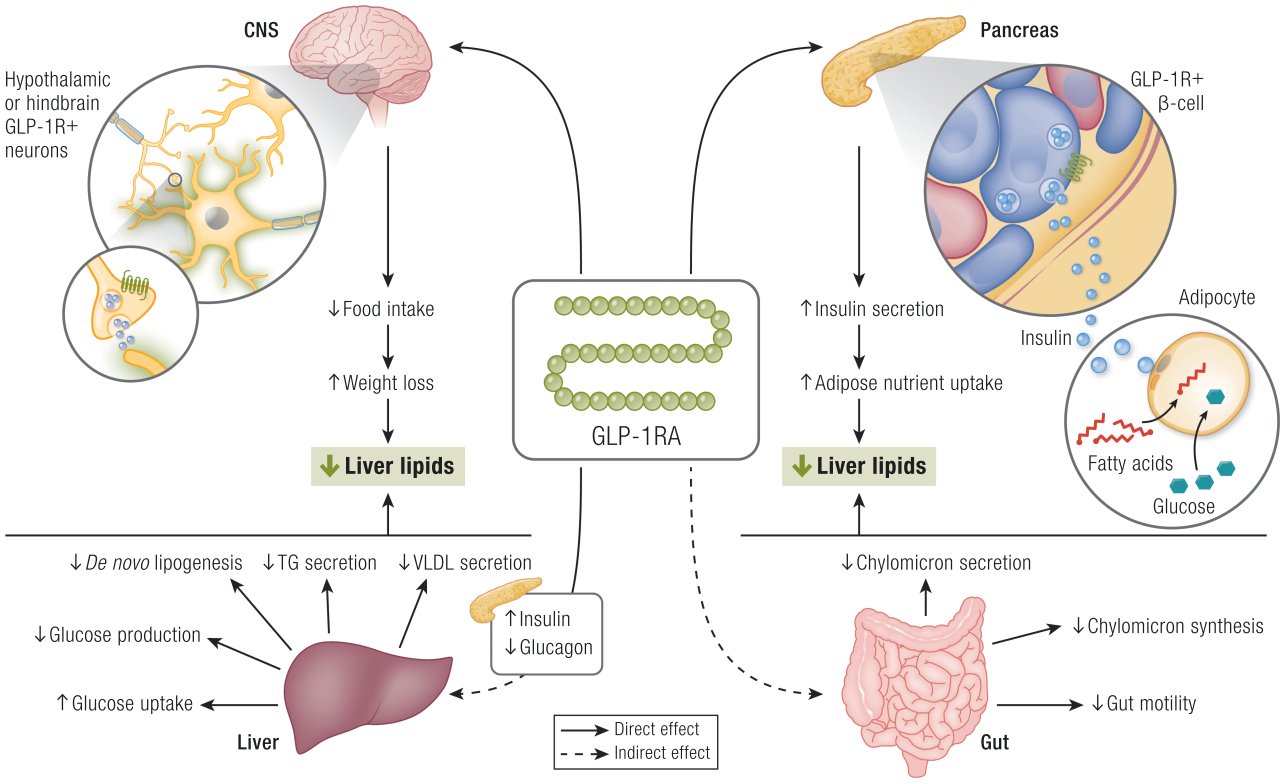

Figure 1. Glucagon-like peptide-1 receptor agonists (GLP-1RA) reduce liver lipids through indirect and direct mechanisms that involve the central nervous system (CNS), pancreas, liver and gut. GLP-1RA activates hypothalamic and hindbrain GLP-1R+ neurons in the CNS. This results in reductions in food intake and subsequent weight loss, which contributes to the reduction in liver lipids. GLP-1 also potentiates insulin secretion via GLP-1R+ pancreatic β cells, resulting in increased adipose nutrient uptake. Adipose insulin signaling provides a buffer for glucose and fatty acids to be stored in adipose tissue and not in liver, thereby reducing liver lipid levels. Although hepatocytes do not express the GLP-1R, GLP-1RA reduces hepatic glucose production, de novo lipogenesis, triglyceride secretion, and very low-density lipoprotein secretion and increases liver glucose uptake due to increase insulin and decreased glucagon signaling from the pancreas. Similarly, enterocytes do not express the GLP-1R, but GLP-1 action is linked to reductions in gut chylomicron synthesis, chylomicron secretion, and gut motility. Collectively, direct and indirect mechanisms link GLP-1R activity to biological pathways within the CNS, pancreas, liver, and gut, reducing hepatic lipid levels and maintaining liver homeostasis. Abbreviations: IELs, intraepithelial lymphocytes; NASH, nonalcoholic steatohepatitis.

of exendin(9-39), complicating understanding of the GLP-1R-dependent pathways controlling enterocyte chylomicron secretion (28). Interestingly, intraperitoneal exendin-4 administration reduces jejunal TG accumulation assessed by biochemical analysis and Oil Red O staining, supporting the possibility that reduced chylomicron secretion may be partially attributable to reduction of lipid absorption (28). Varin et al used mouse genetics to interrogate whether neuronal GLP-1Rs transduce the hypolipidemic actions of GLP-1RA in mice. Surprisingly, disruption of GLP-1R expression in the central and enteric nervous system within the *Wnt1* expression domain or abrogation of autonomic GLP-1Rs within the *Phox2b* expression domain did not attenuate the actions of multiple GLP-1RA to reduce excursion of TRL following olive oil loading in mice (29). Hence, the identity of the precise GLP-1R+ cell types and pathways communicating signals to the enterocyte enabling reduction of chylomicron secretion are not yet understood.

Clinical studies reveal GLP-1RA reduces chylomicron synthesis and secretion. In 15 healthy men (average age 41.8 years) studied using a pancreatic clamp, a single subcutaneous injection of exenatide (10 μg) suppressed the production rate of TRL-ApoB-48, but not TRL-ApoB-100 [a marker of low-density lipoprotein cholesterol (LDL)], determined via deuterated leucine enrichment in lipoproteins (30). Notably, these actions of exenatide were independent of any changes in gastric emptying, as the lipid rich nutrients were delivered to the gut via an intraduodenal tube (30). The actions of GLP-1RA to reduce circulating levels of TRLs are conserved in people with T2D. Analysis of 35 individuals (31 men and 4 women) with impaired glucose tolerance or recent onset T2D given a single dose of exenatide revealed a pronounced reduction in circulating levels of TG, apolipoproteins B-48 and CIII, remnant-like lipoprotein cholesterol, and remnant-like lipoprotein TG serum apoB48 (31).

These actions of GLP-1RA to suppress TRLs remain evident in chronic studies. Treatment of 10 subjects with T2D (average age 48.6 years) with 1.2 mg liraglutide daily for 6 months reduced ApoB48 levels and ApoB48 production rate and enhanced the catabolic rate of ApoB48 (determined via D_8-valine isotopes), findings independent of body weight loss (32, 33). Similarly, 3 weeks of treatment with escalating doses of liraglutide to achieve 1.8 mg daily, reduced postprandial levels of TGs and ApoB48, without changes in gastric emptying rate, in 20 subjects with T2D (34). Comparable findings were observed in 15 people with T2D treated with oral semaglutide for 15 weeks, demonstrating marked reductions in postprandial levels of TGs, VLDL, and ApoB48 (35).

Liver Lipid Flux and VLDL

Consistent with reduction in chylomicron synthesis, GLP-1R agonism reduces circulating levels of VLDL, a lipoprotein secreted from the liver that transports lipids to other tissues. APOE * 3-Leiden mice fed a HFD for 22 weeks had lower plasma TG and rates of VLDL-TG production when treated with exendin-4, associated with reductions in body weight and liver lipids (23). Chronic exendin-4 administration for 7 days reduced plasma TG, cholesterol, VLDL-TG, and VLDL-cholesterol in 60% fructose–fed hamsters (22). Reductions in VLDL were also observed after icv administration of exendin-4 (250 ng) in fructose-fed hamsters, findings abolished by subdiaphragmatic truncal vagotomy (22). VLDL-TG were also lower in liraglutide-treated C57BL/6J mice fed a 4% fructose supplemented diet for 16 weeks (22).

Reduction of circulating levels of VLDL following therapy with GLP-1RA is observed in studies of people with T2D or obesity (25, 35-37). Interpretation of these experiments is often confounded by weight loss and improved glycemia, and the identity of the cellular GLP-1Rs important for indirect reduction of hepatic VLDL secretion is not clear. Notwithstanding the ease of measurement and extensive focus on hepatosteatosis as a modifiable consequence of therapy with GLP-1RA, changes in hepatic fat are less important overall as predictors of major liver events and cardiometabolic outcomes, relative to changes in fibrosis, as discussed further in the following text.

GLP-1RA and Metabolic-associated Fatty Liver Disease

NAFLD is a spectrum of metabolism-related liver conditions, characterized by ectopic hepatic lipid accumulation not attributed to excessive alcohol consumption (38). It affects approximately 25% of the global population (39) and is accompanied by large direct and indirect costs (40) highlighting the need for solutions to reduce this growing health problem. NASH is characterized by hepatosteatosis and hepatic inflammation whereas MAFLD is a contemporary term for fatty liver diseases associated with metabolic dysfunction (41). Despite extensive clinical investigation, progress has been challenging for NASH drug development. GLP-1RA have shown protective effects in preclinical and clinical models of fatty liver disease, supporting the investigational exploration of GLP-1–based therapeutics to treat MAFLD and NASH.

