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A look to the future in non-alcoholic fatty liver disease: Are glucagon-like peptide-1 analogues or sodium-glucose co-transporter-2 inhibitors the answer?

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Abstract

The increasing prevalence of diabetes and non-alcoholic fatty liver disease (NAFLD) is a growing public health concern associated with significant morbidity, mortality and economic cost, particularly in those who progress to cirrhosis. Medical treatment is frequently limited, with no specific licensed treatments currently available for people with NAFLD. Its association with diabetes raises the possibility of shared mechanisms of disease progression and treatment. With the ever-growing interest in the non-glycaemic effects of diabetes medications, studies and clinical trials have investigated hepatic outcomes associated with the use of drug classes used for people with type 2 diabetes (T2D), such as glucagon-like peptide-1 (GLP-1) analogues or sodium-glucose co-transporter-2 (SGLT2) inhibitors. Studies exploring the use of GLP-1 analogues or SGLT2 inhibitors in people with NAFLD have observed improved measures of hepatic inflammation, liver enzymes and radiological features over short periods. However, these studies tend to have variable study populations and inconsistent reported outcomes, limiting comparison between drugs and drug classes. As these drugs appear to improve biomarkers of NAFLD, clinicians should consider their use in patients with NAFLD and T2D. However, further evidence with greater participant numbers and longer trial durations is required to support specific licensing for people with NAFLD. Larger trials would allow reporting of major adverse hepatic events, akin to cardiovascular and renal outcome trials, to be determined. This would provide a more meaningful evaluation of the impact of these drugs in NAFLD. Nevertheless, these drugs represent a future potential therapeutic avenue in this difficult-to-treat population and may beget significant health and economic impacts.

KEYWORDS

cirrhosis, GLP-1 analogue, insulin resistance, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), SGLT2 inhibitor, type 2 diabetes

Rebecca K. Vincent and David M. Williams contributed equally and should be considered joint first author.

1 | INTRODUCTION

Non-alcohol related fatty liver disease (NAFLD) is a global health concern. The prevalence of NAFLD is increasing, affecting 25% of people globally,[1,2] with a higher incidence in those with obesity and/or diabetes.[3] NAFLD is associated with a substantial health economic impact, representing a financial burden of more than $100 bn annually in the United States alone.[1] Moreover, the personal impact of NAFLD should not be underestimated, particularly given its association with comorbidities including diabetes, cardiovascular disease and the multi-system consequences of obesity leading to increased morbidity. All-cause mortality is higher in those with NAFLD compared with the general population,[4] making it an important health concern which needs to be addressed. Despite the burden of NAFLD, there are few effective management strategies to offer patients.

2 | DIAGNOSIS OF NAFLD

Diagnosing NAFLD is challenging, as current disease definitions suggest it is a diagnosis of exclusion, rather than a positive diagnosis. The consensus is that a diagnosis of NAFLD can be made if there is >5% of hepatic fat accumulation in the absence of other causes.[2,5–7] This can make a positive diagnosis of NAFLD challenging because of confounding factors, especially the presence of excess alcohol, and the definition suggests a liver biopsy is required to quantify the degree of fat. It is recognized that NAFLD is an inflammatory spectrum of disease, ranging from hepatic steatosis (often termed NAFLD, which is not strictly so) to steatohepatitis, and ultimately fibrosis and cirrhosis, which has its own sequelae.[3,4] This spectrum and the burden NAFLD places on society and the individual is illustrated in Figure 1.

A definition of NAFLD that is not solely based on histology is lacking, making the diagnosis and staging of NAFLD challenging. It is difficult to distinguish between simple hepatic steatosis and non-alcoholic steatohepatitis (NASH) clinically, although both are an inflammatory state, and it is difficult to predict who will progress to fibrosis and cirrhosis, and who will remain in the earlier steatosis stage. Understanding the mechanism of disease progression is essential to target individuals at greater risk of steatohepatitis to reduce morbidity whilst ensuring cost-effective treatment can be identified and studied.[8] There are several non-invasive methods to identify individuals with NAFLD (eg, NAFLD score, FIB-4 score, alanine transaminase [ALT]: aspartate transaminase [AST] ratio, AST:platelet ratio). There are problems, however, with these scores as they are largely based on blood test results, which have a poor predictive value,[9] and liver function tests, which poorly reflect liver damage.[10] Abdominal imaging and transient elastography can aid the diagnosis of steatosis and fibrosis.[9] Biomarkers such as cytokeratin-18, a protein released during hepatocyte death which correlates with liver dysfunction, have been studied in NAFLD.[9,10] However, the cytokeratin-18 level is raised in any form of liver damage and is therefore not specific to NAFLD. Adiponectin, which has a role in lipid and glucose metabolism by influencing insulin receptors, has also been suggested as a biomarker in NAFLD. In dyslipidaemia and an insulin-resistant state, adiponectin levels are low compared to controls and can be used to assess progression of hepatic steatosis to NASH.[10] Furthermore, leptin has been implicated due to its role in appetite and body weight, and is associated with insulin resistance (IR). Higher levels of leptin have been identified in individuals with NALFD.[10] However, these biomarkers are not routinely used in clinical practice and further work is needed to identify meaningful biomarkers. Diagnosis is often based on a combination of scoring systems, clinical history and risk factors such as diabetes, cardiovascular disease, obesity and metabolic syndrome. Indeed, metabolic profiling has been suggested as a useful tool when determining which patients are more likely to progress to NASH and cirrhosis.[11] Yet, despite knowing that certain patient groups are at risk of NAFLD, there is currently no screening programme to identify those at greater risk of developing steatohepatitis. Furthermore, non-invasive tests are frequently unhelpful, often necessitating liver biopsy.[5,6] However, there are significant risks and patient aversion associated with liver biopsy due to its invasive nature. Nevertheless, if NASH can be proven, then the patient may be suitable for novel treatments, off-label medication or participation in a clinical trial.[6]

