Glucagon-like peptide 1 receptor agonists and other incretin receptor agonists: mechanisms and possible hepatoprotective effects in non-alcoholic fatty liver disease

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Abstract

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretins that stimulate insulin secretion from pancreatic beta cells in response to food ingestion. Modified GLP-1 and GIP peptides are potent agonists for their incretin receptors, and recent evidence shows that the dual GLP-1 and GIP receptor agonist tirzepatide is effective in promoting marked weight loss. It is well recognized that GLP-1 receptor agonists signal in the central nervous system to suppress appetite, increase satiety and thereby decrease calorie intake; but recently many other effects of incretin signalling have been recognised that are relevant to the treatment of non-alcoholic fatty liver disease (NAFLD). This Review provides an overview of the literature supporting the notion that endogenous incretins and incretin-receptor agonist treatments are important not only for decreasing risk of developing NAFLD, but also for treating NAFLD and NAFLD-related complications. We discuss incretin signalling and related incretin-receptor agonist treatments, mechanisms in key relevant tissues affecting liver disease, as well as clinical data from phase-2 randomised controlled trials. Finally, we present future perspectives in this rapidly moving field of research and clinical medicine.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is well recognized as a leading aetiology for chronic liver disease, affecting up to nearly 30% of the global populations. The incidence of NAFLD is rapidly increasing worldwide in parallel to the epidemics of obesity and type 2 diabetes mellitus (T2DM)[1-4].

Although there are no approved pharmacotherapies for NAFLD, the complexity of pathophysiological processes of this common liver disease is clearly reflected by several new drugs under investigation targeting multiple pathological pathways[5].
Because T2DM is closely interrelated to NAFLD and its progressive form, non-alcoholic steatohepatitis (NASH)[6,7], an increasing number of randomized controlled trials in individuals with NAFLD or NASH (irrespective of the presence of T2DM) have focused on the efficacy of newer glucose-lowering agents, such as, for example, glucagon-like peptide-1 receptor agonists (GLP-1RAs)[8].

GLP-1RAs, also known as incretin mimetics, have revolutionized the management of T2DM globally. Convincing evidence has demonstrated the efficacy and safety of GLP-1RAs for the treatment of T2DM. Furthermore, large cardiovascular outcome trials showed that some GLP-1RAs also exert meaningful cardiovascular and renal benefits[9].
Unimolecular peptide-based dual agonists against GLP-1 receptor and the glucose-dependent insulinotropic polypeptide (GIP) receptor (e.g., tirzepatide), as well as dual agonists against GLP-1 and glucagon receptors (e.g., cotadutide) or triple agonists against GLP-1, GIP and glucagon receptors have been gaining much attention recently as novel glucose-lowering agents that may better control glycaemia and body weight[10,11].
In particular, an updated meta-analysis reported that tirzepatide induces dose-dependent improvements in glycaemic control that are clinically important not only versus placebo, but also when compared with once-weekly GLP-1RAs (subcutaneous semaglutide and dulaglutide) or basal insulin regimens[12]. With respect to lowering of body weight, a marked dose-dependent effect is also evident with tirzepatide even when compared with semaglutide and dulaglutide[12]. On this background of evidence, some GLP-1RAs and tirzepatide are becoming attractive therapeutic options for NAFLD or NASH, particularly in individuals with coexisting T2DM or obesity[8,13].

In this narrative Review, we discuss incretin signalling and related incretin-receptor agonist treatments in NAFLD and NASH. We discuss mechanisms in key relevant tissues

that affect liver disease in NAFLD, and clinical data from phase-2 randomized controlled trials. Finally, we present future perspectives in this rapidly moving field of research and clinical medicine.

Potential hepatoprotective actions of incretin receptor agonists on NAFLD

Incretin effects and other gastrointestinal hormones

There are two known incretins: the glucose-dependent insulinotropic polypeptide (GIP) that is produced by K cells of the upper intestine and the glucagon-like peptide 1 (GLP-1) that is produced by L cells of the lower intestine. These two incretins potentiate insulin secretion at plasma glucose concentrations >4 mmol/L[14,15]. The so-called “incretin effect” is defined as the increase in pancreatic insulin secretion after oral glucose ingestion compared with the insulin secretion after an equivalent amount of glucose administered as an intravenous glucose infusion. In healthy individuals the incretin effect is responsible for up to ~70% of insulin secretion after oral glucose ingestion and incretins (predominantly GLP-1 and GIP) are therefore essential for post-prandial glucose regulation[16].

The concept of a molecule that is restricted to GLP-1 and GIP may now be too narrow. It is likely that incretins also include other gastrointestinal hormones, which interact with GIP and GLP-1 during normal meals[17], to affect metabolism relevant to T2DM and NAFLD. Glucagon secretion from pancreatic alpha cells has also been shown to have incretin-like effects in mice[18] and thus glucagon may also act as an insulinotropic hormone in the fed state that would complement insulin rather than oppose insulin action, according the classical model. Besides glucagon, the gastrointestinal tract secretes oxyntomodulin (OXM), cholecystokinin, gastrin, peptide tyrosine–tyrosine and xenin that all display satiating qualities, whilst also improving pancreatic β-cell function or survival; therefore, making these peptides attractive candidates for complementing and amplifying the actions of GLP-1 and GIP[19,20]. OXM is a peptide released post-prandially that activates both the GLP-1 receptor and the glucagon receptor, reduces food intake and increases energy expenditure, thus making it an attractive potential pharmacological agent for inducing weight loss[20], although further research is needed in this area.

GLP-1 and GIP sites of action

Receptors for GLP-1 and GIP are expressed not only in pancreatic β-cells, but also in several extra-pancreatic tissues. In mice, GLP-1 receptor is highly expressed in the lung and duodenum, while GIP receptor is highly expressed in the testis of mice. The expression patterns of GLP-1 and GIP receptors are quite different, suggesting that GLP-1 and GIP have their own physiological activities[21]. GLP-1 receptor mRNA has been detected in the liver in polymerase-chain reaction experiments, but in an important study of primate livers no immunohistochemistry signal with a specific monoclonal antibody was detected in any normal monkey liver samples[22]. Consensus opinion is therefore that hepatocytes (as well as Kupffer cells and stellate cells) do not express the canonical GLP-1 receptor, and that GLP‑1 principally has indirect actions on the liver in NAFLD[23].

Although the principal actions of GLP-1RAs on the liver may be indirect, it may prove possible to amplify the beneficial effects of GLP-1RAs in T2DM, obesity and NAFLD by generating peptides that have both GLP-1 and GIP actions. Furthermore, by combining both GLP-1 and GIP functions with other peptides that activate glucagon receptors (or maybe other gastrointestinal-derived peptide functions), it should also be possible to develop multifunctional peptides. These multifunctional peptides will have widespread benefits not only on the liver, but also on many of the extra-hepatic diseases such as T2DM or cardiovascular disease that are linked to NAFLD as a 'multisystem disease' [24,25]. The beneficial effects of incretin-receptor agonists treatments to attenuate comorbidities associated with NAFLD and T2DM are schematically illustrated in **Figure 1,** and the actions of GLP-1, GIP (and also glucagon) in key tissues/organs that are relevant to the pathogenesis of NAFLD are summarized in **Table 1**.