Endogenous GLP-1 Levels in NAFLD

Endogenous GLP-1 levels are reduced in some human studies of people with NAFLD and NASH. In 52 individuals with biopsy-proven NAFLD (n = 16) and NASH (n = 36), glucose-simulated GLP-1 levels were reduced by ~10% compared to 50 healthy controls (42). There was no difference in glucose-stimulated GLP-1 levels between the NAFLD and NASH groups. Similarly, in a study of 70 individuals with overweight or obesity, liver fat measured using the hepatic/renal echo intensity ratio was inversely correlated with postprandial GLP-1 (43). Moreover, plasma levels of DPP-4 may be increased in people with T2D, obesity, and NAFLD (44), which may further contribute to reduced levels of active GLP-1. The importance of circulating GLP-1 for predicting liver disease severity independent of confounding variables is not established.

GLP-1RA Reduce Hepatic Steatosis

GLP-1RA reduce hepatic steatosis in HFD-fed or genetically obese mice. *ob/ob* mice treated with exendin-4 (10 and 20 µg/kg/day) for 60 days exhibited reductions in hepatic lipids assessed by histological examination and biochemical quantification, an effect associated with weight loss (45). Subsequently, multiple studies in mice and rats with experimental NAFLD have shown similar reductions in hepatic lipid content following treatment with GLP-1RA (46-49). Several studies have demonstrated GLP-RA-dependent reduction of hepatosteatosis while controlling for weight loss. HFD-fed C57BL/6J mice treated with the exendin-4-based GLP-1RA, AC3174 (30 mg/kg/day), for 4 weeks exhibited greater reductions in liver lipids and hepatic enzymes compared to weight-matched pair–fed mice (48). After 4 weeks of semaglutide treatment, HFD-fed C57BL/6J mice had less liver steatosis and reduced hepatic expression of genes important for lipid metabolism (*Srebf1*, *Mlxipl*, *Fas*) and inflammation (*Il6*, *Il1b*). Analysis of a pair-fed control group demonstrated that the steatosis score was not different in pair-fed vs semaglutide-treated animals, suggesting body weight loss as an important contributor to reduction of steatosis (50). Notwithstanding speculation that GLP-1RA may act on the liver to reduce liver fat via a noncanonical GLP-1R (9), the actions of GLP-1RA to reduce hepatosteatosis were completely extinguished in *Glp1r*$^{-/-}$ mice (48).

GLP-1RA reduce hepatic steatosis in humans with T2D or obesity, mirroring findings in preclinical studies. Liraglutide (1.2 mg daily) for 6 months reduced liver fat content from 17.3% to 11.9% (mean 31% decrease) measured by proton magnetic resonance spectroscopy ([^{1}H]-MRS) in 68 individuals (both males and females with average BMI of 35.9 ± 6.8) with uncontrolled T2D; greater weight loss was associated with additional reductions in liver fat (51). Decreased liver fat content (44%), reduced visceral fat, and weight loss were observed in 31 women with polycystic ovary syndrome treated with 1.8 mg per day of liraglutide for 26 weeks; however, the median baseline liver fat content in this group was only 1% (52). An open-label trial with 0.75 mg once-weekly dulaglutide for 24 weeks in 27 people (baseline BMI 29.9) with T2D, NAFLD, and ≥6.0% magnetic resonance imaging proton density fat fraction reported a –32.1% reduction in liver fat content from baseline compared to –5.7% in the standard care control group (53). Dulaglutide-treated individuals lost 4.3 kg of weight compared to 2 kg in the control subjects (53). As a 5% reduction in body weight is sufficient to reduce hepatic steatosis (54), understanding the weight loss-independent actions of GLP-1RA in people with hepatosteatosis remains challenging.

The combination of twice-daily exenatide (10 µg) with pioglitazone (45 mg /day) in 11 individuals with T2D reduced hepatic fat (average baseline 12.1%; average post-treatment 4.7%) to a greater extent than subjects randomized to pioglitazone alone, without changes in body weight after 12 months (55). The exenatide/pioglitazone combination improved HbA1c, liver enzymes, and high-density lipoprotein cholesterol when compared to 11 pioglitazone-treated controls that experienced a 3.7-kg increase in body weight. Although the available data highlight the importance of weight loss for indirect hepatic actions of GLP-1, it is plausible that GLP-1RA also regulate hepatic lipid metabolism through mechanisms regulating TG-rich chylomicrons, VLDL export, hepatic de novo lipogenesis, and/or hepatic mitochondrial oxidation, independent of changes in body weight.

GLP-1RA Reduce Hepatic Inflammation

GLP-1RA reduce systemic and hepatic inflammation in studies of animals and people with and without NASH. Exendin-4 treatment (50 µg/kg/day) for 4 weeks in APOE*3-Leiden. CETP mice fed a Western diet reduced liver inflammation markers (*Tnfa*, *Il1b*, and *Il6*) and infiltration of CD68+, F4/80+, and Mac-1+ cells, determined through immunohistochemistry (56). Nevertheless, simultaneous reductions in body weight and plasma glucose in the exendin-4–treated mice challenge mechanistic interpretation of the anti-inflammatory actions of exendin-4 (56). Semaglutide treatment for 17 weeks at 4, 12, and 60 µg/kg/day in $Ldlr^{-/-}$ mice fed a Western diet reduced liver TGs and messenger RNA (mRNA) levels of genes implicated in inflammation and fibrosis, accompanied by a dose-dependent reduction in body weight (57). Chronic semaglutide (10 µg/kg/day) administration for 18 weeks reduced plasma cytokine levels [Growth related cytokine (GRO), tumor necrosis factor α, interleukin (IL)-6] in high-fat/high-cholesterol diet–fed wild-type control and $Glp1r^{Tie2-/-}$ mice, with a selective knockout of the endothelial and hematopoietic cell GLP-1R (49). Notably, semaglutide reduced liver TGs and RNA biomarkers of inflammation (*Tnf*, *Ccl2*, *Tgfb1*, *Cd3g*, *Il2*) in livers from $Glp1r^{Tie2+/+}$ but not $Glp1r^{Tie2-/-}$ mice. These findings implicate endothelial and/or hematopoietic cell GLP-1Rs as targets for a subset of actions underlying GLP-1RA-mediated reduction of hepatic inflammation (49).

In humans, a 12-week course of liraglutide 1.8 mg daily reduced circulating levels of high-sensitivity C-reactive protein and the chemokine (C-C motif) ligand 2 (CCL-2) in 7 people with biopsy-confirmed NASH (24). Whether these changes occurred independent of reduction in glycemia and body weight observed in the same subjects cannot be determined. Exenatide given acutely or twice daily for 12 weeks reduced mRNA transcripts encoding biomarkers of inflammation measured in circulating white blood cells of people with T2D, independent of changes in body weight (58). Nevertheless, a larger 26-week study did not detect consistent directional changes in markers of inflammation in white blood cells after liraglutide therapy. Moreover, *GLP1R* mRNA transcripts were not detected in human white blood cells (59). Similarly, oral semaglutide reduced C-reactive protein to a much greater extent than empagliflozin over 26 and 52 weeks in subjects with T2D, despite nearly identical weight loss in the 2 treatment arms (60). Collectively, GLP-1RA reduce both systemic and hepatic inflammation in preclinical and clinical studies; however, the mechanisms remain poorly understood (10) and interpretation of the data is often confounded by simultaneous improvement in multiple metabolic parameters, including lipids, glucose, insulin sensitivity, and weight loss.

GLP-1RA and the Reduction of Hepatic Fibrosis

Hepatic fibrosis is the buildup of extracellular matrix that interferes with hepatocyte and overall liver function and often develops secondary to preexisting hepatosteatosis, viral infection, hepatic inflammation, and hepatic injury (61). GLP-1RA reduce levels of key markers of hepatic fibrosis in some but not all animal studies. Liraglutide (40 nmol/kg) administration for 6 weeks in high fat, high fructose, and cholesterol (HFFC)-fed *ob/ob* mice reduced levels of liver α-smooth muscle actin, hydroxyproline, and hepatic mRNA fibrosis markers (*Cola11*, *Col3a1*, *Col4a1*, *Timp1*). Nevertheless, no change in the histology fibrosis score was observed (62). Similarly, 4 weeks of liraglutide (30 nmol/kg twice daily) reduced hepatic inflammation and liver injury in high-fat, high-sucrose diet–fed guinea pigs, without any histological improvement in fibrosis (63). Analysis of methionine-choline–deficient diet–fed mice treated with the long-acting GLP-1 analogue, G8, E22, G36-GLP-1, designated GLP-1-Fc, exhibited a reduction in hepatic expression of only a small subset of fibrosis markers (*Col1a1*, *Tgfb*) while levels of fibroblast activation protein mRNA transcripts were increased by GLP-1-Fc, and hepatic stellate cell numbers were not reduced (64). Conversely, high-fat, high-fructose–fed mice treated with liraglutide 0.4 mg/kg/day for 12 weeks exhibited reduced body weight, decreased markers of hepatic Kupffer and stellate cell activation (ie, α-smooth muscle actin, Col1a1, and galectin-3), yet fibrosis assessed histologically was not reduced (47). Consistent with these findings, the dual peroxisome proliferator-activated receptor α/δ agonist elafibranor, but not liraglutide (0.2 mg/kg twice daily for 8 weeks), reduced hepatic fibrosis in high-fat, high-sucrose diet–fed C57BL/6J and $Lep^{ob/ob}$ mice with metabolic liver disease (65). In contrast, a 4-week infusion of liraglutide (570 µg/kg/day via osmotic minipumps) in methionine-choline–deficient diet–fed mice reduced hepatic RNA biomarkers of fibrosis (*Timp1*, *Serpine1*, *Mmp13*, *Col1a1*, *Col1a2*, *Col1a3*) and decreased the number and size of collagen fibers and the extent of fibrosis assessed by Sirius Red staining of liver sections, without changes in glycemia or body weight (66). Liraglutide (50 µM) reduced human stellate cell activation and directly reduced stellate cell proliferation in stellate cell cultures and precision cut human liver slices ex vivo (67). However, the direct or indirect mechanisms through which GLP-1RA may reduce fibrosis remain unclear as the canonical GLP1R has not been detected in hepatocytes or Kupffer or stellate cells (49).