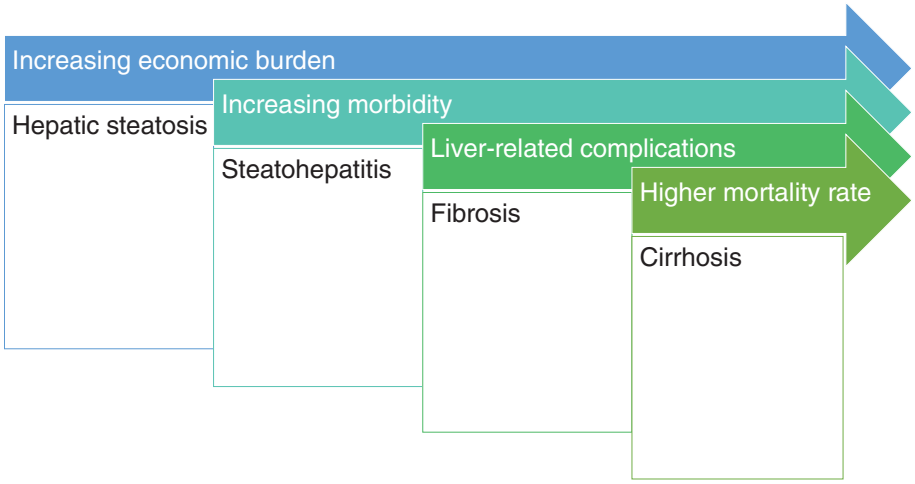

FIGURE 1 A schematic linking the disease spectrum of non-alcoholic fatty liver disease and how as the disease progresses it affects the individual and society

3 | PATHOPHYSIOLOGY OF NAFLD

The situation becomes increasingly complicated when considering the pathophysiology of NAFLD because of its multifactorial nature and association with other comorbidities. Liver fat accumulation results from an imbalance among fatty acid influx (adipose tissue lipolysis), hepatic *de novo* lipogenesis and lipid disposition (fatty acid oxidation) and VLDL secretion from the liver.[12] Studies in humans have shown that adipose tissue lipolysis, hepatic *de novo* lipogenesis and diet contribute to free fatty acid (FFA) accumulation in the liver, with diet playing the smallest part.[13] The progression of NAFLD involves an interplay between cellular stress responses (lipotoxicity and increased oxidative stress)[14] and hepatic lipid flux, with varying degrees of cytotoxic potential, to which individual patients respond differently,[9] and inflammation. Additionally, the relationship between gut and pancreatic-released hormones, gut microbiota, and IR in muscle, adipose tissue and liver is implicated in the pathophysiology.[15,16] Although multifaceted, the key steps in the development and progression of NAFLD are the development of IR,[3,4] a high fat diet and obesity.[9] Obesity contributes to the pathophysiology by causing adipocyte hypertrophy and hypoxia, leading to macrophage influx and pro-inflammatory state.[16] Obesity, however, is not the sole, or indeed key contributory factor to the development and progression of NAFLD as it can also be seen in lean individuals.[13] IR which develops as part of the inflammatory state causes hepatic steatosis, further dysregulation in adipose tissue leading to increased FFAs[17] and sensitization of the liver to metabolic attacks.[18,19] Hepatic lipotoxicity is caused by increased long-chain fatty acids, ceramides and diacylglycerol stored within the liver, causing the release of reactive oxygen species by the liver and contributing to inflammation and consequently hepatic fibrosis and hepatocyte apoptosis. Furthermore, increasing hepatic steatosis renders the liver resistant to insulin, exacerbating the situation.[17] There is, however, a bidirectional relationship between hepatic steatosis and IR, with each fuelling each other.[17] These processes ultimately lead to a pro-inflammatory state and hepatocyte injury.[9]

As a result of their shared aetiology, NAFLD is intrinsically linked with metabolic syndrome and type 2 diabetes (T2D).[1] In fact, individuals with T2D have a five times greater risk of NAFLD and progression to NASH than people without T2D.[20] Yet, hepatic steatosis is partly an adaptive and protective response in which lipotoxic FFAs are stored as more stable components.[21] However, with on-going hepatic insult, and contributing factors, such as T2D and genetic predisposition, this protective response is overwhelmed, leading to hepatocyte damage and fibrosis.[4] Hepatic IR is caused by pro-inflammatory cytokines (tumour necrosis factor [TNF]α, interleukin [IL]-6), endoplasmic reticulum stress, pro-inflammatory pathways, such as the JUN and NF.kB pathway, and lipid metabolites, which further exacerbate IR. Additionally, there is a complex relationship between IR, glucagon and hepatic sensitivity to glucagon. Glucagon exerts several direct hepatic effects including decreasing hepatic fat, as demonstrated by studies investigating glucagon antagonists for T2D.[22] However, people with cirrhosis demonstrate a fasting hyperglucagonaemia possibly due to failure of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) to suppress glucagon. This is associated with deranged liver enzymes and increased hepatic FFA flux due to lipolysis in adipose tissue, complicating the picture.[23] Interestingly, GLP-1 analogues reduce glucagon secretion and hepatic fat accumulation by blocking the endoplasmic reticulum stress response.[24] Indeed, the GLP-1 analogue liraglutide reduced postprandial triglyceride and apolipoprotein B48 elevations in people with T2D after a fat-rich meal.[25] The effect of GLP-1 analogue use is discussed further below, and the pathophysiology of NAFLD is summarized in Figure 2.

4 | TREATMENT CHALLENGES IN NAFLD

As the global burden of NAFLD is considerable, identifying a point in the disease spectrum at which it becomes financially viable and efficacious to treat is crucial. Similar thought was followed in the management of hepatitis C as the market flooded with several treatments, all of which had varying success based on different viral genotypes, but all were expensive. This needed to be evaluated in terms of which treatments would provide the greatest benefit balanced against the cost of the drug. Benefit could be in terms of, for example, positive societal outcomes or health expenditure. Health technology assessments also examined the best time to treat an individual with hepatitis C. Given the burden NAFLD places on the economy, healthcare system and the patient, further discussion to establish whether treating individuals with hepatic steatosis or NASH is cost-effective or appropriate would be welcome.