GLP-1 synthesis, secretion and regulation of levels

GLP-1 is produced by post-translational processing of proglucagon by proprotein convertase subtilisin-kexin type 1 (PCSK1) or PCSK3 (also known as furin) and exists in two equally bioactive forms, namely glycine extended GLP1 (GLP-1 7-37) and amidated GLP-1 (GLP-1 7-36)[14,15]. N-terminally truncated GLP-1(7-37) and GLP-1(7-36)NH2 are the two 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 inter-prandial states and circulating levels of GLP-1 increase ~2 to 3-fold after meal ingestion[23]. Intestinal L cells are located with increasing density from the duodenum to the colon[26]. Within minutes of ingestion of nutrients there is a rise in GLP-1 secretion and the increase in GLP-1 concentration is very short lived, as GLP-1 (and GIP)

is metabolised quickly by the dipeptidyl peptidase-4 (DPP-4) enzyme, giving GLP-1 a short half-life of only ~1-2 minutes in the circulation[15]. Thus, only 10-15% of gastrointestinal-produced GLP-1 reaches the systemic circulation[15].

GLP-1 receptor agonist activity

The GLP-1 receptor is a G protein-coupled receptor that is expressed in the pancreas, kidneys, lungs, heart, gastrointestinal tract and in other organs[22]. As discussed in more detail below, GLP-1RAs are effective in decreasing liver fat content in individuals with NAFLD or NASH; and although less certain, there is some evidence that GLP-1RAs might also benefit liver fibrosis in NAFLD. It seems likely that GLP-1RAs are effective in NAFLD because of an indirect effect of these agents on other organs/tissues (beyond the liver) that may influence NAFLD. It is not known how GLP-1RAs may decrease liver fibrosis, but some experimental studies have suggested that GLP-1RAs could regulate extracellular matrix homeostasis by inhibiting the effects of reactive oxygen species and the mitogen-activated protein kinase (MAPK) signalling pathway to inhibit the formation of basic leucine zipper transcription factor, ATF-like (BATF)/JUN heterodimers[27]. However, whether this mechanism also operates in human livers is uncertain[28].

In the pancreas, GLP-1 is also a strong inhibitor of glucagon secretion (also strictly glucose dependent), which is possibly mediated by a direct effect on the pancreatic α-cells. However, this inhibitory effect is also likely to occur via the paracrine effects of increased levels of somatostatin and insulin from neighbouring δ-cells and β-cells, respectively[14,15]. In pancreatic β-cells, stimulation of GLP-1 receptors results in glucose-dependent insulin secretion, highlighting the important point that the effects of GLP-1 only occur when plasma glucose concentrations are greater than fasting levels[14,15]. There has been considerable research to investigate the effects of GLP-1RAs in different organs/tissues in the last decade[29]. In individuals with T2DM, supraphysiological doses of GLP-1 can normalize the endogenous insulin response during a hyperglycaemic clamp[29]. GLP-1RA treatment in patients with T2DM leads to better glycaemic control, body weight reduction, and improvement in cardiovascular risk factors, which are associated with improved micro-vascular and macro-vascular complications associated with T2DM[29].

Some GLP-1RAs have particularly weak effects with respect to influencing body weight (e.g., albiglutide), whereas other compounds have more pronounced effects (e.g., semaglutide), even when their glucose-lowering effects are similar[30]. This discrepancy of

effects on body weight has sparked interest in characterizing the mechanisms of action of these agonists. There is a key role for the arcuate nucleus within the hypothalamus, area postrema (AP), and nucleus tractus solitarii (NTS) for the influence of systemically administered GLP-1RAs on appetite, satiety, calorie intake and body weight[30]. GLP-1RAs seem to be effective at preventing meal initiation by suppressing the activity of NPY/agouti-related peptide (AgRP) producing neurons in the arcuate nucleus and inducing meal termination in the lateral parabrachial nucleus (PB)[31,32]. Signals reaching the PB originate from the arcuate nucleus of the hypothalamus and brain stem (AP and NTS). POMC/CART neurons expressing GLP-1 receptors activate PB neurons and directly or indirectly suppress NPY/AgRP neurons, thus leading to disinhibition of suppressive signals to the PB[31,32] (main effects of GLP-1RAs in brain are excellently summarised in [30]). Recent evidence suggests important metabolic effects of GLP-1 receptor agonism in the brain. For example, exenatide causes greater augmentation in insulin secretion in lean compared with obese subjects, although the brain response to food pictures was observed only in obese subjects[33]. Exenatide also regulates brain glucose metabolism in the post-absorptive state[34], and liraglutide alters brain activity related to desirable foods in subjects with T2DM[35]. In mice, it has been shown that liraglutide induces activation of hypothalamic neurons and its downstream metabolic effects are mediated by tanocyte transport (specialized ependymoglial cells) into the medio-basal hypothalamus[36], thereby emphasising the notion that hypothalamic tanocytes may be key regulators of metabolism. That said, it should be noted that not all GLP-1RAs have the same effects to suppress appetite and whether these effects in the brain occur with all GLP-1RAs is uncertain. Thus, it is conceivable that differential activation of these pathways by different GLP-1RAs may explain their differential effects at inducing weight loss in subjects treated with this class of drugs. Recent evidence also suggests that a GLP-1 receptor-mediated central effect in the brain may directly mediate changes in glucose metabolism. For example, gut-innervating, GLP1-receptor-expressing vagal afferents relay anorexigenic signals to parabrachial nucleus neurons that control meal termination. Moreover, GLP-1 receptor vagal afferent activation improves glucose tolerance, and its inhibition increases plasma glucose levels independent of food intake[37]. The main effects of GLP-1 and the crosstalk between the central and peripheral nervous systems relevant to metabolic diseases have been extensively discussed by Moscogiuri et *al*[38].

Promoting weight loss is very important in NAFLD, as regardless of the subject's initial body mass index or waist circumference, weight loss occurs because of a net negative energy balance where whole body energy requirements are not met by energy intake. Total energy expenditure consists of three different components[39]. These components are: (i) resting metabolic rate, (ii) diet-induced thermogenesis; and (iii) physical activity-induced thermogenesis[39]. All of these components decrease with weight loss. GLP-1 promotes satiety through activation of GLP-1 receptors in the hypothalamus and brainstem, which reduces food intake, thus inducing weight loss via a decrease in dietary energy intake[14,15]. When diet-induced weight loss occurs with overweight or obesity, this weight loss comprises ~70%–75% fat mass and ~25%–30% soft tissue lean mass (or "fat-free mass" when bone mineral is included)[40]. Moreover, the contribution of loss of lean mass remains relatively constant across a wide range of weight losses[41]. Therefore, the main metabolic benefit of weight loss due to GLP-1RA treatment is mediated mostly via loss of fat mass, rather than a loss of lean mass.

Weight loss has a very powerful effect on the liver to decrease liver fat content; an effect that has been known about for many years. For example, there is a marked and rapid perioperative benefit to shrink the liver with calorie restriction prior to bariatric surgery[42]. A total weight loss of at least 5% has been shown to achieve overall liver volume reduction by approximately 10%[42]. A 5% weight loss will also decrease visceral adipose tissue by approximately 10% and more relevant to NAFLD will decrease hepatic triglyceride by approximately 40%[43]. In fact, even very small amounts of weight loss are beneficial to the liver to decrease liver fat. In a recent clinical trial, testing the effect of synbiotic treatment to decrease liver fat measured by magnetic resonance spectroscopy in patients with NAFLD, we were able to show that only 1 kg of weight loss (~1% decrease) was associated with a ~2% decrease in liver fat content[44]. Thus, GLP-1 receptor agonist-induced marked weight loss has a profound effect to decrease liver fat that is very relevant to people with NAFLD; and importantly, this occurs, regardless of their individual overall baseline levels of body fat.

When weight loss occurs, there is an increase in adipose tissue triglyceride lipolysis as triglyceride stored in adipose or ectopic sites is hydrolysed. Adipose tissue triglyceride lipolysis increases non-esterified fatty acid and glycerol fluxes to the liver[45,46]. When plasma insulin levels are low and glucagon levels are high e.g., with persisting net negative energy imbalance, there is a consequent increase in both hepatic fatty acid

oxidation (producing a rise in ketone bodies) and gluconeogenesis (increasing hepatic glucose output and fasting plasma glucose concentrations). In contrast, in the post-prandial state, the presence of nutrients in the ileum (which is rich in neuroendocrine L cells) releases GLP-1, which acts via GLP-1 receptors to increase pancreatic insulin secretion, delay gastric emptying and, importantly, promote satiety by acting in the hypothalamus and brainstem[14,15].