The degree of hepatic fibrosis is a primary predictor of cardiovascular disease, liver transplant, and death in people living with NASH (68). Whether treatment with GLP-1RA reduces the rate of development or reverses fibrosis in people with MAFLD, with or without T2D, remains uncertain. Observational data of subjects with T2D treated with a range of GLP-1RA (exenatide, lixisenatide, liraglutide, and dulaglutide) for 24 months did not reveal evidence for reduced fibrosis assessed using serial analysis of the Fibrosis-4 Index (69). Among 26 subjects randomized to receive liraglutide 1.8 mg daily (vs 26 randomized to placebo) for 48 weeks, 2 (9%) in the liraglutide group vs 8 (36%) in the placebo group exhibited biopsy-proven progression of fibrosis (70). Moreover, a larger and longer randomized controlled trial of semaglutide administration over 72 weeks in subjects with NASH, the majority with concomitant stage F2 or F3 fibrosis, revealed no differences in histological fibrosis scores in subjects treated with semaglutide (71). Hence, the available preclinical and clinical data examining the actions of GLP-1RA on progression or resolution of fibrosis are inconsistent, and no clear conclusions can yet be drawn.

GLP-1RA and the Treatment of NASH

GLP-1RA reduce hepatic steatosis and inflammation in preclinical models of NASH. Many histological, biochemical, and clinical features associated with NASH are reproduced in mice using diets that contain HFFC. C57BL/6J mice fed a HFFC

diet for 29 weeks developed severe NASH and were subsequently treated with liraglutide (40 nmol/kg/day) for 6 weeks, achieving a 9% reduction in body weight (62). Liraglutide reduced liver triacylglycerol (TAG) content, plasma alanine aminotransferase (ALT), steatosis, and inflammation scores but not levels of type I collagen, a marker of fibrosis (62). Similarly, treatment of HFFC-fed *ob/ob* mice with liraglutide for 8 weeks reduced body weight, liver TAGs, plasma ALT, and hepatic markers of inflammation (*Ccl2, Cd68* mRNA), and fibrosis (liver hydroxyproline and *Col1a1*, *Col3a1*, *Col4a1* mRNAs) but did not improve the fibrosis score (62). Related findings were described in studies of HFFC-fed C57BL/6JRj mice treated with liraglutide (0.4 mg/kg/day) for 12 weeks (47). Liraglutide decreased body weight, plasma ALT, and aspartate aminotransferase (AST), NAFLD activity score (NAS), liver TAGs, the extent of hepatocyte ballooning, markers of Kupffer cell (galactin-3), and hepatic stellate cell activation (α-smooth muscle cell actin), without changes in fibrosis.

Identity of a subset of cellular sites of GLP-1R expression important for the hepatoprotective actions of GLP-1RA came from studies of high-fat, high-cholesterol diet–fed $Glp1r^{Tie2-/-}$ mice, with inactivation of the GLP-1R in endothelial and hematopoietic/immune cell lineages (49). Semaglutide (10 μg/kg/day for 18 weeks) reduced liver TGs, fibrosis, and mRNA biomarkers of inflammation (*Tnf*, *Ccl2*, *Tgfb1*, *Cd3g, Il2*) in $Glp1r^{Tie2+/+}$ control mice, but these actions were attenuated in $Glp1r^{Tie2-/-}$ mice, despite comparable weight loss in both groups (49). Hence, a subset of the beneficial actions of GLP-1RA in mouse models of NASH may reflect weight loss–independent effects through GLP-1Rs within the Tie2 cellular domain.

Clinical Studies of GLP-1RA in NASH

GLP-1RA– and GLP-1–based coagonists (72) are being investigated for the treatment of people with NASH. In a small multicenter trial examining the efficacy of liraglutide, 23 individuals with overweight and obesity, with and without T2D, and biopsy-confirmed NASH were treated with daily injections of liraglutide (1.8 mg) for 48 weeks. As highlighted in the previous discussion, 9 of the 23 individuals (39%) had resolution of NASH without worsening of fibrosis compared to only 2 of 22 individuals (9%) in the control arm (70). The improvement in NASH histology was associated with a decreased rate of fibrosis progression, a 5% reduction in body weight, and a 0.5% reduction in HbA1c (70). Notably, weight loss achieved via diet and lifestyle interventions over 12 months, in the range of ≥7%, has been linked to improvements in liver NASH histology, but not fibrosis, in individuals with NASH (73). A placebo-controlled trial examined the efficacy of once-daily semaglutide at 3 doses (0.1, 0.2, or 0.4 mg per day) vs placebo over 72 weeks in 320 individuals with histology-confirmed NASH. Histological entry criteria for enrollment encompassed NAS ≥ 4, ≥1 steatosis, ≥1 hepatocyte ballooning, ≥1 lobular inflammation, and F1-F3 fibrosis stage without cirrhosis. The primary endpoint was resolved NASH without worsening of fibrosis in the subgroup with F2-F3 fibrosis (71). Participants were living with obesity or overweight (mean body weight of 98.4 kg); 62% had T2D; the average age was 55; and 90, 72, and 158 had stage F1, F2, and F3 fibrosis, respectively. Compared to the 17% of individuals with resolved NASH in the placebo group, NASH resolution was higher in the groups treated with 0.1, 0.2, or 0.4 mg semaglutide, with 40%, 36%, and 59% of participants, respectively, exhibiting biopsy-proven resolved NASH without worsening of fibrosis (71). Nevertheless, the percentage of study subjects with improvement of at least 1 liver fibrosis stage with no worsening of NASH was not consistently different, perhaps partly reflecting the unexpectedly high rate of fibrosis resolution (33%) in the placebo group. Worsening of fibrosis was detected in 5% to 10% of semaglutide-treated subjects and 19% of the placebo-treated group. The mean body weight loss was −5%, −9%, and −13% in the 0.1, 0.2, and 0.4 mg semaglutide groups, respectively, compared to −1% in the placebo group. ALT and AST assessed over the 72 weeks exhibited dose-dependent reductions in the semaglutide group (27% and 30% reduction in ALT and AST, respectively, in the 0.1 mg group; 58% and 48% reduction in the 0.4 mg group, respectively) compared to placebo (19% reduction from baseline levels) (71).

The safety and efficacy of once-weekly semaglutide (2.4 mg) was also examined in a 48-week Phase 2, randomized placebo-controlled trial, in 71 adults with biopsy-confirmed NASH and compensated cirrhosis, with a BMI ≥ 27 kg/m^2 and HbA1c ≤ 9.5% (74). Subjects randomized to semaglutide did not exhibit improvement in hepatic fibrosis or resolution of NASH, although semaglutide-treated subjects exhibited reduced liver enzymes, hepatic fat, circulating TG and LDL, and body weight.

A Phase 3 clinical trial (NCT04822181) is assessing 2.4 mg once-weekly semaglutide in 1200 individuals with noncirrhotic NASH, using endpoints that include (1) resolution of NASH without worsening fibrosis, (2) improvement in liver fibrosis without worsening of steatohepatitis, and (3) time to first liver-related clinical event.

GLP-1R-Dependent Mechanisms Contributing to Resolution of NASH

Evidence for Hepatic GLP-1R Expression

The detection of a functional GLP-1R within liver cells, including hepatocytes, has been controversial and challenging due to very low levels of hepatic GLP-1R expression. Indeed, using ^{125}I-GLP-1(7-36)amide as the radioligand, GLP-1R binding was not detected using in vitro autoradiography in sections from non-neoplastic human liver (75). Similarly, analysis of monkey liver by immunohistochemistry using the validated Mab 3F52 monoclonal anti-GLP-1R antibody did not detect GLP-1R+ liver cells (76). Moreover, reports of GLP-1R expression in hepatocytes have been difficult to interpret or reproduce, due in part to the nonspecificity of many commercially available reagents for detection of the immunoreactive GLP-1R protein (9, 46, 77, 78) and failure to simultaneously detect the presence of full-length *GLP1R*/*Glp1r* mRNA transcripts or canonical GLP-1R signaling responses within hepatocytes (22, 46, 62, 78-80).