Although there are several therapeutic targets which can be exploited in the management of NAFLD, current UK guidelines only recommend pioglitazone or vitamin E in those with advanced fibrosis.[26] Thiazolidinediones, such as pioglitazone, increase adiponectin release from ectopic fat, which reduces the release of pro-inflammatory cytokines. Both rosiglitazone and pioglitazone were studied, but concern surrounding the cardiovascular side effects associated with their use limited progress. Nevertheless, pioglitazone improves the histological features observed in NAFLD.[27] However, there remains a paucity of evidence to support the above guidance and additional pharmacological options, or indeed surgical interventions, in the management of NAFLD are needed.

Therapies which reduce body weight and/or IR affect NALFD incidence or progression. One meta-analysis observed that metformin use in people with T2D and NAFLD improved liver enzymes, body mass index (BMI) and measures of IR but not histological measures, such as hepatic steatosis, inflammation or fibrosis.[28] A review assessing statins in NAFLD reported that most studies demonstrated improved lipid profiles and liver enzymes, although changes in fibrosis measures were inconsistent.[29] Interestingly, two recent meta-analyses and systematic reviews demonstrated that bariatric surgery was effective in improving hepatic steatosis in the majority of patients, and hepatic fibrosis in almost a third of participants.[30,31] In experimental studies, other treatments, for example, n-3 polyunsaturated fatty acids,[32] L-carnitine,[33] limonoids[34] and polyphenols,[35] were shown to reduce hepatic inflammation and liver enzymes.

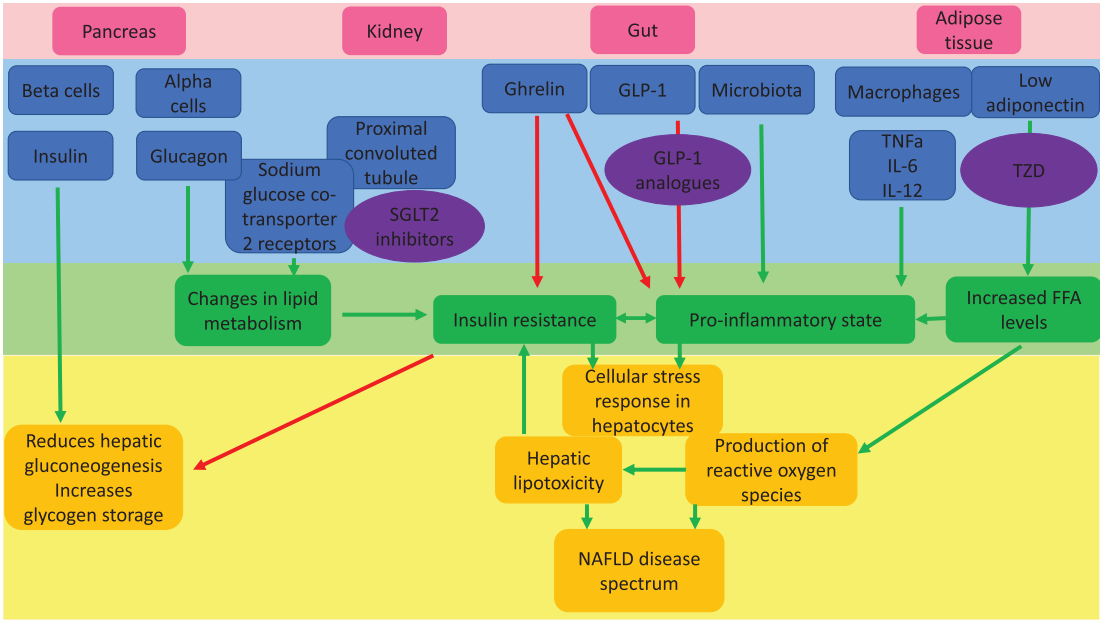

FIGURE 2 The pathophysiology of non-alcoholic fatty liver disease (NAFLD). The organs and tissues involved are listed, with the hormones and inflammatory mediators released listed below. These affect lipid and insulin metabolism and contribute to an inflammatory state, leading to hepatic cellular stress and the development of NAFLD. The green arrows represent a positive effect whereas the red arrows represent an inhibitory effect. Therapeutic targets are included in purple. GLP-1, glucagon-like peptide-1; IL-6, interleukin-6; IL-12, interleukin-12; SGLT2, sodium-glucose co-transporter-2; TZDs, thiazolidinediones; TNFα, tumour necrosis factor-α

For most patients with NAFLD, however, the mainstay of treatment is lifestyle modification, which is infrequently successful. This highlights the need to identify effective alternatives for NAFLD alongside lifestyle modification by targeting novel pathways.

5 | GLUCAGON-LIKE PEPTIDE-1 ANALOGUES

Glucagon-like peptide-1 analogues have developed considerably since the isolation of the exendin-4 peptide from the *Heloderma suspectum* (Gila Monster) lizard in 1992.[36] The peptide GLP-1 is normally secreted within minutes in response to oral glucose following cleavage of the pre-proglucagon gene. The first action of GLP-1 to be characterized was the "incretin effect", whereby an oral glucose load leads to greater pancreatic insulin secretion than an intravenous glucose infusion at the same plasma glucose level. This is because GLP-1 augments insulin secretion and modulates glucagon secretion, being secreted in response to oral rather than intravenous glucose.[37] Importantly, people with T2D demonstrate a reduced incretin effect, with reduced insulin secretion in response to the incretins GLP-1 and GIP.[38] Over the last 20 years therapy for T2D has progressed swiftly, with the development of several injectable and oral GLP-1 analogues. Key side effects associated with GLP-1 analogue use include nausea, vomiting, diarrhoea, pancreatitis and local injection site reactions.