Notably, for individuals with metabolic diseases (such as NAFLD and T2DM), there are benefits of weight loss that also occur in those who are non-obese. Weight loss improves insulin sensitivity both in the skeletal muscle and in the adipose tissue, and increases insulin clearance rate, without affecting insulin secretion from the pancreas[47]. These positive metabolic effects emphasise that weight loss has multiple benefits in people with NAFLD and T2DM, regardless of their initial levels of adiposity. Over the past decade, it has also become clear that NAFLD is a 'multisystem disease' that not only affects the liver, but also increases the risk of developing important extra-hepatic complications, such as T2DM, cardiovascular disease and chronic kidney disease[24,25]. Thus, it is also important to also note that modest weight loss (e.g., ~5%), even in people without obesity (albeit with metabolic dysfunction), can improve cardiometabolic risk factors, such as dysglycaemia, insulin resistance, atherogenic dyslipidaemia, and high blood pressure[47].

GIP secretion, signalling and activity

Post-translational processing of the precursor protein pro-GIP at residue 65 by proprotein convertase subtilisin/kexin type 1 (PC1/3) in intestinal K cells, gives rise to the established 42-amino-acid form of GIP [GIP(1-42)]. The pro-GIP peptide sequence also contains a consensus cleavage site for PC2 at residues 52-55, resulting in a COOH-terminal truncated GIP isoform, GIP(1-30)[48]. GIP signalling increases pancreatic insulin secretion acting via binding to the GIP receptor to potentiate glucose-dependent insulin secretion through cyclic adenosine 3′,5′-monophosphate (cAMP) protein kinase A (PKA) and exchange protein (directly activated by cAMP 2 [Epac2] signalling)[49]. Additionally, GIP also has antiapoptotic functions that are mostly mediated by the activation of cyclic AMP response element-binding protein (CREB) and the suppression of both p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK)[50]. Although GIP expression has been detected in the brain, including hippocampus, thalamus, cerebellum, brainstem, and cortex in rats [51] and in hypothalamus in humans[52], it remains controversial

as to whether GIP has any clinically relevant effects on appetite. Specifically, both positive and negative effects of GIP on appetite have been described[53].

GIP also has potentially important effects in adipose tissue that may be relevant to amelioration of NAFLD, producing a stimulatory effect on lipoprotein lipase and pre-adipocyte differentiation[53]. Nutrient intake is a strong stimulant of GIP release, and in euglycaemic or hypoglycaemic states GIP does not influence pancreatic insulin secretion, but stimulates glucagon secretion; thus, these data suggest that GIP supports the maintenance of optimal plasma glucose levels[54,55]. Studies indicate that disturbed GIP signalling in both obesity and T2DM is associated with impairments of fat metabolism and liver fat accumulation, but the precise mechanisms are still poorly understood[56]. GIP directly induces energy accumulation in adipocytes by increasing lipoprotein lipase activity, stimulation of lipogenesis, as well as by enhancing plasma membrane GLUT4 expression and glucose uptake[57]. Thus, GIP not only potentiates pancreatic insulin secretion, but it also increases the anabolic action of insulin in the adipose tissue, thus facilitating lipogenesis and preadipocyte differentiation[53]. This effect in adipose tissue could also be beneficial in NAFLD, with direct effects in adipose tissue having indirect benefits to protect the liver.

Combined GLP-1 and GIP receptor agonism to benefit NAFLD and other cardiometabolic diseases

When combined with GLP-1 receptor agonism, the effect of GIP receptor agonism enhances the actions of GLP-1RA treatment alone. It has been reported that dual receptor agonist activity has relevant therapeutic effects to induce marked weight loss, better glycaemic control and improved plasma lipid profile[58]. Dual GLP-1 and GIP receptor agonists achieve better glycaemic control and weight loss, compared with single selective GLP-1RAs, such as exenatide or liraglutide[59]. A unimolecular dual incretin receptor agonist can be derived from an intermixed sequence of GLP-1 and GIP, that has enhanced antihyperglycaemic and insulinotropic efficacy, relative to single GLP-1RAs; and this improved effect occurs across different species[59]. These dual receptor agonists are also more effective than single GLP-1RAs at improving adiposity-induced insulin resistance and pancreatic insulin secretion[59]. Additionally, it has been shown that both the half-life and the side effect profile of these mono-agonists and dual agonist molecules can be improved by chemical manipulation of the peptide sequences. For example, site-specific lipidation or PEGylation increases the half-life of biological action, and these

modified peptides provide comparable pharmacology and enhanced efficacy relative to similarly modified selective GLP-1RAs[59]. These modifications with less frequent administration also decrease peak drug exposure and reduce adverse gastrointestinal effects[59] that are a common problem with the use of GLP-1RAs.

One concern with weight loss induced by GLP-1 or GIP receptor agonists is that weight loss induced by these agents could also cause further skeletal muscle loss in a group of patients who may already have skeletal muscle dysfunction or sarcopenia[60] and who could be at further risk from losing more skeletal muscle mass[61]. Skeletal muscle has a key role in systemic insulin sensitivity and is the most important tissue for whole-body insulin-stimulated glucose uptake. Whilst it remains uncertain whether any benefit from losing body fat (particularly in visceral fat depots) is negated by skeletal muscle loss, this is an area of important research. Recently, using a phase-sensitive bioimpedance analyser, ultrasonography to assess the thickness of visceral adipose tissue, and analysis of muscle strength and anthropometric variables, the effects of a 6-month treatment with subcutaneous semaglutide on body composition was investigated in 40 patients with T2DM[62]. Importantly, 95% of these patients were able to tolerate a clinically meaningful dose of 0.5 mg/week. In this real-life setting, the investigators found that semaglutide induced significant weight loss, predominantly due to a reduction in fat mass and visceral adipose tissue as expected with a mild decline in the fat-free mass index and skeletal muscle mass that was not associated with a loss of muscle strength. It is noteworthy that these changes occurred rapidly after 3 months treatment and this effect was sustained and did not worsen at 6 months. As these T2DM patients were not advised about any relevant change to their lifestyle or nutritional behaviours, it remains uncertain whether an increase in exercise for example in the form of aerobic exercise or resistance training would have offset these relatively small changes. Relevant targets for preserving lean mass with weight loss with incretin receptor agonists have recently been reviewed[63]. Whilst it is beyond the scope of this review to discuss these targets, they include, for example, growth hormone, activin type II receptor inhibition, urocortin 2 and urocortin 3[63]. However, in the meantime and in the absence of available evidence to know what to target specifically, it would seem sensible to warn patients treated with these drugs about the potential harmful effects of losing muscle mass with weight loss, and recommend an increase in physical activity, if feasible.