Cellular domains of *Glp1r* transcriptional activity were examined using a reporter mouse expressing the tandem dimer (td) Tomato protein under the control of the *Glp1r* promoter. Hepatic tdTomato reporter expression was observed within endothelial cells of liver sinusoids, central veins, and intrahepatic branches of the portal vein but not in hepatocytes of male mice (81). Nevertheless, the same intrahepatic tdTomato+ vascular structures were not GLP-1R immunopositive using a reasonably validated commercially available antibody (Abcam ab218532) (81). Richards et al examined transcriptional

domains of *Glp1r* expression using transgenic mice expressing *Cre* under the control of *Glp1r* regulatory sequences to enable activation of fluorescent protein reporters such as tdTomato red fluorescent protein (82). *Glp1r* promoter-controlled fluorescence was not detected within hepatocytes but was localized to fibers adjacent to the portal vein.

Glp1r mRNA transcripts were detected using single cell RNA-seq in a subset of murine hepatic endothelial cells (83). Consistent with these findings, targeting of the floxed *Glp1r* allele within endothelial and hematopoietic lineages from $Glp1r^{Tie2-/-}$ mice produced a marked reduction of hepatic *Glp1r* mRNA expression (49). Fluorescent-activated cell sorting identified *Glp1r* mRNA transcripts corresponding to a full-length functional GLP-1R within a subset of intrahepatic γδ T cells (49). Since hepatocytes, hepatic stellate cells, and most immune cells do not express the canonical GLP-1R, the cellular mechanisms linking GLP-1R activation to reduction of liver steatosis, inflammation, and fibrosis beyond weight loss remain unclear. In the following text, we discuss how putative GLP-1R+–expressing cell types may indirectly affect hepatic steatosis and NASH (Fig. 2).

Central Nervous System Actions of the GLP-1R, Weight Loss, Energy Balance, and NASH

Weight loss likely contributes substantially to the benefits of GLP-1RA in NASH, mediated by reductions in food intake, a process controlled by multiple widely distributed CNS GLP-1R+ cells (11, 29, 84, 85). Inhibition of gastric emptying, which is often transient in the context of chronic use of long-acting GLP-1RA (86), may also contribute to initial reduction of appetite, mediated in mice through intestinofugal myenteric neurons projecting to abdominal sympathetic ganglia (87). Although GLP-1RA promote beiging and activation of brown fat leading to weight loss in animals, these findings have not yet been reproduced in humans (11). Weight loss alone is associated with improvements in hepatosteatosis (88), supporting the hypothesis that weight loss contributes substantially to resolution of fatty liver and NASH in people treated with GLP-1RA.

Achievement of weight loss is increasingly viewed as an effective adjunctive strategy for NASH (89). In 8 people living with obesity and T2D, loss of ~8 kg following consumption

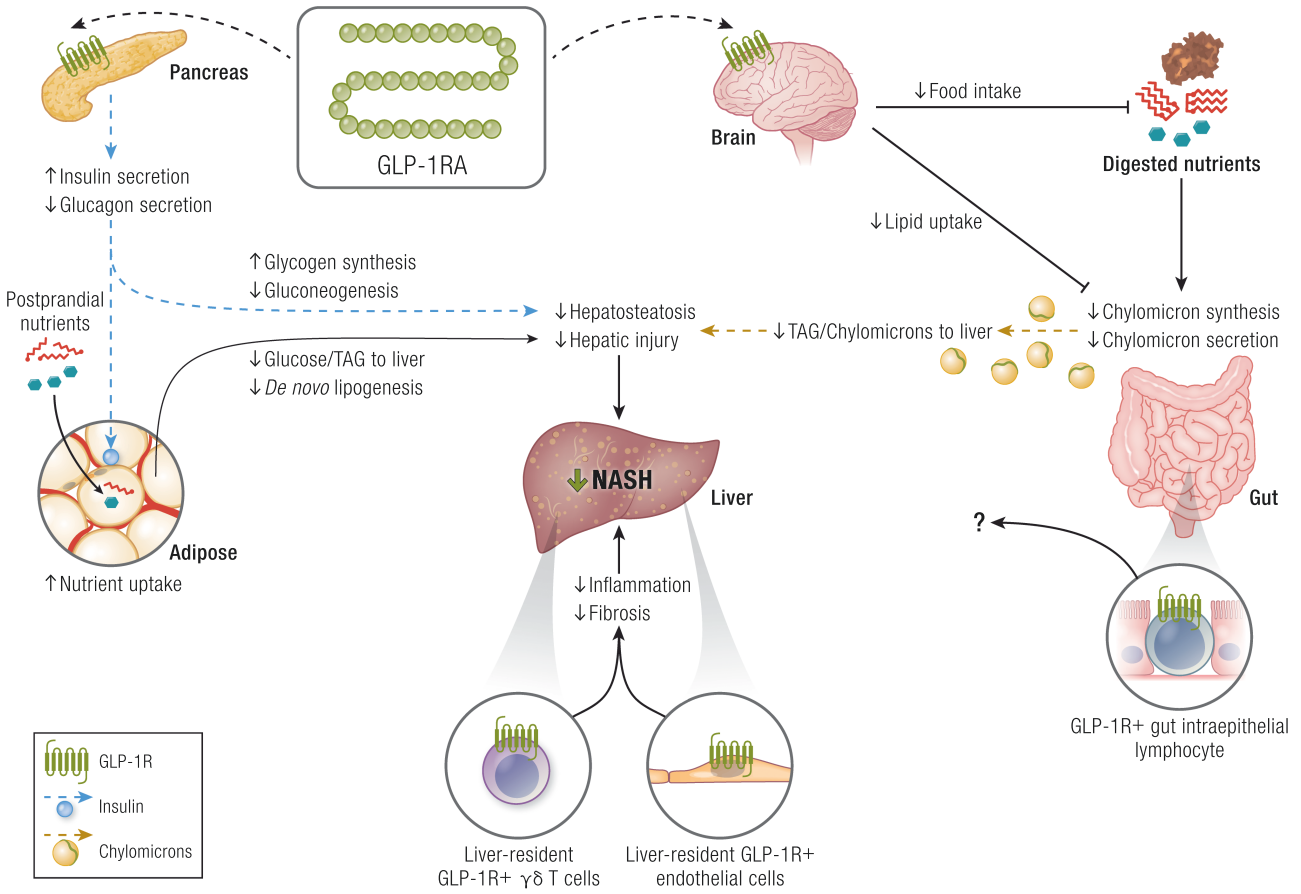

Figure 2. Glucagon-like peptide-1 receptor agonists (GLP-1RA) reduce nonalcoholic steatohepatitis (NASH) through GLP-1R+ pancreatic β cells and central nervous system (CNS) neurons in the brain. GLP-1RA potentiate pancreatic insulin secretion that signals to insulin receptors within adipose tissue and liver. Liver insulin signaling increases glycogen synthesis and reduces gluconeogenesis. Insulin signaling in adipose tissue initiates the uptake of postprandial nutrients (eg, glucose and fatty acids), which reduces the amount of lipids that are sent to the liver and de novo lipogenesis. GLP-1RA also signal through GLP-1R+ CNS neurons to reduce food intake, thereby reducing the amount of glucose, protein, and fatty acids that enter and are absorbed via the gut. Some evidence supports a role for CNS-dependent activation of GLP-1Rs to reduce lipid uptake. Reductions in food intake and lipid uptake reduce chylomicron synthesis and secretion, resulting in less triacylglycerol and chylomicrons reaching the liver. Since neither hepatocytes nor enterocytes express the canonical GLP-1R, current evidence suggests a role for the brain, as well as liver-resident γδ T cells, as possible GLP-1R-dependent cell types responsible for reductions in NASH with GLP-1RA therapy. The importance of GLP-1R+ liver-resident endothelial cells and gut-resident intraepithelial lymphocytes for hepatic lipid metabolism or hepatic inflammation has not been established.

of a hypocaloric 3% fat diet was associated with an 81% reduction in intrahepatic lipid as determined by [^{1}H]-MRS (90). Similarly, Lazo et al observed a 50.8% reduction in hepatic fat, determined by [^{1}H]-MRS, associated with a 8.3% reduction in body weight after 12 months of intensive lifestyle intervention to induce a minimum of 7% weight loss (54). This was achieved through reduced food intake (1200-1800 kcal per day) and increased physical activity (175 min/week) in 46 individuals with T2D and overweight or obesity (54). Greater weight loss was correlated with further reductions in hepatic steatosis. However, biomarkers of liver injury (ALT, AST, γ-glutamyl transferase) and circulating cytokines (IL-8, IL-10, tumor necrosis factor α) were not different after 12 months of intensive lifestyle intervention (54). Analysis of 88 people with histologically proven NASH who lost greater than 5% of their body weight by following a 52-week low-fat hypocaloric diet and an exercise walking regimen revealed that 51 (58%) of the study subjects achieved resolution of NASH (91).