Whilst GLP-1 receptors are most prominently expressed in the pancreas and central nervous system, they have also been observed in the lungs, kidneys, heart, peripheral nervous system, gastrointestinal tract, adipose tissue and liver. This implies that GLP-1 analogues may have other important metabolic effects beyond glycaemic modulation. Various trials have demonstrated significant improvements in important metabolic outcomes associated with GLP-1 analogue use, including reduced mortality, reduced major adverse cardiovascular events,[39] reduced progression of renal disease,[40] improved weight loss[41] and improved lipid profiles.[42] Consequently, GLP-1 analogues broadly improve metabolic health, particularly in people with T2D and an impaired incretin hormone response.

6 | NAFLD AND GLP-1 ANALOGUES

As discussed above, drugs used to treat NAFLD generally aim to reduce hepatic inflammation and oxidative stress to reduce progressive fibrosis and cirrhotic liver disease. GLP-1 analogues have the potential to improve hepatic outcomes in people with T2D, by improving risk factors for NAFLD and direct hepatic mechanisms to reduce inflammation and oxidative stress.

7 | NON-DIRECT HEPATIC EFFECTS OF GLP-1

7.1 | Glycaemic and lipid control

The first licensed use of GLP-1 analogues was for exenatide in 2005 in people with poorly controlled T2D, with five GLP-1 analogues (exenatide,

liraglutide, lixisenatide, dulaglutide and semaglutide) currently licensed for T2D. These drugs are effective and associated with glycaemic control (glycated haemoglobin [HbA1c] improvements of ~9–18 mmol/mol (0.8%-1.6%).[37] This is important, as changes in HbA1c are recognized as an independent risk factor for the development and reversal of NAFLD in people with T2D.[19] Additionally, several trials investigating GLP-1 analogues have observed improvements in markers of dyslipidaemia typically associated with diabetes, such as lower LDL cholesterol and triglyceride levels.[43] Indeed, improved lipid profiles improve mortality and cardiovascular outcomes, and decelerate the progression of NASH.[44] This may be a result of GLP-1 analogues improving adipose and hepatic tissue insulin sensitivity. Indeed, "cross-talk" between adipose tissue and the liver may be responsible for the reduced IR and improved glycaemic control associated with GLP-1 analogues by enhancing insulin's antilipolytic role and reducing plasma FFAs.[45]

7.2 | Body weight

Glucagon-like peptide-1 analogues are a licensed therapy for obesity, with liraglutide (Saxenda) approved for use in 2014 for people with a BMI ≥30 kg/m^2 or ≥27 kg/m^2 and an obesity-related comorbidity. GLP-1 analogues act peripherally to reduce gastric emptying and centrally to stimulate hypothalamic neurones, which suppress appetite. Liraglutide use in obesity is associated with an additional mean weight loss of 5.9 kg over 56 weeks.[46] Whilst not yet approved for weight loss, other GLP-1 analogues in clinical use for T2D have shown promising weight loss effects.[41] This may have significant clinical impact, as almost 40% of people who attain a weight loss ≥1% total body weight per year achieve NAFLD remission.[47]

8 | DIRECT EFFECTS OF GLP-1 ON THE LIVER

Improved metabolic risk factor control would improve hepatic IR, halting or even reversing NASH. However, studies demonstrating GLP-1 receptors within the liver raise the possibility of direct hepatic action. The latter is supported by studies observing reduced hepatic glucose production and enhanced glucose uptake associated with intravenous infusion of GLP-1 analogues.[45]

As detailed above, progression of fatty to fibrotic liver disease is mediated by pro-inflammatory cytokines and inflammation, resulting in hepatic and adipose IR exacerbating hepatic inflammation. Increased IR impairs suppression of lipolysis, resulting in increased FFA levels and hepatocyte damage. Interestingly, administration of GLP-1 analogues to people with NASH improves hepatic and adipose insulin sensitivity, enhances insulin-mediated suppression of lipolysis and lessens hepatic *de novo* lipogenesis.[43] Nevertheless, the confounding stimulation of insulin secretion by GLP-1 analogues to overcome hepatic IR indirectly should not be overlooked, and further studies evaluating this area are important.

As discussed, increased serum adiponectin level is the mechanism associated with thiazolidinedione use in NASH, as higher adiponectin correlates with improved insulin sensitivity and histological recovery in hepatic steatosis.[48] Similarly, liraglutide use in people with NASH increased serum adiponectin and decreased serum leptin levels, reducing the leptin:adiponectin ratio.[43] This has important implications as adiponectin regulates hepatic fatty acid oxidation, reducing *de novo* lipogenesis.[49] Moreover, elevated GLP-1 levels in vagotomized mice directly prevented hepatic VLDL overproduction and improved IR measures.[50] This occurs because GLP-1 analogues such as exendin-4 increase peroxisome proliferator-activated receptor-α (PPARα) expression, which is typically reduced in people with NAFLD, stimulating FFA oxidation.[51] Moreover, GLP-1 analogues reduce fatty acid-related hepatocyte apoptosis by reducing endoplasmic reticulum-mediated cell death and hepatic FFA accumulation.[52]

Glucagon-like peptide-1 analogues also appear to improve hepatic gluconeogenesis and IR. In dogs, exenatide increased hepatic glucose turnover by ~30% because of increased whole-body glucose disposal and hepatic glucose uptake in hyperglycaemic or euglycaemic conditions.[53] This is the mechanism highlighted by pre-clinical studies to explain improvements in glycaemic control associated with GLP-1 analogue use.[54] Therefore, GLP-1 analogues may break the inflammatory cycle associated with fatty liver disease to limit the development of advanced fibrotic disease, thereby supporting the role of the liver in moderating glycaemic control.[49]