Recognition of a wider incretin concept beyond GIP and GLP-1 is facilitating the development of novel gut hormone-derived agents that may not only benefit glucose metabolism in T2DM but may also benefit NAFLD. It is likely that multiagonist agents will evolve over time. These agents may include classes of specially tailored drugs that combine the amino acid sequences of key metabolic hormones into one single entity with enhanced potency and sustained action. Successful examples of this strategy already include multiagonist agents targeting the receptors not only for GLP-1 and GIP but also including the glucagon receptor[64]. Agents such as tirzepatide that combine both GLP-1 and GIP receptor agonist functions are already showing considerable promise in the treatment of obesity[65]. Preclinical data have shown that the affinity of tirzepatide for GIP receptors is equal to the affinity of native GIP for GIP receptors, whereas tirzepatide binds GLP-1 receptors with affinity approximately five times weaker than native GLP-1 binding to GLP-1 receptors[66]. Thus, GIP activation appears to synergistically act with GLP-1 receptor activation to allow greater weight reduction than that achieved with GLP-1 receptor monoagonism[66]. Signalling studies have also shown that tirzepatide mimics the actions of native GIP at the GIP receptor but shows bias at the GLP-1 receptor to favour cAMP generation over β-arrestin 1 (βARR1) recruitment, coincident with a weaker ability to drive GLP-1 receptor internalization compared with GLP-1[67]. Experiments in primary islets reveal βARR1 limits the insulin response to GLP-1, but not GIP or tirzepatide, thereby suggesting that this biased agonism of tirzepatide enhances pancreatic insulin secretion[67]. Thus, in summary, dual incretin receptor agonist activity could be clinically very important in NAFLD, because it is a disease with consequences beyond the liver[24,25]. The effects of both endogenous incretins and exogenous incretin receptor agonists on key tissues influencing both liver disease processes in NAFLD and metabolic syndrome are schematically shown in **Figure 2.**

Randomized controlled trials in patients with NAFLD or NASH

The potential effects of GLP-1RAs for specifically treating NAFLD or NASH have been recently investigated in an ever-increasing number of randomized controlled trials (RCT) that enrolled individuals with and without pre-existing T2DM.

Table 2 summarizes the principal phase-2 active-controlled or placebo-controlled RCTs (published up to 31 July 2022) that used either a GLP-1RA or a dual GLP-1 and GIP receptor agonist for the specific treatment of patients with NAFLD or NASH. No RCTs were available for dual agonists against GLP-1 and glucagon receptors or triple agonists

against GLP-1, GIP and glucagon receptors. In all these randomized controlled trials the diagnosis of liver disease was based on either liver biopsy or magnetic resonance-based techniques (i.e., magnetic resonance-protein density fat fraction [MRI-PDFF] or magnetic resonance spectroscopy); and there were at least 15 individuals per each treatment arm of interest[68-80].

GLP-1 receptor agonists

There is currently a dozen active-controlled or placebo-controlled phase-2 RCTs that used exenatide (two studies), liraglutide (six studies), semaglutide (three studies) or dulaglutide (one study) to specifically treat NAFLD or NASH (**Table 2**). Overall, these RCTs included 1,041 overweight or obese middle-aged individuals with NAFLD or NASH (55% men; mean age 54 years; BMI 32 kg/m^2), most of whom had coexisting T2DM (approximately 80% of total), and who were treated for a median period of 26 weeks (only three RCTs had a follow-up length ≥48 weeks). Three RCTs were conducted in Europe (UK, France and Netherlands), five in Asia (China, Singapore and India), one in the United States, whereas two RCTs included international cohorts of individuals with NASH. Notably, only two of these RCTs included subjects with biopsy-confirmed NASH, whereas the remaining 10 RCTs used magnetic resonance-based techniques for testing the effects of GLP-1RAs on NAFLD/NASH. GLP-1RA treatment was usually well tolerated with a rate of adverse events not exceeding that of either placebo or reference treatment, except for a greater frequency of gastrointestinal disorders (such as nausea, decreased appetite, vomiting or diarrhea). However, these gastrointestinal disorders were transient and mild-to-moderate in severity across published RCTs.

Many of these RCTs have been also included in our previous meta-analysis that incorporated phase-2 RCTs published until December 2020[81]. As shown in **supplementary Figure 1**, the meta-analysis showed that compared to placebo or reference therapy, GLP-1RA treatment for a median of 26 weeks was associated with an improvement in the absolute percentage of liver fat content, as assessed by magnetic resonance-based techniques (pooled weighted mean difference: -3.92%, 95% confidence interval [CI] -6.3 to -1.6%; p<0.0001)[81]. Notably, this meta-analysis also showed that in the two placebo-controlled RCTs using liver biopsy for diagnosing NASH, the treatment with GLP-1RAs (once-daily subcutaneous liraglutide 1.8 mg for 48 weeks, or subcutaneous semaglutide at dose of 0.1 mg, 0.2 mg or 0.4 mg/day for 72 weeks) resulted in a higher percentage of subjects with histologic resolution of NASH with no

worsening of fibrosis, compared with placebo (pooled random-effects odds ratio 4.06, 95% CI 2.5-6.6; p<0.0001). Conversely, there was no significant difference in the percentage of those with improvement in fibrosis stage without worsening of NASH (pooled random-effects odds ratio 1.50, 95% CI 0.98-2.3; p=0.060) (**supplementary Figure 2A** and **2B**)[81]. The meta-analysis also confirmed that GLP-1RA treatment was associated with bodyweight reduction (up to ~5 kg). In particular, treatment with semaglutide resulted in a marked, dose-dependent reduction in body weight (−13% with semaglutide 0.4 mg/day vs. −9% with semaglutide 0.2 mg/day vs. −5% with semaglutide 0.1 mg/day vs. −1% with placebo)[81]. After the publication of this meta-analysis, Alkhouri *et al*. published an open-label, proof-of-concept clinical trial testing the efficacy of a 24-week treatment with semaglutide alone (2.4 mg once weekly) or in combination with the farnesoid X receptor agonist cilofexor and/or the acetyl-coenzyme A carboxylase inhibitor firsocostat in patients with NASH[79]. The authors reported that compared with semaglutide monotherapy, combination treatments (especially semaglutide + firsocostat) resulted in greater improvements in hepatic steatosis measured by MRI-PDFF, and non-invasive tests of liver fibrosis. However, it is important to note that the improvements were not greater in the triple combination vs. the double combination groups.

Taken together, the aforementioned findings mostly derived from phase-2 RCTs suggest that treatment with GLP-1RAs, especially subcutaneous semaglutide (which tested its effects on primary histological endpoints assessed by liver biopsy, i.e., the “gold standard”, on the largest placebo-controlled RCT published to date), may exert beneficial effects on NAFLD and NASH. Larger phase 3 controlled trials of GLP-1RAs in individuals with biopsy-confirmed NASH are now needed to answer further questions regarding their long-term beneficial effects on liver fibrosis and NASH resolution, and to confirm safety. As reported in **Table 3**, a phase 3 placebo-controlled RCT in approximately 1,200 patients with biopsy-confirmed NASH is ongoing, to investigate the efficacy and safety of once-weekly semaglutide versus placebo over 240 weeks (NCT04822181). If these promising results will be confirmed in phase-3 RCTs, it is reasonable that GLP-1RAs will become an important therapeutic option for individuals with NAFLD or NASH (alone or more likely in combination with other drugs), especially in individuals with coexisting obesity or T2DM.

Dual GLP-1 and GIP receptor agonism

As also reported in **Table 2,** Gastaldelli *et al*. assessed the effect of a 52-week treatment with once-weekly subcutaneous tirzepatide versus once-daily insulin deglutec on liver fat content in individuals with T2DM[80]. In a sub-study of the open-label, parallel-group, phase-3 SURPASS-3 trial, these authors randomly assigned 296 participants to active treatment (tirzepatide 5 mg, n=71; tirzepatide 10 mg, n=79; tirzepatide 15 mg, n=72; insulin degludec, n=74). The primary efficacy endpoint was the change from baseline in liver fat content as assessed by MRI-PDFF at week 52, using pooled data from tirzepatide 10 and 15 mg arms versus insulin degludec. From an overall mean baseline liver fat content of 15.7%, the absolute reduction in liver fat content at week 52 was significantly greater for the pooled tirzepatide 10/15 mg arms versus the insulin degludec arm (-8.1% vs. -3.4%). The estimated treatment difference versus insulin degludec was -4.7% (95% CI -6.72 to -2.70; p<0.0001). The proportions of participants with ≥30% relative decrease from baseline in liver fat content at week 52 (i.e., a treatment response that has been associated with some improvements in liver histology) were nearly two to three times higher in each tirzepatide arm versus insulin degludec arm. All tirzepatide doses also markedly reduced body weight (by ~ 8–11 kg) and abdominal visceral fat depots from baseline at week 52, while insulin degludec increased both[80]. Thus, these data provide evidence of a possible hepatoprotective effect of tirzepatide. However, the lack of liver biopsy data does not allow to confirm or refute any beneficial effect of tirzepatide on individual histological features of NASH.