Surgical interventions leading to weight loss are also associated with improvements in biopsy-confirmed NASH. Histological analyses of paired biopsies (pre- and postlaparoscopic banding-mediated weight loss) revealed reduced hepatosteatosis, lobular inflammation, fibrosis, Mallory bodies, and ballooning degeneration in 36 subjects (25 females, 11 males) with obesity who lost on average 34.0 kg of weight (52% of body weight) over ~25.6 months following laparoscopic banding (92). Similarly, in a prospective study of individuals with severe obesity and biopsy-proven NASH, resolution of NASH without progression of hepatic fibrosis was reported in 81% of people examined after 1 year and 84% of subjects studied 5 years after bariatric surgery (93). Collectively, weight loss achieved through lifestyle, pharmaceutical, or surgical intervention is an effective strategy to reduce histologically confirmed NASH.

The results of preclinical (46-48, 56, 62) and clinical (70, 71) studies using GLP-1RA demonstrate that reductions in hepatosteatosis, inflammation, and liver injury are generally associated with weight loss. For example, obese mice fed a NASH-inducing diet (40% fat, 20% trans fat, 2% cholesterol, and 22% fructose) for 36 weeks followed by treatment with 0.4 mg/kg/day liraglutide for 12 weeks exhibited lower NASH-associated histology scores (steatosis, lobular inflammation, hepatocyte ballooning) compared to baseline. The improvements in liver histology were associated with a 2.3 g reduction in body weight (47). Newsome et al observed marked improvements in NASH in human subjects, ages 18 to 75 years, 62% with T2D, without worsening of fibrosis following treatment with semaglutide, 0.1, 0.2, or 0.4 mg per day for 72 weeks (71). These improvements were associated with dose-dependent reductions in body weight of –4.84%, –8.91%, and –12.51%, respectively, from baseline. Whether the improvement in NASH reflects mechanistic contributions beyond weight loss is difficult to ascertain in clinical studies.

GLP-1R agonism reduces food intake, thereby enabling weight loss, through multiple GLP-1R+ regions in the CNS (11), predominantly within nuclei localized to the hypothalamus and hindbrain (94-96). Daily icv administration of GLP-1 (3 nmol) in rats reduced daily food intake and resulted in a 15% reduction of body weight after 7 days (97). Conversely, rats injected with 30 nmol of the GLP-1R antagonist, exendin(9-39) by the icv route increased food intake and body weight after 4 days (97). The use of site-specific peptide injections, mouse genetics, and chemogenetics support the concept that widely distributed accessible GLP-1Rs localized to multiple regions of the CNS, not protected by the blood-brain barrier, contribute to the GLP-1R-dependent control of food intake (11, 29, 84, 85). Interestingly, weight reduction observed in mice treated with dulaglutide (0.05 mg/kg) was partially attenuated in mice with disruption of GLP-1Rs in domains of the autonomic nervous system targeted by *Phox2b*-Cre, including the nodose ganglion (29). Moreover, chemogenetic studies of gut-brain GLP-1R–dependent circuits further highlight the pharmacological contributions of multiple GLP-1R+ circuits, both within and external to the CNS, for the pharmacological control of food intake (98).

Considerable evidence from studies in mice and rats links GLP-1R activation to enhanced sympathetic nervous system activity, increased beiging of white adipose tissue, and enhanced activity of brown adipose tissue (BAT) (99-101), resulting in increased energy expenditure. Increased cold-induced BAT activity has been correlated with relative reductions in hepatic lipid accumulation in adult humans (102, 103). Nevertheless, metabolic studies of individuals treated with liraglutide or semaglutide have not detected evidence for increased energy expenditure in the context of GLP-1RA-induced weight loss in humans (104, 105). Whether GLP-1RA prevent a greater reduction in energy expenditure in the context of reduced food intake and weight loss is uncertain and requires more careful study.

The liver is innervated by autonomic nervous system nerve fibers important for the central GLP-1R-dependent control of hepatic lipid metabolism in fructose-fed mice and hamsters (22). Hamsters with a subdiaphragmatic truncal vagotomy did not exhibit weight loss or reduction in circulating VLDL levels when treated with icv exendin-4, implying the importance of the parasympathetic branch of the vagus nerve in the pharmacological response to central GLP-1R agonism (22). icv exendin-4 administration also lowers gut chylomicron production in hamsters through peripheral adrenergic receptor and central melanocortin-4 pathways (28). In contrast, widespread genetic inactivation of the GLP-1R within the CNS and enteric nervous system targeted by *Wnt1*-Cre did not abrogate the GLP-1R–dependent inhibition of postprandial TG excursions in mice (29).

Hepatic innervation by both sympathetic and parasympathetic fibers help maintain metabolic homeostasis. icv GLP-1 administration enhances hepatic Akt phosphorylation and lowers levels of hepatic TG in HFD-fed insulin-resistant mice studied under hyperinsulinemic euglycemic clamp conditions (15). Conversely, icv administration of the GLP-1R antagonist Ex-9 impaired the insulin-mediated suppression of hepatic glucose production, collectively supporting a role for CNS GLP-1Rs in modulating hepatic metabolism. The importance of brain-liver signaling for metabolic actions of GLP-1 relevant to humans with liver disease is more challenging to ascertain.

GLP-1, Insulin, Adipose Tissue, Muscle, and Hepatic Lipid Metabolism

Sustained weight loss due to GLP-1RA therapy improves insulin sensitivity in rodents and humans. In the absence of weight loss, GLP-1RA might potentiate glucose-stimulated

insulin secretion in people with hyperglycemia, perhaps contributing to excessive hepatic lipid deposition. Theoretically, sustained hyperinsulinemia secondary to GLP-1R agonism might also augment peripheral insulin signaling to store glucose and fat in liver, white adipose tissue, and muscle. In states of hepatic insulin resistance, such as obesity or NASH, hepatic insulin signaling is selectively impaired and redirects glucose away from glycogen synthesis and toward pathways of lipogenesis, further exacerbating NAFLD.

Interestingly, *Glp1r*$^{-/-}$ mice exhibit decreased hepatic glycogen accumulation and attenuated phosphorylation of GSK-3β in the liver, impaired insulin-mediated suppression of hepatic glucose production and enhanced exercise-stimulated glucose production independent of insulin action (13). Consistent with these findings, icv administration of the GLP-1R antagonist exendin (9-39) impaired, whereas icv GLP-1 enhanced, the insulin-mediated suppression of hepatic glucose production in mice (15). Collectively, these findings suggest that GLP-1 may modulate hepatic insulin action indirectly through the brain and neural communication. GLP-1RA administration is associated with reductions in the enzymes implicated in the lipogenic pathway in preclinical studies (22, 23, 45, 62), actions likely indirect due to lack of *Glp1r/GLP1R* expression in hepatocytes.

Insulin signaling in white adipose tissue plays an integral role in storing TGs, indirectly diverting fatty acids away from the liver. During fasting conditions, white adipocytes undergo lipolysis, a process that liberates fatty acids and glycerol from TGs that are shuttled into the bloodstream as an energy source. Conversely, postprandial insulin receptor activation on white adipocytes increases both glucose and fatty acid uptake from the bloodstream. Importantly, insulin diminishes white adipocyte lipolysis, reducing the amount of adipocyte-derived nonesterified fatty acids in the bloodstream. However, lipolysis is not blunted postprandially in insulin-resistant white adipocytes, thereby releasing fatty acids into the bloodstream that can be sequestered by the liver. As GLP-1R activation promotes insulin signaling under conditions of hyperglycemia, this may blunt adipose tissue lipolysis, thereby reducing the shuttling of free fatty acids to the liver and reducing substrate used for de novo lipogenesis. Exendin-4 treatment for 7 days was associated with reductions in plasma free fatty acid levels and hepatic *Srebf1* gene expression, independent of reduction in food intake, in hamsters (22). After 12 weeks of therapy with liraglutide in 7 people with biopsy-proven NASH, GLP-1R agonism led to reductions in hepatic de novo lipogenesis (24); however, the mechanistic contributions of insulin signaling, adipose tissue, and neural inputs are difficult to assess in these experiments. Moreover, the canonical GLP-1R is not detected in the majority of murine or human adipocytes (106, 107).

Skeletal muscle also sequesters glucose and fatty acids postprandially, reducing the amount of lipids and glucose available to the liver. There is little evidence that GLP-1 directly modulates muscle glucose uptake as GLP-1Rs are not expressed in skeletal muscle cells (9); however, infusion of native GLP-1 augments muscle microvascular blood flow in rats and healthy humans (108, 109). Activation or blockade of the CNS GLP-1R regulates hepatic glucose production in HFD-fed mice; however, no effect of enhanced or reduced CNS GLP-1R signaling on skeletal muscle glucose uptake was detected in the same experiment (15). Similarly, GLP-1 infused icv at a rate of 0.01 μg/min did not increase skeletal muscle glucose uptake in insulin-clamped HFD-fed mice (15). Consistent with the preclinical data, there was no difference in median change in glucose disposal in response to low or high insulin administered via a clamp in 7 subjects with biopsy-proven NASH following 12 weeks of liraglutide treatment (24). Hence, it seems unlikely that changes in muscle glucose uptake impact hepatic lipid accretion in the context of enhanced GLP-1R signaling.