9 | POTENTIAL ROLE OF MULTI-AGONISTS IN NAFLD

Multi-agonists of GLP-1, GIP and/or glucagon receptor have been developed and investigated for treatment of diabetes and obesity. Co-agonism of the glucagon receptor with incretin hormone agonists may seem counterintuitive due to their opposing glycaemic effects, especially because a function of GLP-1 is to suppress pancreatic glucagon release.[55] However, stimulation of the glucagon receptor has several non-glycaemic effects such as enhancing energy expenditure, hepatic lipid oxidation and improving weight loss amongst other factors.[56] Indeed, reduced glucagon receptor signalling increases hepatic fat accumulation,[57] and use of glucagon antagonists in people with T2D increases hepatic fat.[22] One trial observed that people with T2D and NAFLD had higher fasting glucagon levels than controls without NAFLD.[58] Some hypothesize that reduced hepatic glucagon receptor signalling in NAFLD results in a pancreatic feedback mechanism, resulting in augmented compensatory glucagon secretion.[59]

The hepatic benefits associated with dual GLP-1/glucagon receptor agonism appear to have direct and indirect mechanisms.[60] Hepatic glucagon receptor signalling reduces hepatic fat and improves mitochondrial turnover, whilst enhanced GLP-1 signalling reduces food intake and body weight and improves glycaemic control.[61] Co-agonist treatment in mice significantly improves obesity, diabetes and measures of hepatic inflammation and fibrosis.[62] Thus, the potentially harmful changes in glycaemic control associated with glucagon receptor agonism are curtailed by enhanced GLP-1 signalling, whilst supporting further hepatic benefits through the mechanisms discussed previously.

10 | EVIDENCE FOR GLP-1 ANALOGUES AND MULTI-AGONISTS IN LIVER DISEASE

10.1 | Exenatide

One trial explored the effect of exenatide on ectopic fat stores, observing significant reductions in epicardial and liver fat content over 26 weeks.[63] Other studies have demonstrated additional improvements in glycaemic control, body weight, triglycerides and hepatic enzymes in people treated with exenatide and insulin.[64,65] Indeed, exenatide use supported histological resolution of NASH in one pilot study.[66] However, most of these trials had only short-term follow-up and lacked a placebo-controlled group.[63,64]

10.2 | Liraglutide

The LEAN-J pilot study[67] and LEAN trial[68] evaluated the effect of liraglutide in the treatment of biopsy-proven NASH. Liraglutide use was associated with improved weight loss, visceral fat content, hepatic enzymes and hepatic inflammation on histology. Whilst encouraging, it would be useful to know the longer-term impact on NASH outcomes, as the trial extended to 48 weeks. Moreover, participant numbers were small, with only 71 participants combined. However, a strength of the LEAN study was that it defined NASH resolution histologically and used two independent pathologists, features lacking in most trials to date. The CGH-LiNASH trial is an ongoing study to compare changes in body weight, hepatic fat content and liver enzymes in participants with NASH receiving liraglutide 0.6 to 3.0 mg or bariatric surgery.[69]

10.3 | Lixisenatide

We are unaware of completed or ongoing prospective trials investigating lixisenatide in people with NAFLD. A previous systematic review of randomized controlled trials using lixisenatide for people with T2D concluded that its use improved the likelihood of ALT normalization in obese patients over 29 weeks, but did not significantly affect serum AST, alkaline phosphatase or bilirubin.[70] However, interpretation of this review should be cautious as it included trials which did not aim to determine the impact of lixisenatide in NAFLD. Included trials recruited people with T2D (with or without NAFLD) and excluded those with severe liver disease.

10.4 | Dulaglutide

We are not aware of published trial evidence investigating dulaglutide in NASH. The D-LIFT trial aimed to investigate the effect of dulaglutide 0.75 to 1.5 mg once weekly for 24 weeks on hepatic fat measurements in people with T2D using magnetic resonance imaging-derived proton density fat fraction. The study enrolled 60 participants and concluded in February 2020.[71] We look forward to the publication of the results from this trial.

10.5 | Semaglutide

The SEMA-NASH trial recently completed in March 2020 investigated semaglutide 0.1 to 0.4 mg daily in people with NASH over 72 weeks.[72] Preliminary results indicate good outcomes, with NASH resolution in most participants receiving the highest dose of semaglutide.[73] This study is a dedicated fatty liver disease trial evaluating semaglutide in 320 participants with NASH with or without T2D. The trial results may change the way we view NASH as a separate disease entity to T2D, although with overlapping pathophysiological mechanisms. Of course, should this trial demonstrate superiority, there would be a case for major treatment changes in in this cohort. Completed trials investigating GLP-1 analogues in NASH are shown in Table 1.

10.6 | Multi-agonists

Evidence supporting the use of dual/triple agonists in liver disease is largely preclinical. One early study found that use of a dual GLP-1/glucagon receptor agonist in mice resulted in significant weight loss, improved lipid metabolism and hepatic steatosis.[74] Subsequent studies have demonstrated that dual agonists are associated with improved hepatic histopathological changes in rodents.[75,76] Interestingly, a recent study investigated the effect of individual or combination administration of GLP-1, GIP and glucagon receptor agonists in mice. The study observed that both dual GLP-1/glucagon receptor agonists and triple GLP-1/GIP/glucagon receptor agonists resulted in greater histological improvements in NAFLD disease activity compared with liraglutide. Moreover, GIP or glucagon receptor analogues alone did not influence liver lipids or histology.[77]

Whilst dual GLP-1/GIP receptor agonists have shown promise in several features of metabolic syndrome,[78] few focused studies have investigated their role in people with liver disease. In a *post hoc* analysis of a phase II trial for diabetes, people with T2D administered the dual GLP-1/GIP receptor agonist tirzepatide showed improvements in biomarkers of NASH, including liver enzymes and adiponectin.[79] However, an unknown proportion of participants had NASH, and hepatic fat was not measured, limiting the analysis. Similar results have been observed with the use of triple agonists in mice, with improved body weight and steatohepatitis.[80] Whilst preclinical evidence is encouraging, focused human trials exploring multi-agonists would be welcomed.