Research agenda and future perspectives

Table 3 summarizes the main ongoing phase-2 and phase-3 RCTs (available on ClinicalTrials.gov at July 31, 2022) assessing the efficacy and safety of GLP-1RAs, dual GLP-1 and GIP receptor agonists, or other incretin receptor agonists, such as dual GLP-1 and glucagon receptor agonists or triple GLP-1/GIP/glucagon receptor agonists for the treatment of subjects with NAFLD or NASH.

The liver expresses glucagon receptors and cotadutide is a novel dual GLP-1 and glucagon receptor agonist that has been shown to decrease body weight and improve glycaemic control, serum liver enzymes and non-invasive fibrosis biomarkers in patients with overweight/obesity and T2DM, as well as histologic features of NASH and fibrosis in mice[82-84]. Whilst the actions of cotadutide to reduce body weight, food intake and improve glycaemic control are mediated mainly through its GLP-1RA activity, it has been experimentally reported that the actions of cotadutide on the liver to reduce lipid content,

increase glycogen flux and improve mitochondrial turnover and function are directly mediated via liver glucagon receptor signalling[84]. Thus, (and perhaps surprisingly since glucagon stimulates hepatic gluconeogenesis), glucagon receptor agonist signalling would seem to be beneficial, at least when combined with GLP-1 and GIP receptor signalling. Moreover, metabolomic and transcriptomic analyses have implicated beneficial effects on lipogenic, fibrotic and inflammatory pathways, as unique therapeutic effects of glucagon receptor agonism. Interestingly, in two experimental mouse models, cotadutide has also been shown to attenuate liver fibrosis to a greater extent than liraglutide or obeticholic acid, despite adjusting the dose to achieve similar weight loss in these mouse models[84]. Thus, cotadutide, via direct hepatic glucagon agonism and extra-hepatic (GLP-1 receptor) effects, could be a promising therapeutic option for the treatment of NASH. In this regard, a phase 2b placebo-controlled RCT is currently ongoing to evaluate the efficacy of cotadutide in patients with biopsy-confirmed NASH and fibrosis (NCT05364931).

TB001 is also a dual GLP-1 receptor/glucagon receptor agonist that has affinity towards the glucagon receptor[85]. Similarly, in rodent models, TB001 retarded the progression of liver fibrosis with remarkable potency, selectivity and low toxicity[85]. TB001 treatment dose-dependently attenuated liver injury and collagen accumulation. In addition to decreased levels of extracellular matrix (ECM) accumulation during hepatic injury, activation of hepatic stellate cells was also inhibited via suppression of transforming growth factor-beta expression, as well as downstream the Smad signalling pathway. Moreover, TB001 attenuated liver fibrosis mainly through blocking downstream activation of proinflammatory nuclear factor kappa B/NF-kappa-B inhibitor alpha (NFκB/IKBα) pathways, as well as c-Jun N-terminal kinase (JNK)-dependent induction of hepatocyte apoptosis. Thus, the effects of TB001 treatment in pre-clinical models are similar to those of cotadutide and led further support to the notion that dual GLP-1 receptor/glucagon receptor agonism may be a promising therapeutic approach to treating liver fibrosis [85]. For this reason, other dual GLP-1 and glucagon receptor agonists are currently being studied, such as BI456906 in a phase 2b placebo-controlled RCT (NCT04771273), and efinopegdutide in a phase 2a open-label, active-comparator-controlled RCT (NCT04944992). Finally, triple receptor agonists that also include GIP receptor signalling agonists are also showing promise and need further testing. HM15211 is a triple GLP-1/GIP/glucagon receptor agonist that showed promising results in a phase 1b/2a RCT in obese individuals with NAFLD, with a maximal 88% reduction of liver fat content at

12 weeks with the highest dose[86]. A phase 2b placebo-controlled RCT is now ongoing to evaluate the efficacy of HM15211 in patients with biopsy-proven NASH (NCT04505436).

Another potential therapeutic effect that needs testing is to enhance GLP-1 receptor agonism and increase GLP-1 signalling to both increase insulin secretion and weight loss effects. Entinostat (MS-275), a histone deacetylase inhibitor, acts in such a way by upregulating expression of genes involved in endocytosis, cAMP signalling, PI3K-Akt signalling and insulin secretion[87].

Conclusions

Strong evidence supports the concept that NAFLD is a 'multisystem disease' and as such requires a multidisciplinary and holistic approach to its treatment. Treatment with a GLP-1RA that decreases appetite, reduces calorie intake, promotes net negative energy balance and induces weight loss is likely to be beneficial in individuals with NAFLD, regardless of their personal levels of overall adiposity.

Emerging evidence suggests that treatment with a dual GLP-1 and GIP receptor agonist, tirzepatide, may prove even more potent than a GLP-1RA alone, to ameliorate NAFLD. It is plausible that the generation of peptides that are multifunctional and mimic the properties of several gastrointestinal peptides may prove to be a very effective strategy for treating NAFLD, although confirmation is awaited by ongoing clinical trials. Moreover, considering the multiple pathways implicated in NAFLD pathophysiology, the combination of a GLP-1RA with different agents (e.g., a farnesoid X receptor agonist, or an Acetyl-CoA carboxylase inhibitor [NCT04971785], a fibroblast growth factor-21 analogue [NCT05016882], or a sodium-glucose cotransporter-2 inhibitor [NCT04639414 and NCT05140694]), might prove to be the best approach to specifically treat NAFLD.

It has become increasingly evident that NAFLD not only adversely affects the liver, but also increases the risk of developing extra-hepatic complications, such as T2DM, cardiovascular disease, chronic kidney disease and certain extra-hepatic cancers (such as, for example, colorectal and breast cancers)[24,25]. Therefore, multifunctional gastrointestinal peptide agonists acting not only directly on the liver, but also on organs affected by NAFLD, could be a potentially exciting therapeutic approach to modifying disease outcomes related to these other diseases beyond the liver. This area of research is also likely to gain momentum over the next decade.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms “non-alcoholic fatty liver disease”, “NAFLD”, “non-alcoholic steatohepatitis”, “NASH”, “incretins”, “glucagon-like peptide-1 (GLP-1)”, “glucose-dependent insulinotropic polypeptide (GIP)”, “GLP-1 receptor agonists”, “incretin receptor agonists”, “GLP-1 receptor agonist”, “glucagon receptor agonist”, “dual or co-agonist”, “exenatide”, “liraglutide”, “dulaglutide”, “semaglutide”, “cotadutide” and “tirzepatide” from database inception to July 31, 2022. Articles were also identified through searches of the authors’ own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

Contributors section: all authors contributed equally to the manuscript.

Figure legends

Figure 1. Effects of incretin receptor agonists to attenuate the spiral of worsening comorbidities associated with NAFLD and type 2 diabetes mellitus.

GLP-1 receptor agonists have proven efficacy to benefit type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and chronic kidney disease (CKD). Glucagon-like peptide-1 (GLP-1) receptor agonists are effective in facilitating weight loss and weight loss also benefits NAFLD. Combined GLP-1 receptor and glucose-dependent insulinotropic polypeptide (GIP) receptor agonists may be more effective at promoting weight loss (than GLP-1 receptor agonists alone). Thus, combined GLP-1 and GIP receptor agonists may prove to be very effective treatments for NAFLD or NASH.