GLP-1, Immune Cells, and Hepatic Inflammation

The mechanisms underlying GLP-1RA-dependent reductions in liver inflammation are unclear, as the majority of immune cells do not express the canonical GLP-1R (9, 10). Enteroendocrine L cells respond to gut or systemic infection or sterile inflammation by increasing the secretion of GLP-1 (110-114). Intestinal IELs are the major GLP-1R+ immune cells in the gut that interact with locally produced GLP-1 to suppress gut inflammation (27). Deletion of the integrin β7 protein, which directs immune cells to the gut increased GLP-1 secretion in HFD-fed mice, associated with improved glucose homeostasis and reduced atherosclerosis (115). Whether augmented or deficient GLP-1R+ IEL activity can regulate gut permeability, nutrient absorption, microbiota composition, and local or systemic inflammation, which, in turn, impacts the extent of hepatic inflammation requires further investigation.

Mouse liver expresses a very low level of *Glp1r* mRNA transcripts (46, 49) localized to hepatic endothelial cells and CD8+ γδ T cells (49). Interestingly, mice with Tie2-Cre–mediated inactivation of *Glp1r* in endothelial and hematopoietic cells exhibit marked reduction of *Glp1r* expression in the liver (49). γδ T cells receive input from the gut microbiota that contributes to development of HFD-induced obesity and NAFLD in mice (116, 117). Induction of NAFLD in *Tcrd* null mice deficient in γδ T cells protected against hepatic steatosis, lowered ALT levels, and improved insulin sensitivity (116), whereas glucose intolerance and ALT levels increased after reintroduction of wild-type γδ T cells. Whether γδ T cell GLP-1R expression is important for the actions of GLP1RA in humans with NASH requires additional analysis.

The invariant natural killer T (iNKT) cell is an immune cell type also linked to NASH. iNKT cells recognize CD1d that binds to lipid antigens and contributes to hepatocyte cytotoxicity (118). Liraglutide-dependent activation of murine iNKT cells induced fibroblast growth factor 21 (FGF21) secretion from inguinal and BAT depots to increase thermogenesis in HFD-fed mice, although GLP-1R was not directly localized to mouse iNKT cells (101). Notably, the actions of liraglutide to reduce body weight were diminished in HFD-fed CD1d and Jα18 knockout mice that lack functional iNKT populations. Whether iNKT cells mediate the anti-inflammatory actions of GLP-1RA in the human liver has not been studied.

GLP-1RA also increase levels of hepatic and circulating FGF21 in mouse experiments, findings associated with reduction of hepatic glucose output, reduced liver fat, and decreased inflammation (119, 120). In contrast, 12 weeks of liraglutide administration to subjects with T2D and NAFLD reduced body weight and liver fat and decreased circulating levels of FGF21 (121). The importance of FGF21 as a

downstream target for GLP-1RA in humans with NASH is unclear as GLP-1RA do not consistently induce circulating levels of FGF21 in people (122, 123).

GLP-1–based Co-agonists and Combination Therapy for the Treatment of Hepatosteatosis and NASH

Considerable progress has been made toward improving the therapeutic efficacy of GLP-1RA via development of unimolecular co-agonists containing GIP or GCG, or use of GLP-1RA in combination with amylin agonists (Fig. 4) (72). Furthermore, as the majority of drugs under development for NASH directly target cells within the liver, it seems likely that GLP-1RA will be useful elements of combination therapy for tackling distinct complementary pathways in NASH therapeutics. For example, the combination of semaglutide plus the FXR agonist cilofexor (30 and 100 mg) and/or firsocostat (20 mg), the liver-directed acetyl CoA-carboxylase inhibitor, produced greater reduction in liver enzymes, hepatic fat, and noninvasive imaging and biochemical biomarkers of fibrosis, relative to semaglutide alone, in an exploratory 24-week Phase 2 trial (124). Real-world data scrutinizing changes in liver enzymes in new users of glucose-lowering drugs suggest that both sodium-glucose cotransporter-2 (SGLT-2) inhibitors and, to a lesser extent, GLP-1RA, are associated with a reduced risk of transaminase elevations in people with T2D (125). An open label 52-week randomized trial combining the SGLT-2 inhibitor luseogliflozin 2.5 mg once daily, with 0.5 mg of once-weekly semaglutide is underway in Japanese people with NASH and T2D, with primary efficacy endpoints to be assessed using liver biopsy (126). Herein, we discuss the effects of co-agonist drugs that are in the therapeutic pipeline for NAFLD and NASH (Fig. 3).

GIPR:GLP-1R Co-agonists

Tirzepatide (LY3298176) is a unimolecular GIPR and GLP-1R co-agonist administered once weekly for the treatment of T2D that may also be effective in lowering markers of liver injury and fibrosis. A Phase II clinical trial in people with T2D (starting HbA1c 7.0-10.5%, with or without stable metformin therapy) administered 1, 5, 10, or 15 mg of tirzepatide once weekly for 26 weeks (127, 128). Tirzepatide reduced serum ALT by 5 to 10 U/L by study end, and greater reductions in ALT were observed with tirzepatide relative to people treated with dulaglutide. Dose-dependent reductions in TG, ApoC-III, ApoB, and LDL-C levels were observed with tirzepatide (129). The levels of keratin-18, an indirect biomarker of apoptosis, and procollagen-3, a biomarker for fibrosis, were also reduced in people treated with tirzepatide (128). In the SURPASS Phase 3 trials for people with T2D, tirzepatide reduced plasma lipid levels and body weight, actions that may indirectly contribute to improvements in NASH biomarkers (130). A substudy of the SURPASS-3

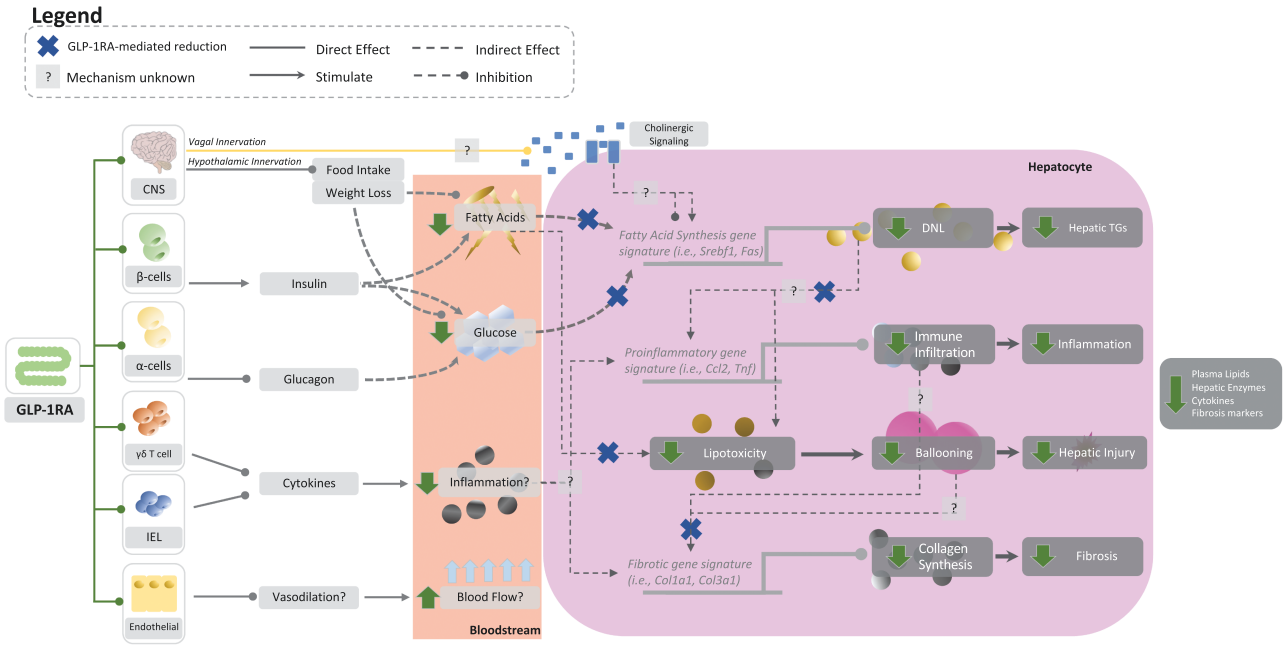

Figure 3. Glucagon-like peptide-1 receptor agonist (GLP-1RA)-dependent mechanisms in the reduction on hepatic triglycerides, inflammation, injury, and fibrosis. GLP-1RA signals to various GLP-1R+ cell types such as the central nervous system (CNS), pancreatic islets, and immune and endothelial cells. Vagal innervation in the CNS is critical to key liver lipid processes, but whether cholinergic signaling is responsible for this is currently unclear. Hypothalamic GLP-1R signaling reduces food intake, leading to subsequent weight loss and reductions in glucose and fatty acids. Further, β-cell insulin secretion promotes fatty acid and glucose uptake into insulin sensitive tissues, reducing macronutrient inductions of fatty acid synthesis gene signature and lipid accretion in the liver and subsequent de novo lipogenesis (DNL) and intrahepatic triglycerides (TGs). The GLP-1RA reduction in hepatic TGs has also been linked indirectly to reductions in immune infiltration and inflammation in the liver; however, the mechanisms underlying this are currently unclear. GLP-1RA also reduces the accumulation of lipids that lead to lipotoxicity partly due to the reductions in DNL, reducing ballooning as well as other markers of hepatic injury. Lastly, the reduction in hepatic lipids, inflammation and injury are all linked to reductions in the fibrosis gene signature, leading to reductions in collagen synthesis, extracellular matrix synthesis, and fibrosis. GLP-1RA are also linked to reductions in plasma lipids, inflammatory mediators, hepatic injury enzymes, and fibrosis markers. Other GLP-1R+ cell types such as α cells and glucagon signalling, immune cell types such as γδ T cells, and intraepithelial lymphocytes (IELs) via cytokines and endothelial cell regulation of blood flow may be other mechanisms that may directly or indirectly regulate liver disease, but the mechanisms and the functions of these cells types are less established.