10.7 | Dipeptidyl peptidase-4 inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitor use in T2D is common, with several drugs in this class used in clinical practice, such as saxagliptin,

TABLE 1 Summary of completed trials using glucagon-like peptide-1 analogues for the treatment of fatty liver disease

Drug (trial name)	Participants	Control	Trial duration	Primary outcome	Results	Changes in liver enzymes
Exenatide 5 −10 mcg[64]	n = 60 NAFLD + obesity	Intensive insulin therapy	12 weeks	Compare changes in glycaemic control, lipids and liver enzymes	HbA1c: −1 mmol/mol (0.1%)[a] FPG: −0.21 mmol/L[a] Triglycerides: −0.06 mmol/L[a]	AST: −13.9 U/L[a] ALT: −30.3 U/L[a] γGGT: −9.8 U/L[a]
Exenatide 5 −10 mcg twice daily[66]	n = 8 NASH + T2D	No control group	28 weeks	Number of participants with improved histology Change in NAFLD activity score	3/8 (37.5%) improved histology Mean change in NAFLD activity score − 1.5[b]	AST: Not reported ALT: −24 U/L[b] γGGT: Not reported
Liraglutide 0.9 mg/d (LEAN-J)[67]	n = 19 NASH	No control group	24 weeks	Number of participants with improved histology Changes in visceral fat and liver enzymes	6/10 (60%) showed improved histology Visceral fat area: −2.8 cm^{2b}	AST −10.8 U/L[b] ALT −7.3 U/L[b] γGGT −6.2 U/L[b]
Liraglutide 1.8 mg/d (LEAN)[68]	n = 52 NASH + obesity	Placebo	48 weeks	Resolution of NASH without worsening fibrosis	Resolved NASH in 9/23 (39%) vs. 2/22 (9%) in placebo	AST −6.7 U/L[a] ALT −10.7 U/L[a] γGGT −22.8 U/L[a]
Dulaglutide 0.75−1.5 mg weekly (D-LIFT)[71]	n = 60 NASH + T2D	Standard treatment for T2D	24 weeks	Change in liver fat, liver enzymes and measures of hepatic fibrosis	Results awaited	Results awaited
Semaglutide 0.1-0.4 mg weekly (SEMA-NASH)[72,73]	n = 320 NASH	Placebo	72 weeks	Resolution of NASH without worsening fibrosis vs. placebo	NASH resolved in 33/56 (59%) receiving 0.4 mg daily vs. 10/58 (17%) in placebo[c]	Results awaited

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; FPG, fasting plasma glucose; γGGT, gamma-glutamyl transpeptidase; HbA1c, glycated haemoglobin; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; T2D, type 2 diabetes.

[a]Results presented after adjustment for control or placebo group.

[b]Results presented as absolute values as no control group in study.

[c]Preliminary results only, full results unavailable at time of writing.

sitagliptin, linagliptin and vildagliptin. Side effects include gastrointestinal disturbance, nasopharyngitis and skin lesions.[81] The DPP-4 enzyme degrades GLP-1 and GIP and therefore DPP-4 inhibitor use prolongs the action of endogenous incretins. Given the results associated with GLP-1 analogues in this cohort, DPP-4 inhibitors may improve NAFLD outcomes also. However, DPP-4 inhibitor use in T2D is neutral with respect to body weight changes and cardiovascular protection.[81] Nevertheless, DPP-4 inhibitor use in animal models with fatty liver disease has demonstrated positive results, with reduced proinflammatory measures such as TNFα, IL-6, adipose tissue inflammation and hepatic steatosis.[82] However, results of DPP-4 inhibition in people with NAFLD have provided mixed results.

Saxagliptin improved measures of IR, inflammatory markers and liver enzymes in people with NAFLD and T2D.[83] Similarly, addition of saxagliptin and dapagliflozin to metformin in people with T2D significantly reduced liver and adipose fat content, and improved liver enzymes.[84] Vildagliptin was found to improve liver enzymes and reduced fasting hepatic triglycerides by 27% in people with T2D over 6 months.[85] This was supported by a subsequent trial which observed improved fatty liver grading on ultrasonography, improved liver enzymes and lipids, and reduced body weight associated with vildagliptin use in people with NAFLD.[86] Whilst linagliptin has shown promise in rodent models of NAFLD,[87] we are not aware of any completed human trials evaluating its use in NAFLD. Results from trials investigating the effect of sitaglipitin on NAFLD are mixed. One trial found significantly greater reductions in hepatic steatosis and NAFLD activity score associated with sitagliptin in people with NASH over 1 year.[88] Similarly, sitagliptin use resulted in greater hepatic and total body fat content reductions than glimepiride in people with T2D despite similar improvements in glycaemic control.[89] However, other studies observe sitagliptin was not associated with improved fibrosis score, NAFLD activity score, liver enzymes, serum adiponectin or lipid profiles over 24 weeks in people with biopsy-proven NASH.[90] Similarly, sitagliptin did not improve liver fat content, liver enzymes or lipids in people with NAFLD and T2D or prediabetes over 24 weeks.[91]

Despite these hopeful results, randomized controlled trials with longer follow-up and larger participant numbers are lacking. However, DPP-4 inhibitors appear to be safe in NAFLD, although the clinical benefit of these medications in the treatment of NAFLD is debated.