Figure 2. Effects of endogenous incretins and exogenous incretin receptor agonists on key tissues influencing metabolic syndrome and liver disease processes in NAFLD.

It is most likely that incretin receptor agonists are effective in NAFLD because of an indirect effect of incretins on other organs/tissues that influence NAFLD. In the pancreas, glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists (GLP1RAs) are a strong inhibitor of glucagon secretion (which is also strictly glucose dependent), which is possibly mediated by a direct effect on the pancreatic α-cells. However, this inhibitory effect is also likely to occur via the paracrine effects of increased levels of somatostatin and insulin from neighbouring δ-cells and β-cells, respectively. GLP-1 signalling has a key role to influence appetite, satiety and calorie intake via actions in the arcuate nucleus within the hypothalamus, area postrema (AP), and nucleus tractus solitarii (NTS) that results in decreased calorie intake and weight loss. Weight loss is principally due to loss of adipose tissue fat, and this decreases flux of adipose tissue-derived long-chain fatty acids (LCFAs) and glycerol to the liver (LCFAs from adipose tissue are a powerful stimulus and substrate for hepatic *de novo* lipogenesis and glycerol is a substrate for hepatic gluconeogenesis). Thus, weight loss facilitated by incretin-effects has a powerful effect on the liver to facilitate reduction in hepatic lipid accumulation. As a result of decreased nutrient intake with GLP-1RA treatment, decreased dietary fat intake also results in decreased chylomicron synthesis and consequently decreased chylomicron uptake in liver, resulting in a reduction in the exogenous dietary supply of fatty acids to the liver for hepatic lipid synthesis. In contrast to GLP-1, glucose-dependent insulinotropic

polypeptide (GIP) also has anti-apoptotic functions that are mostly mediated by the activation of cyclic AMP response element-binding protein (CREB) and the suppression of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK). GIP expression has also been detected in the brain, including hippocampus, thalamus, cerebellum, brainstem, and cortex in rats and in the hypothalamus in man. However, it is controversial as to whether GIP has any clinically relevant effects on appetite, and both positive and negative effects of GIP on appetite have been described. Nutrient intake is also a strong stimulant of GIP release, and GIP stimulates glucagon secretion from the pancreas. GIP also directly induces energy accumulation in adipocytes by increasing lipoprotein lipase activity, stimulation of hepatic lipogenesis, as well as by enhancing plasma membrane GLUT-4 expression and glucose uptake. Unlike GLP-1, GIP also increases the anabolic action of insulin in adipose tissue, facilitating lipogenesis and pre-adipocyte differentiation. Thus, GLP-1 and GIP both act indirectly to benefit NAFLD and dual agonist incretin treatment has a powerful therapeutic effect to induce weight loss, improve glycaemic control and plasma lipids and therefore benefit the features of metabolic syndrome (MetS) besides liver disease in NAFLD.

Supplementary Figure 1. Forest plot and pooled estimates of the effect of different GLP-1RAs on the absolute percentage of liver fat content as assessed by magnetic resonance-based techniques as compared with placebo. Data are derived from Mantovani et al. Metabolites 2021;11:73.

Supplementary Figure 2. Forest plot and pooled estimates of the effects of GLP1-RA treatment on either (A) histologic resolution of NASH with no worsening of liver fibrosis; or (B) improvement in liver fibrosis with no worsening of NASH. Data derived are from Mantovani et al. Metabolites 2021;11:73.

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Table 1. Potential actions of glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and glucagon in key tissues and organs relevant to NAFLD.

	Liver	Gastrointestinal tract	Brain	Adipose tissue	Pancreas	Cardiovascular system
GLP-1	(indirect action) ↓ inflammation via reduction in T cell responses and oxidative stress ↓ glucose production	↓ gastric emptying and motility ↓ chylomicron synthesis and secretion	↓ appetite & inflammation ↑ neuroprotection & memory	↑ glucose uptake ↑ lipolysis*	↑ insulin secretion & beta cell proliferation ↓ glucagon secretion & beta cell apoptosis	↑ vasodilation, endothelial cell function, myocardial contractility, diastolic function, cardiac output & protection ↓ blood pressure
GIP	↓ glucagon-stimulated glucose production	↓ gastric emptying, motility & gastric acid secretion	↓ & ↑ appetite (uncertain effects)	↑ lipogenesis, lipoprotein lipase, Pre-adipocyte differentiation	↑ insulin secretion & beta cell proliferation ↓ beta cell apoptosis ↑ & ↓ glucagon secretion[#]	↑ endothelial cell function
Glucagon	↑ thermogenesis, energy expenditure, glycogenolysis & gluconeogenesis	↓ gastric emptying, & motility ↑ gall bladder contraction	↓ appetite	↑ thermogenesis, energy expenditure & lipolysis	↑ insulin secretion	↑ cardiac output, cardioprotection & contractility

Content adapted from[14,15,23,29,49,53,88]. *Effects on adipocytes are indirect and mediated via weight loss. [#]Both increases and decreases of glucagon secretion have been reported.

Table 2. Principal phase 2 or phase 3 placebo-controlled or active-controlled randomized clinical trials testing different GLP-1RAs or dual GLP-1 and GIP receptor agonist tirzepatide for the specific treatment of patients with NAFLD or NASH (as assessed by either magnetic resonance-based techniques or liver biopsy).

Author, Year, Country (ref.)	Study Characteristics	Interventions (n), Study Length	Primary Hepatic Outcome Measures	Major Adverse Effects
GLP-1 receptor agonists (n= 12 trials)				
Armstrong *et al*. 2016, UK [68]	Phase 2 placebo-controlled RCT Patients with biopsy-confirmed NASH and fibrosis Mean age: 51 years; male sex: 60%; BMI: 36 kg/m^2; pre-existing T2DM: 33%; ALT: 71 IU/L; AST: 51 IU/L; fibrosis F3-F4 (on histology:) 52%	A. Liraglutide 1.8 mg/day (n=26) B. Placebo (n=26) Length: 48 weeks	Liraglutide was associated with greater histological resolution of NASH than placebo: 39% vs. 9%, p=0.019 9% of patients in the liraglutide group versus 36% of patients in the placebo group had progression of liver fibrosis (p=0.040)	Moderate gastro-intestinal adverse events in liraglutide vs. placebo: 81% vs. 65%, respectively
Dutour *et al.* 2016, France [69]	Phase 2 placebo-controlled RCT Patients with T2DM, 95% of whom had NAFLD assessed by MRS Mean age: 52 years; male sex: 48%; BMI: 36 kg/m^2; pre-existing T2DM: 100%; HbA1c: 7.5%; ALT: 29 IU/L; AST: 22 IU/L	A. Exenatide 5-10 mcg bid (n=22) B. Placebo (n=22) Length: 26 weeks	Liver fat content was significantly reduced after exenatide compared with placebo (−23.8±9.5% vs. +12.5±9.6%, p=0.007)	Not reported
Yan *et al*. 2019, China [70]	Phase 2 active-controlled RCT Patients with T2DM and NAFLD assessed by MRI-PDFF	A. Liraglutide 1.8 mg/day (n=24) B. Insulin glargine 0.2 IU/kg/day (n=24)	In the liraglutide and sitagliptin groups, liver fat content significantly decreased from baseline to week 26 (liraglutide: from 15.4±5.6% to 12.5±6.4%, p<0.001; sitagliptin: from 15.5±5.6% to 11.7±5.0%, p=0.001)	Not reported