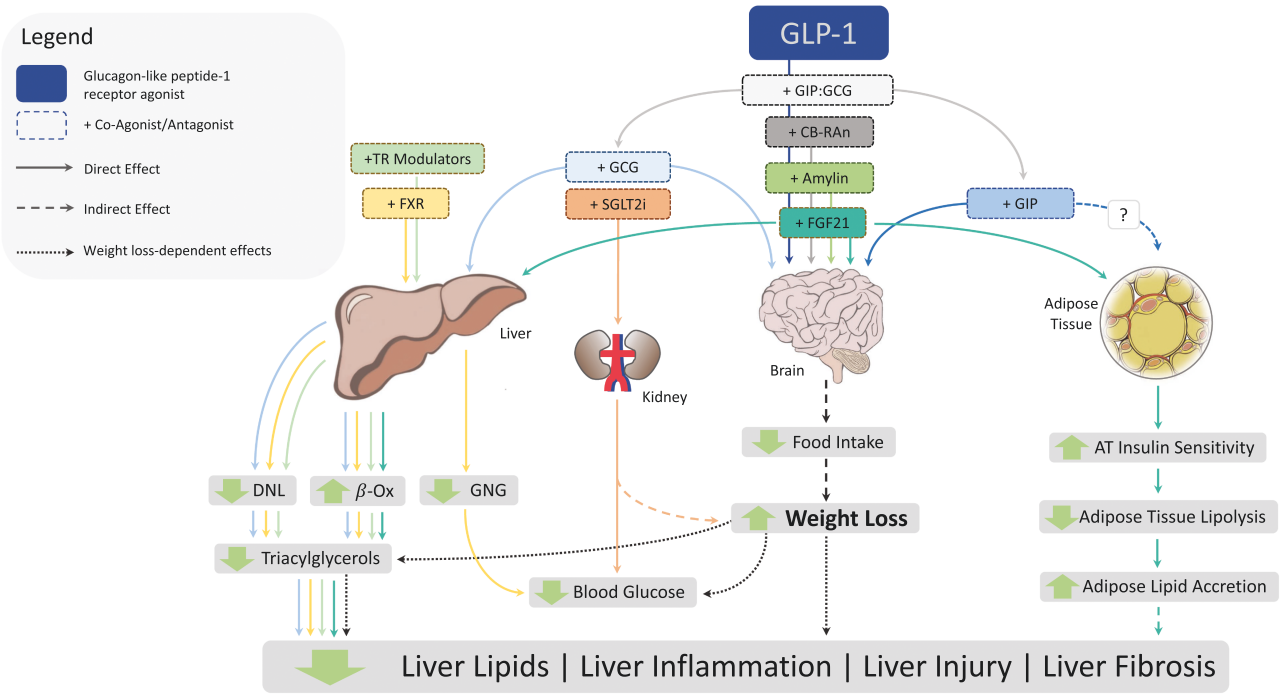

Figure 4. Combination therapy with glucagon-like peptide-1 receptor (GLP-1R) agonism to reduce liver lipid accretion, inflammation, injury, and fibrosis. GLP-1R agonists (GLP-1RA) signal to the brain to reduce food intake and induce subsequent weight loss. Glucagon receptor (GCGR) agonists reduce triacylglycerols by reducing de novo lipogenesis (DNL) and increasing β-oxidation (β-Ox) in liver. Degradation-resistant glucose-dependent insulinotropic polypeptide receptor (GIPR) agonists act on the brain to reduce food intake and increase lipid accretion via insulin action in adipose tissue, acting as a buffer to divert lipids away from liver. Thus, GCGR-GLP-1R and GIPR-GLP-1R co-agonists, as well as unimolecular GLP-1R:GIPR:GCGR triagonists, may leverage the effects of these peptide hormones to improve liver health. Farnesoid X receptor (FXR) agonists (1) decrease DNL and β-Ox to reduce triacylglycerols and (2) abate gluconeogenesis (GNG) to lower blood glucose levels. Thyroid receptor (TR) modulators also work to reduce hepatic de novo lipogenesis and β-Ox to reduce triglycerides (TGs). Sodium-glucose cotransporter 2 inhibitors (SGLT2i) curb glucose reabsorption in kidneys to reduce blood glucose levels and promote weight loss through indirect mechanisms. Cannabinoid receptor antagonists (CB-RAn) and amylin receptor agonists act on the brain to reduce food intake with subsequent weight loss. fibroblast growth factor 21 (FGF21) combination treatments may also act on the liver to reduce TGs, reduce food intake through CNS signaling to increase weight loss, but also increase adipose tissue (AT) insulin sensitivity, leading to reduced AT lipolysis and subsequent increases in AT lipid accretion, thereby reducing liver lipids. Collectively, direct and indirect actions of GLP-1RA and molecular partners may act through potentiation of weight loss, regulation of liver TAGs, and adipose tissue lipid accumulation to reduce hepatic lipid accumulation, inflammation, injury, and fibrosis.

cohort treated with tirzepatide revealed a marked reduction in hepatic fat and both visceral and subcutaneous adipose tissue, consistent with the weight loss experienced in tirzepatide-treated subjects (131)

A Phase 2 clinical trial examining the efficacy of tirzepatide in people with NASH is underway; the primary endpoint is the percentage of people with absence of NASH with no worsening fibrosis after 52 weeks (NCT04166773).

Glucagon Receptor:GLP-1R Co-agonists

Increased de novo lipogenesis and attenuated mitochondrial fatty acid oxidation potentiate hepatic lipid accumulation in NAFLD and NASH (132), whereas GCG directly increases fatty acid oxidation and decreases hepatic TG synthesis and secretion (133). Glucagon receptor (GCGR) activation also reduces appetite and body weight, contributing to the effectiveness GCGR-GLP-1R co-agonists in preclinical models of NASH (72). Both the GCG agonist g1437 and the GCGR-GLP-1R co-agonist cotadutide, but not liraglutide, reduced de novo lipogenesis in mouse hepatocytes (62). Furthermore, g1437 and cotadutide increased *Ppargc1a/PPARGC1A* gene expression and mitochondrial biogenesis in murine and human hepatocytes and restored mitochondrial respiration in hepatocytes isolated from mice with experimental NASH (62). C57BL6/J mice fed a HFFC diet for 29 weeks to induce NASH and treated with 10 nmol/kg cotadutide for 42 days lost ~10% body weight and exhibited lower hepatic lipid levels (TAGs, cholesterol esters, and free fatty acids), reductions in plasma ALT, and histological reductions in steatosis, inflammation, liver injury, and fibrosis (62). These beneficial findings were mirrored in *ob/ob* mice fed a HFFC diet for 8 weeks and then treated for 6 weeks with 30 nmol/kg cotadutide (62).

Cotadutide has been studied in 834 adults with T2D (HbA1c of 7-10.5%) inadequately controlled with metformin and a BMI ≥ 25 kg/m^2 (NCT03235050). Study subjects were treated with either 100, 200, or 300 µg cotadutide; 1.8 mg liraglutide; or placebo daily for 54 weeks. Similar reductions in HbA1c and percentage weight loss were observed in cotadutide vs liraglutide treatment groups; however, the extent of weight loss was greater in the 300 µg cotadutide arm compared to liraglutide (–5.01% vs 3.44%, respectively) (134). Notably, 100, 200, and 300 µg cotadutide reduced plasma ALT levels by 7.52%, 12.01%, and 14.15%, respectively, compared to 3.21% reduction in the liraglutide group. Similarly, plasma AST levels were reduced by 1.77%, 6.22%, and 9.14% compared to a 0.35% increase in the liraglutide group. Ongoing clinical trials that assess GCGR-GLP-1R co-agonists include a 12- week clinical trial with pemvidutide (ALT-801) in subjects with NAFLD and overweight or obesity with or without T2D (NCT05006885). Efinopegdutide administered once weekly

[MK-6024, previously known as JNJ-64565111 (135)], is also being studied 24 weeks, using a primary outcome of reduction in liver fat, with semaglutide as an active comparator, in people living with NAFLD (NCT04944992).

ALT-801 (pemvidutide) is a GLP-1/GCGR receptor dual agonist that improves NASH outcomes in preclinical studies. C57BL6/J mice (with biopsy-confirmed NAS score after 29 weeks on a HFFC diet) were treated with ALT-801 (either 5 or 10 nmol/kg) for 12 weeks (136) and compared to mice treated with semaglutide (10 nmol/kg) or elafibranor (78 µg/kg). ALT-801 lowered hepatic lipids, liver weight, biomarkers of liver injury (plasma ALT and AST), the inflammation marker galactin-3, the fibrosis marker Col1a1, and the NAS score (136). Mechanistic interpretation of the benefits of ALT-801, currently being studied in separate human trials for NASH and obesity indications, is confounded by accompanying weight loss.