10.8 | Sodium-glucose co-transporter-2 inhibitors

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are licensed for the treatment of T2D. The commonest side effects associated with their use are an increased frequency of genital and urinary tract infections and, rarely, diabetic ketoacidosis. These drugs inhibit SGLT2 in the proximal convoluted tubule, causing a glucose-mediated osmotic diuresis and natriuresis to improve glycaemia, weight loss and blood pressure.[92] Thus, these drugs improve all-cause mortality and cardiovascular outcomes and reduce progression of chronic renal disease in people with diabetes.[40,92] There is therefore potential to improve outcomes in people with NAFLD. Moreover, as these drugs are already used for people with T2D, introducing them for new indications such as NAFLD is a relatively quick and inexpensive process compared with producing new therapies, if there is benefit associated with their use. In contrast to the GLP-1 receptor, SGLT2 expression is limited to the proximal convoluted tubule and glucagon-secreting pancreatic α cells.[93] This implies the mechanisms by which SGLT2 inhibitors affect liver outcomes are indirect and different from GLP-1 analogues. This has been observed in the context of cardiovascular outcomes, with an anti-inflammatory role of GLP-1 analogues and improved haemodynamic effects associated with SGLT2 inhibitor use, beyond the impact of these drugs on glycaemic control.

Firstly, use of SGLT2 inhibitors is associated with weight loss ~3 kg, with a preferential reduction in visceral fat rather than water loss after 4 weeks of treatment.[41] Secondly, these drugs are also associated with HbA1c improvements of ~5 to 11 mmol/mol (0.5%-1.0%), improving hepatic outcomes.[19] Thirdly, SGLT2 inhibitor use is associated with a shift in lipid metabolism to reduce serum triglycerides and increase LDL levels.[94] The shift in lipid metabolism is probably a result of a reversal of the high insulin:glucagon ratio frequently seen in people with T2D. This occurs as a result of SGLT2 inhibitor-mediated glycaemic improvements causing a relative drop in serum insulin, and blockade of SGLT2 in the pancreatic α cell, resulting in increased glucagon secretion.[93,95] The reduced insulin:glucagon ratio promotes a shift from hepatic carbohydrate to fatty acid metabolism to reduce liver triglyceride content and β-oxidation of hepatic FFAs, with subsequent ketone formation potentially causing euglycaemic ketoacidosis.[95] Certainly, glucagon has an important role in NAFLD development, and the hormonal shift in insulin and glucagon induced by SGLT2 inhibitors may mediate NAFLD remission.[96] Reduced glucagon receptor expression or use of glucagon receptor antagonists increases hepatic fat levels.[22,57]

A recent systematic review of trials in people with T2D and NAFLD treated with SGLT2 inhibitors observed consistent improvements in NAFLD outcomes. These included improved liver enzymes, liver fat content, measures of liver fibrosis and risk factors including BMI, HbA1c and lipids.[97]

11 | EVIDENCE FOR SGLT2 INHIBITORS IN LIVER DISEASE

11.1 | Dapagliflozin

One non-randomized trial found that dapagliflozin use was associated with reduced BMI, body fat mass, reduced liver enzymes and increased serum adiponectin.[98] However, the study was limited by a lack of data regarding hepatic fat changes during the study. The DEAN study is an ongoing phase III trial aiming to recruit 100 participants with NASH and T2D to investigate the impact of dapagliflozin 10 mg daily on histological liver changes over 12 months. The study is expected to be completed in November 2021.[99]

TABLE 2 Summarizes completed trials using sodium-glucose co-transporter-2 inhibitors for the treatment of fatty liver disease

Drug (trial name)	Participants	Control	Trial duration	Primary outcome	Results	Changes in liver enzymes
Dapagliflozin 5 mg[98]	n = 11 NASH + T2D	No control group	24 weeks	Changes in BMI, body fat mass and liver enzymes	BMI −3.7 kg/m^{2}[a] Body fat mass −6.1 kg[a]	AST: −26 U/L[a] ALT: −29 U/L[a] γGGT: −31 U/L[a]
Empagliflozin 10 mg (E-LIFT)[100]	n = 50 NAFLD + T2D	Standard treatment for T2D	20 weeks	Change in LFC	Mean liver fat reduction −4.9% vs. 0.9% in controls	AST: −7.7 U/L[b] ALT: −10.9 U/L[b] γGGT: −11.0 U/L[b]
Empagliflozin 25 mg (EmLiFa)[101]	n = 84 T2D	Placebo	24 weeks	Change in LFC	Absolute change of −1.8%[b] Relative change of −22%[b]	AST: Not reported ALT: −7% from baseline[b] γGGT: −7% from baseline[b]
Canagliflozin 100 mg[105]	n = 5 NASH + T2D	No control group	24 weeks	Histopathological changes	Improved histopathological features in all participants	AST: −6 U/L[a] ALT: −13.6 U/L[a] γGGT: −13 U/L[a]
Canagliflozin 100 mg[106]	n = 10 NASH + T2D	No control group	12 weeks	Changes in serum ALT	-	AST −17.6 U/L[a] ALT −23.9 U/L[a] γGGT −16.0 U/L[a]
Canagliflozin 100 mg[107]	n = 35 NAFLD	No control group	26 weeks	Changes in liver enzymes and lipid profile	LDL cholesterol −6.0 mg/dL[a] HDL cholesterol +1.5 mg/dL[a] Triglycerides −44.4 mg/dL[a]	AST −16.9 U/L[a] ALT −33.8 U/L[a] γGGT −24.4 U/L[a]
Canagliflozin 100 mg[109]	n = 20 NAFLD + T2D	No control group	52 weeks	Change in LFC	Mean LFC reduction −5.5%[a]	AST −9 U/L[a] ALT −21 U/L[a] γGGT −40 U/L[a]
Ipragliflozin 50 mg[110]	n = 43 NASH or NAFLD + T2D	No control group	24 weeks	Change in body weight and liver enzymes	**NASH** Body weight −1.4 kg[a] **NAFLD** Body weight −1.4 kg[a]	**NASH** AST −34.5 U/L[a]; ALT −32.0 U/L[a]; γGGT −33.0 U/L[a] **NAFLD** AST −10.5 U/L[a]; ALT −18.5 U/L[a]; γGGT −14.5 U/L[a]
Ipragliflozin 50 mg[111]	n = 66 NAFLD + T2D	Pioglitazone	24 weeks	Changes in liver-to-spleen attenuation ratio	Liver-to-spleen ratio +0.01[b]	AST −1.0 U/L[b] ALT −2.5 U/L[b] γGGT +5.1 U/L[b]
Ipragliflozin 50 mg[112]	n = 44 NAFLD + T2D	Metformin + pioglitazone	24 weeks	Changes in LFC, visceral fat, NAFLD liver fat score	Fatty liver index −10.85[b] Visceral fat area −33.2 cm^{2}[b] NAFLD liver fat score −0.55[b]	AST +3.4 U/L[b] ALT −3.1 U/L[b] γGGT −9.3 U/L[b]
Luseogliflozin 2.5 mg[113]	n = 32 NAFLD + T2D	Metformin	26 weeks	Changes in the liver-to-spleen ratio and visceral fat area	Liver-to-spleen ratio +0.26[b] Visceral fat area −31.4 cm^{2}[b]	AST: Not reported ALT −18.5 U/L[b] γGGT: Not reported
Luseogliflozin 2.5 mg[114]	N = 40 NAFLD + T2D	No control group	24 weeks	Change in HbA1c and LFC	HbA1c: −3 mmol/mol (0.29%)[a] LFC: −5.8%[a]	AST −8.8 U/L[a] ALT −12.3 U/L[a] γGGT −14.2 U/L[a]