	Mean age: 44 years; male sex: 69%; BMI: 29.8 kg/m^2; pre-existing T2DM: 100%; HbA1c: 7.7%; ALT: 43 IU/L; AST: 33 IU/L	C. Sitagliptin 100 mg/day (n=27) Length: 26 weeks		
Khoo *et al*. 2019, Singapore [71]	Phase 2 active-controlled RCT Non-diabetic patients with obesity and NAFLD assessed by MRI-PDFF Mean age: 41 years; male sex: 90%; BMI: 33 kg/m^2; pre-existing T2DM: 0%; ALT: 88 IU/L; AST: 48 IU/L	A. Liraglutide 3.0 mg/day (n = 15) B. Lifestyle modifications (diet + exercise) (n = 15) Length: 26 weeks	Both treatment groups showed significant and similar reductions in liver fat content at 26 weeks (-8.1±13.2 vs. -7.0±7.1%)	Nausea, abdominal discomfort and diarrhoea in the liraglutide group
Liu *et al*. 2020, China [72]	Phase 2 active-controlled RCT Patients with T2DM and NAFLD assessed by MRI-PDFF Mean age: 48 years; male sex: 50%; BMI: 28 kg/m^2; HbA1c: 8.3%; ALT: 38 IU/L; AST: 28 IU/L	A. Exenatide 1.8 mg/day (n = 38) B. Insulin glargine 0.2 IU/kg/day (n = 38) Length: 24 weeks	Liver fat content was significantly reduced after exenatide treatment (change of liver fat: −17.6±12.9%) compared to insulin glargine	Adverse events were comparable between the two groups
Bizino *et al*. 2020, Netherlands [73]	Phase 2 placebo-controlled RCT Patients with T2DM and NAFLD assessed by MRS Mean age: 60 years; male sex: 59%; BMI: 32 kg/m^2; pre-existing T2DM: 100%; HbA1c: 8.3%; ALT: 14 IU/L; AST: 33 IU/L	A. Liraglutide 1.8 mg/day (n=23) B. Placebo (n=26) Length: 26 weeks	Reduction in liver fat content was not different between the two treatment arms (liraglutide: from 18.1±11.2% to 12.0±7.7%; placebo: from 18.4±9.4% to 14.7±10.0%; estimated treatment effect -2.1% [95% CI -5.3 to 1.0])	There were no serious drug-related adverse events

Kuchay *et al*. 2020, India [74]	Phase 2 placebo-controlled RCT Patients with T2DM and NAFLD assessed by MRI-PDFF Mean age: 47 years; male sex: 70%; BMI: 29.7 kg/m^2; HbA1c: 8.4%; ALT: 69 IU/L; AST: 47 IU/L	A. Dulaglutide 1.5 mg/week (n=32) B. Placebo (n=32) Length: 24 weeks open-label trial (add-on to usual care)	Dulaglutide resulted in a control-corrected absolute reduction in liver fat content of -3.5% (95% CI -6.6 to -0.4; p=0.025) and relative reduction of -26.4% (95% CI -44.2 to -8.6; p=0.004) compared to placebo Absolute changes in liver stiffness on Fibroscan (-1.31 kPa [-2.99 to 0.37]; p=0.12) were not significant between the two treatment arms	There were no serious drug-related adverse events
Guo *et al*. 2020, China [75]	Phase 2 placebo-controlled RCT Patients with T2DM (treated with metformin) and NAFLD assessed by MRS Mean age: 52 years; male sex: 56%; BMI: 28.7 kg/m^2; pre-existing T2DM: 100%; HbA1c: 7.4%; ALT: 32 IU/L; AST: 28 IU/L	A. Liraglutide 1.8 mg/week (n=32) B. Once-daily insulin glargine (n=32) C. Placebo (n=32) Length: 26 weeks	Liraglutide resulted in a control-corrected absolute reduction in liver fat content of -6.3% (p<0.05) and relative reduction of -24% (p<0.05). Although the reduction in liver fat content was greater with liraglutide than insulin glargine, it was not significantly different between the two treatment arms (-6.3% vs. -3.4%; p >0.05)	There were no serious drug-related adverse events. Only mild-to-moderate gastrointestinal events were reported in the liraglutide group
Zhang *et al*. 2020, China [76]	Phase 2 active-controlled RCT Patients with T2DM (treated with metformin) and NAFLD assessed by MRS Mean age: 51 years; male sex: 47%; BMI: 27.3 kg/m^2; HbA1c: 8.1%; ALT and AST: not reported	A. Liraglutide 1.8 mg/week (n=30) B. Pioglitazone 30 mg/day (n=30) Length: 24 weeks open-label trial (add-on to usual care)	Liraglutide resulted in a control-corrected absolute reduction in liver fat content of -4.0% (95% CI -6.6 to -0.4; p<0.05) and relative reduction of -17% (p<0.05). This reduction in liver fat content was greater with liraglutide than pioglitazone	There were no serious drug-related adverse events. Only mild-to-moderate gastrointestinal events were reported in the liraglutide group
Newsome *et al*. 2021, Multinational cohort [77]	Phase 2 placebo-controlled RCT	A. Semaglutide 0.1 mg/day (n=80)	Among patients with stage F2 or F3 fibrosis, the percentage of patients in whom NASH resolution was achieved with no worsening of fibrosis was 40% in the 0.1-mg	There were no serious drug-related adverse events. Nausea, constipation, and vomiting were higher in the 0.4-mg group than in the placebo group

	Patients with biopsy-confirmed NASH and fibrosis Mean age: 55 years; male sex: 41%; BMI 35.7 kg/m^2; pre-existing T2DM: 62% (HbA1c 7.3%); ALT: 54 IU/L; AST: 43 IU/L	B. Semaglutide 0.2 mg/day (n=78) C. Semaglutide 0.4 mg/day (n=82) D. Placebo (n=80) Length: 72 weeks	group, 36% in the 0.2-mg group, 59% in the 0.4-mg group, and 17% in the placebo group (p<0.001 for semaglutide 0.4 mg vs. placebo) Improvement in fibrosis stage occurred in 43% of the patients in the 0.4-mg group and in 33% of the patients in the placebo group (p=0.48)	
Flint *et al*. 2021, Multinational cohort [78]	Phase 2 placebo-controlled RCT Patients with NAFLD assessed by MRI-PDFF and magnetic resonance elastography Mean age: 60 years; male sex: 70%; BMI ≥30 kg/m^2: 84%; pre-existing T2DM: 73%; ALT: 37 IU/L; AST: 30 IU/L	A. Semaglutide 0.4 mg/day (n=34) B. Placebo (n=33) Length: 72 weeks	Semaglutide significantly reduced liver fat content compared with placebo and more subjects achieved a ≥30% reduction in liver fat content with semaglutide at weeks 24, 48 and 72 (with an estimated treated ratio of 0.50 at week 72) Changes in liver stiffness were not different between the two groups	Gastrointestinal adverse events (diarrhoea and nausea) were reported by more patients in the semaglutide than placebo group
Alkhouri *et al*. 2022, USA [79]	Phase 2 active-controlled RCT Patients with NASH assessed either by liver biopsy or by MRI-PDFF ≥10% and Fibroscan®-measured liver stiffness ≥7 kPa Mean age: 56 years; male sex: 30%; BMI 35 kg/m^2: 84%; pre-existing T2DM: 55%; ALT: 49 IU/L; AST: 40 IU/L	A. Semaglutide 2.4 mg/week (n=21) B. Semaglutide 2.4 mg/week and once-daily cilofexor 30 mg (n=22) C. Semaglutide 2.4 mg/week and once-daily cilofexor 100 mg (n=22)	Compared with semaglutide monotherapy, combination treatments resulted in greater improvements in liver steatosis measured by MRI-PDFF (least-squares mean of absolute changes: ranging from −9.8% to −11.0% vs.−8.0%; the difference was statistically significant only between the semaglutide and semaglutide + firsocostat groups) as well as in non-invasive tests of liver fibrosis	Treatments were well tolerated – the incidence of adverse events was similar across groups (73-90%) and most events were gastrointestinal in nature