Long-acting Amylin Analogs and GLP-1RA Co-administration

Combination treatment with the long-acting amylin analog cagrilintide (AM833) and once-weekly semaglutide warrants further exploration for NASH due to the potent reductions in food intake and weight loss observed in clinical studies. In a Phase 2 trial studying 706 people with a BMI ≥ 30 kg/m^2 or a BMI ≥ 27 kg/m^2 with hypertension or dyslipidemia, cagrilintide once weekly at doses of 2.4 and 4.5 mg achieved a 9.5% and 10.6% reduction in body mass compared to baseline, respectively (137). A subsequent dose-ranging study of the combination of 2.4 mg semaglutide with 0.16 to 4.5 mg of cagrilintide once weekly for 20 weeks in people with a BMI of 27 to 39.9 kg/m^2 reported up to a 17.1% weight loss with the cagrilintide/semaglutide combination (138). Whether the addition of agents such as cagrilintide will bring additional benefits to reduction of NAFLD or NASH beyond those achieved with weight loss is uncertain.

GLP-1R:GIPR:GCGR Triagonists

Unimolecular triagonists exhibit simultaneous activity on the GLP-1, GIP, and GCG receptors and reduce hepatosteatosis and markers of liver injury in preclinical studies. Mice fed a high-fat and sucrose diet for 16 weeks exhibited ~ 25% reduction in body weight after 20 days of triagonist treatment (3 nmol/kg/day), reflecting elevated energy expenditure and reduced food intake, compared to just 15% weight loss with a GLP-1R/GIPR co-agonist (139). Hepatic lipid content was decreased in the GLP-1R/GIPR co-agonist group and to an even greater extent in the triagonist-treated group. Interestingly, the weight loss achieved with triagonist treatment was abolished in *Gcgr*$^{-/-}$ mice, but not in *Glp1r*$^{-/-}$ or *Gipr*$^{-/-}$ mice (139). Similar results were achieved using SAR441255, a triagonist that reduces markers of liver injury in diet-induced obese mice and nonhuman primates. SAR441255 treatment (30 µg/kg), twice per day over 28 to 42 days, reduced body weight by ~14% and ~13% in obese C57BL/6NHsd mice and obese diabetic cynomolgus monkeys, respectively (140). In comparison to obese vehicle-treated control mice, 30 µg/kg SAR441255 administration for 28 days resulted in reductions in AST (282 vs 117 U/L), ALT (256 vs 50 U/L), and liver weight (1.59 vs 0.97 g), similar in magnitude to reductions observed in obese mice treated with a dual GLP-1R/CGCR agonist (140). Plasma lipids were not reduced; however, ALT was lower in obese cynomolgus monkeys treated for 6 weeks with a once-daily injection of SAR4441255 (11 µg/kg). Collectively, unimolecular triagonists reduce hepatosteatosis and markers of liver injury in preclinical models; whether these effects will translate to people living with NASH requires additional investigation.

GLP-1RA, NASH, and Cardiovascular Disease

Treatment with long-acting GLP-1RA is associated with reductions in cardiovascular morbidity and mortality in people with T2D (141), which may have implications for treating people living with NASH as cardiovascular events are the leading cause of mortality in individuals with NAFLD and NASH (142). Cardiovascular disease risk may be exacerbated by perturbations in hepatic lipid metabolism of chylomicrons and apoproteins, circulating lipid species that are generally attenuated by treatment with GLP-1RA. Multiple preclinical and clinical studies demonstrate acute and sustained reduction in TG-rich chylomicrons, apoproteins, and VLDL and LDL cholesterol following GLP-1RA therapy (143). As enterocytes and hepatocytes do not express the canonical GLP-1R, the weight loss–independent mechanisms through which GLP-1RA reduce postprandial lipemia remain poorly understood (9).

GLP-1RA reduce the rates of myocardial infarction in several cardiovascular outcome trials (CVOTs) and decrease the rates of stroke, cardiovascular death, hospitalization for heart failure, and all-cause mortality by at least 10% in meta-analyses of long-acting GLP-1RAs (141). The mechanisms linking GLP-1RA to reduction of cardiovascular events may include risk factor modification (decreases in blood pressure, glucose, body weight, and postprandial lipids), reduction of inflammation and atherosclerosis, and direct protective effects on the heart and blood vessels (143, 144). Given the high rates of MAFLD in people with T2D and overweight or obesity, it seems likely that a substantial proportion of individuals with T2D and MAFLD were studied in the GLP-1RA CVOTs. Whether GLP-1RA can reduce rates of major adverse cardiovascular events in people living with NASH with or without T2D is not established. Moreover, the majority of CVOTs reporting the safety of GLP-1RA generally exclude subjects with known liver disease, do not break down the proportion of study subjects with MAFLD, and do not report analyses of primary and secondary outcomes by the presence or absence of MAFLD.

Liver fibrosis stage has been linked to a greater risk of severe liver-related and cardiovascular events in people living with NAFLD and NASH (145, 146) and is the strongest predictor of mortality in people with MAFLD, notably in subjects with stages 3 and 4 fibrosis (68). Long-term sufficiently powered studies of individuals with NASH at risk for developing cardiovascular disease are required to assess the cardiovascular safety of GLP-1–based therapies. The time to first major cardiovascular event is a prespecified secondary outcome in the Effect of Semaglutide in Subjects With Noncirrhotic Non-alcoholic Steatohepatitis (ESSENCE) trial (NCT 04822181) that will enroll 1200 subjects with histological evidence of NASH and hepatic fibrosis, randomized to treatment with once-weekly semaglutide (2.4 m) or placebo for up to 240 weeks.

Weight Loss-dependent and Independent Effects of GLP-1RA in NASH

Since hepatocytes do not express canonical GLP-1Rs, the available evidence suggests weight loss may be the primary driver of mechanisms linking sustained GLP-1R activation

to improvements in NASH. GLP-1R agonism in multiple widely distributed GLP-1R+ neuronal populations (84), including hypothalamic and hindbrain neurons, reduces food intake (11). Weight loss, evident in both preclinical and clinical studies, is associated with reductions in hepatic steatosis, injury, inflammation, and, in some studies, fibrosis. Conversely, GLP-1RA may reduce hepatic inflammation and fibrosis through mechanisms independent of weight loss (66, 147). Semaglutide therapy resulted in similar weight loss in mice with or without hematopoietic and endothelial cell-specific deletion of the *Glp1r* (*Glp1r*$^{Tie2-/-}$). However, liver TGs and the extent of hepatic inflammation and fibrosis markers were reduced to a lesser extent in *Glp1r*$^{Tie2-/-}$ mice (49). These findings were associated with detection of a small population of GLP-1R+ intrahepatic γδ T cells and endothelial cells; however, the precise functional importance of these cell types for the beneficial actions of GLP-1RA in NASH requires further study.

Limitations, Uncertainties, and Future Directions for GLP-1RA in NASH

A substantial number of preclinical studies link therapy with GLP-1RA to reduction of hepatic steatosis and decreased inflammation. Notably, many studies do not include standardized histological assessments of inflammation, liver injury, and fibrosis, and the predictive value of mouse models for human NASH therapeutics continues to be debated (148, 149). Although GLP-1RA consistently reduce hepatic steatosis and inflammation, the magnitude of improvement in fibrosis detected in trials with liraglutide and semaglutide in people with NASH has been more modest and inconsistent (70, 71). It may be reasonable to explore the utility of GLP-1RA combination therapies, where the second component of the combination directly targets fibrosis (150). Beyond combination therapy achieved through the use of co-agonists such as tirzepatide, cotadutide, and pemvidutide (Fig. 4) (11), preclinical studies in animals with MAFLD have demonstrated the feasibility of combining GLP-1RA with Farnesoid X receptor agonists such as obeticholic acid (151), SGLT-2 inhibitors (152), and cannabinoid receptor antagonists (153) (Fig. 4). Complementary mechanisms also suggest the possibility of combining GLP-1RA with thyromimetics for NASH therapeutics (154). GLP-1RA may be ideally suited for combination therapy in NASH, as the majority of its mechanisms do not directly target the liver.

Pivotal insights informing the success of using GLP-1RA for NASH will derive in part from results of the ongoing Phase 3 clinical trial assessing semaglutide 2.4 mg once weekly in people with NASH. This trial will also report secondary outcome measures of liver-related clinical events, including time to first major adverse cardiovascular event, critical for understanding semaglutide action in the NASH population at high risk for cardiovascular disease. The results of CVOTs using GLP-1RA demonstrate a reasonable balance of safety and efficacy in people with cardiovascular disease and T2D (141), and safety studies of semaglutide in people with obesity are ongoing (155). Although the available preclinical and clinical data strongly supports the investigational use of GLP-1RA in people with NASH, the available efficacy and safety database in humans with NASH is limited. The ultimate utility, ideal population, duration of therapy, and safety of sustained GLP-1R agonism for resolution of NASH and fibrosis is not yet known and will be determined by the results of ongoing outcome-driven trials.

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