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; γGGT, gamma-glutamyl transpeptidase; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LFC, liver fat content; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2D, type 2 diabetes.

[a]Results presented as absolute values as no control group in study.

[b]Results presented after adjustment for control or placebo group.

11.2 | Empagliflozin

The E-LIFT trial observed greater improvements in liver fat reduction compared to control and improvements in several liver enzymes with empagliflozin use.[100] The recently published EmLiFa trial supported these results with improvements in liver fat and increased likelihood of NAFLD resolution associated with empagliflozin use in people with recently diagnosed and well-controlled T2D.[101] This was a relatively well-designed study and was placebo-controlled, with uric acid and adiponectin levels collected to explain the findings. However, it lacked follow-up (24 weeks) and included only 84 participants. An analysis of data pooled from five trials investigating empagliflozin use reported improved liver enzymes with empagliflozin use, with greater reductions in those with higher baseline ALT levels.[102]

There are ongoing studies investigating empagliflozin. One study aims to recruit 60 participants with NASH and T2D to determine liver fat changes associated with empagliflozin alone and in combination with pioglitazone over 6 months.[103] Another trial aims to determine whether empagliflozin improves hepatic fat measurements in 12 to 17-year-old obese participants with NAFLD over 26 weeks.[104] We anticipate the results of these trials with great interest.

11.3 | Canagliflozin

One small study investigating canagliflozin in participants with NAFLD observed improved histopathological signs of NAFLD in all cases after 24 weeks.[105] Further studies have since observed improvements in liver enzymes, liver fat content and triglycerides associated with canagliflozin.[106–108] Each of these studies is limited by small participant numbers (n = 5-35) and a lack of control group and therefore, whilst indicative of a benefit in NAFLD, larger controlled trials with longer follow-up are needed to support canagliflozin use in NAFLD. We are not aware of ongoing trials using canagliflozin in NAFLD at the time of writing.

11.4 | Other SGLT2 inhibitors

Two Japanese studies investigating ipragliflozin in NAFLD participants with T2D observed improvements in HbA1c, body weight, liver enzymes, visceral fat and hepatic steatosis.[110,111] A recent Korean study observed that ipragliflozin use was associated with improved hepatic and visceral fat content and liver enzymes.[112] These trials are presented in Table 2.

In one randomized-controlled trial, participants randomized to luseogliflozin had greater improvements in liver:spleen ratio and visceral fat area than participants receiving metformin.[113] A subsequent trial found measures of hepatic fat and enzymes improved with luseogliflozin, although markers of fibrosis were unchanged.[114]

An ongoing trial aims to compare the effect of tofogliflozin 20 mg daily and pioglitazone 15 to 30 mg daily, alone and in combination, on changes in hepatic fat content over a 48-week trial.[115] Completed trials investigating SGLT2 inhibitors in NAFLD are presented in Table 2.

12 | CONCLUSIONS

There is an important population burden of NAFLD, which is likely to increase with the rising prevalence of T2D. Whilst most people with NAFLD may be asymptomatic, a substantial proportion of these people develop progressive liver disease, resulting in cirrhosis or hepatocellular carcinoma. This is associated with significant personal and economic cost. Nevertheless, there is a lack of current evidence-supported treatment available and no licensed therapies for NAFLD exist. However, guidance supports use of pioglitazone in NAFLD, which corroborates the potential for other diabetes therapies to treat such patients.

Trials to date would support the use of GLP-1 analogues or SGLT2 inhibitors in people with NAFLD and T2D. With ongoing research, further licensing and guidance may be extended to include these drugs specifically for the treatment of NAFLD. Novel drugs classes, including GLP-1/GIP/glucagon receptor dual or triple agonists, are being investigated and show great promise. Variable reported outcome measures in clinical trials challenge comparison between drugs and drug classes.

Nevertheless, trials evaluating the impact of SGLT2 inhibitors and GLP-1 analogues in NAFLD lack data surrounding major adverse hepatic events such as development of liver cirrhosis or hepatocellular carcinoma. This contrasts with cardiovascular and renal outcome studies which report on major events affecting these systems. This is likely a consequence of the relatively limited follow-up and would be of major interest given the burden that these stages of liver disease represent. Larger studies with greater patient numbers and duration with more consistently reported outcomes including hepatic fat changes would be welcome.

CONFLICT OF INTEREST

R.K.V. and D.M.W. have no conflicts of interest to declare. M.E. received financial support for consultancy from Novartis, Merck Sharp & Dohme Corp. and Novo Nordisk and has served on the speaker's bureau for Novartis, Lilly, Boehringer Ingelheim, Merck Sharp & Dohme Corp., Novo Nordisk, Janssen and Takeda.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study

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