		D. Semaglutide 2.4 mg/week and once-daily firsocostat 20 mg (n=22) E. Semaglutide 2.4 mg/week plus once-daily cilofexor 30 mg and firsocostat 20 mg (n=21) Length: 24 weeks		
Dual GLP-1 and GIP receptor agonists (n= 1 trial)				
Gastaldelli *et al*. 2022, Multinational cohort [80]	Sub-study of phase 3 SURPASS-3 trial Patients with T2DM (treated with metformin alone or combined with a SGLT2 inhibitor) and NAFLD assessed by MRI-PDFF Mean age: 56 years; male sex: 58%; BMI 33.5 kg/m^2; pre-existing T2DM: 100%; HbA1c: 8.2%; ALT: 30 IU/L; AST: 22 IU/L	A. Tirzepatide 5 mg/week (n=71) B. Tirzepatide 10 mg/week (n=79) C. Tirzepatide 15 mg/week (n=72) D. Once-daily insulin degludec (n=74) Length: 52 weeks	From an overall mean baseline liver fat content of 15.7%, the absolute reduction in liver fat content at week 52 was significantly greater for the pooled tirzepatide 10 mg and 15 mg groups (-8.1%) versus the insulin degludec group (-3.4%). The estimated treatment difference versus insulin degludec was -4.7% (95% CI -6.7 to -2.7; p<0.0001) The proportions of participants with at least a 30% relative decrease from baseline in liver fat content at week 52 were higher in each tirzepatide group (ranging from about 67% to 81% for tirzepatide doses) versus the insulin degludec group (32%)	Gastrointestinal adverse events were reported by more patients in the tirzepatide than in the insulin degludec group

NB: In this table, we did not include post-hoc analyses of randomized controlled trials that tested the effects of these compounds or other incretin receptor agonists (e.g., cotadutide) on plasma aminotransferase levels in patients with T2DM (irrespective of their NAFLD status at baseline), or that used liver ultrasonography or blood biomarkers/scores for testing the effects of these compounds on NAFLD status.

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; MRS, magnetic resonance spectroscopy; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; RCT, randomized controlled trial; T2DM, type 2 diabetes mellitus.

Table 3. Principal ongoing randomised clinical trials assessing the efficacy and safety of GLP-1 receptor agonists, dual GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) receptor agonists, dual GLP-1 and glucagon receptor agonists or triple GLP-1/GIP/glucagon receptor agonists for the specific treatment of patients with NAFLD or NASH.

NCT Number	Trial Acronym	Current Status	Study Participants	Interventions	Study Characteristics	Estimated Sample Size (n)	Primary Hepatic Outcome Measures	Start Date	Estimated Completion Date
GLP-1 receptor agonists									
NCT04822181	ESSENCE	Recruiting	NASH on liver biopsy	Semaglutide vs. placebo	Phase 3, randomised, double-blind, placebo-controlled trial	1200	(1) Histological resolution of NASH and no worsening of liver fibrosis after 72 weeks of treatment; (2) Improvement in liver fibrosis and no worsening of NASH after 72 weeks of treatment (3) Time to first liver-related clinical event (composite endpoint) after 240 weeks of treatment	April 1, 2021	May 2028
NCT05016882	-	Recruiting	NASH on liver biopsy	Semaglutide *plus* NNC0194-0499* vs. placebo	Phase 2, randomised, double-blind, active and placebo-controlled, double-dummy, parallel group, multinational trial	672	(1) Improvement in liver fibrosis and no worsening of NASH after 52 weeks of treatment	August 31, 2021	September 2024
NCT04971785	-	Recruiting	NASH-related compensated cirrhosis on liver biopsy	Semaglutide *plus* cilofexor/firsocostat vs. placebo	Phase 2, randomised, double-blind, double-dummy, placebo-controlled trial	440	(1) Percentage of participants who achieve ≥1 stage improvement in liver fibrosis without worsening of NASH after 72 weeks of treatment;	August 9, 2021	March 2024

							(2) Histological resolution of NASH after 72 weeks of treatment		
NCT04639414	COMBATT2 NASH	Recruiting	NASH on liver biopsy	Semaglutide *plus* empagliflozin vs. placebo and empagliflozin alone vs. placebo	Phase 4, randomised, double-blind placebo-controlled, trial	192 patients with type 2 diabetes	(1) Histological resolution of NASH without worsening of fibrosis after 48 weeks of treatment	March 26, 2021	December 2023
NCT05140694	-	Not yet recruiting	NAFLD on Fibroscan equipped with controlled attenuation parameter (CAP)	Dulaglutide vs. empagliflozin vs. empagliflozin *plus* dulaglutide	Phase 4 randomised, active-comparator controlled, parallel-group trial	135 patients with type 2 diabetes	(1) Changes of CAP score after 24 weeks of treatment	March 1, 2022	December 2025
NCT03648554	REALIST	Not yet recruiting	NASH on liver biopsy	Dulaglutide vs. placebo	Phase 4, multicenter, open, prospective, randomised, controlled dietary reinforcement trial	93 patients with type 2 diabetes	(1) Histological regression of NASH (defined as decrease of at least 2 points in the NAS measured on three components: steatosis, lobular inflammatory foci and hepatocyte ballooning) without worsening of fibrosis after 52 weeks of treatment	September 1, 2019	March 2024
Dual GLP-1 and GIP receptor agonists									
NCT04166773	SYNERGY-NASH	Recruiting	NASH on liver biopsy	Tirzepatide vs. placebo	Phase 2b randomised, double-blind, placebo-controlled trial	196	(1) Histological resolution of NASH with no worsening of liver fibrosis after 52 weeks of treatment	November 19, 2019	December 2023
Dual GLP-1 and glucagon receptor agonists									

NCT05364931	PROXYMO-ADV	Not yet recruiting	NASH with fibrosis on liver biopsy	Cotadutide (MEDI0382) vs. placebo	Phase 2b/3 randomised, double-blind, placebo-controlled trial	1860	(1) Histological resolution of NASH with no worsening of liver fibrosis after 48 weeks of treatment (2) Histological resolution of NASH with no worsening of fibrosis and improvement of liver fibrosis by at least one stage without worsening of NASH after 84 weeks of treatment	May 19, 2022	May 2025
NCT04771273	-	Recruiting	NASH on liver biopsy	BI456906 vs. placebo	Phase 2b, multicenter, double-blind, parallel-group, randomised trial	240	(1) Percentage of patients with histological improvement of NASH (defined as NAS reduction of 2 or more points) after 48 weeks of treatment	April 27, 2021	March 2024
NCT04944992	-	Active, not recruiting	NAFLD on magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF)	Efinopegdutide (MK-6024/HM12525A/JNJ-64565111) vs. semaglutide	Phase 2a, randomised, active-comparator-controlled, open-label trial	130	(1) Mean relative reduction from baseline in liver fat content as measured by MRI-PDFF after 24 weeks of treatment	August 4, 2021	October 2022
Triple GLP-1/GIP/glucagon receptor agonists									
NCT04505436	-	Recruiting	NASH on liver biopsy	HM15211 vs. placebo	Phase 2b, randomised, double-blind, placebo-controlled, parallel group trial	217	(1) Histological resolution of NASH with no worsening of liver fibrosis after 48 weeks of treatment	July 31, 2020	November 2025

NB: the last research using https://clinicaltrials.gov/ was performed at 31 July 2022. *NNC01940499 is a new subcutaneously administered, fibroblast growth factor-21 analogue.

Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, non-alcoholic steatohepatitis.