Review article: the impact of liver-directed therapies on the atherogenic risk profile in non-alcoholic steatohepatitis

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Funding information
National Institutes of Health, Grant/Award Number: R01 DK 105961

Summary

Background: Patients with non-alcoholic fatty liver disease (NAFLD), the most common cause of chronic liver disease, are at higher risk of cardiovascular disease (CVD) and associated mortality. Therefore, it is important to understand how new therapies for non-alcoholic steatohepatitis (NASH) may impact CVD risk factors in these patients.

Aims: To summarise the effects of drug therapies on lipid and lipoprotein levels in patients with NASH and provide insight into the potential mechanisms for the observed changes.

Methods: PubMed searches of the literature were performed and results were compiled.

Results: Recent clinical trials have highlighted the safety and efficacy of drug candidates for the treatment of NASH. Several agents have shown improvements in the histological features of NASH and liver function. Pioglitazone, a drug that is currently available for type 2 diabetes and may be useful for NASH, exhibits beneficial effects on lipids. However, agents such as farnesoid X receptor agonists, which are in development for NASH, may adversely affect circulating lipids and lipoproteins.

Conclusions: NASH is a multi-system disease with a disproportionate CVD burden. Current and future drugs for NASH have had variable impact on the atherogenic risk profile. Potential co-administration of a statin may help mitigate the negative impact of some of these therapies on lipid and lipoprotein levels.
1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) encompasses a histopathologic spectrum ranging from hepatic steatosis alone (fat content >5%), to steatosis accompanied by liver inflammation with or without fibrosis (nonalcoholic steatohepatitis or NASH), to cirrhosis of the liver. NAFLD is a common cause of liver disease in developed countries, largely due to the increased prevalence of comorbidities such as obesity and type 2 diabetes (T2D). Similar to obesity and T2D, patients with NAFLD have a higher risk of atherosclerotic cardiovascular disease (ASCVD, CVD), and CVD is the leading cause of death among patients with NAFLD. While weight loss and exercise regimens have demonstrated short-term success in treatment of hepatic steatosis and NASH, these are only achievable in <10% of patients. Therefore, drug therapies for NASH have been proposed. Due to the higher risk of ASCVD in these patients, however, it is important to understand how new therapeutics, currently used or in development, may favourably or unfavourably impact CVD risk factors in NASH patients.

Recent published literature have demonstrated that many pharmaceutical agents including diuretics, anti-diabetic agents, hormone therapies, steroids, anti-psychotics, and anti-inflammatory agents, elicit direct effects on lipoprotein metabolism thereby affecting circulating lipid and lipoprotein levels. Many of these medications have favourable effects, however, there are a number of drug classes that adversely impact circulating lipids and lipoproteins—for example, some raise low-density lipoprotein cholesterol (LDL-C) levels, which are considered to be a risk marker and causative agent for ASCVD. There has been concern that these agents may thereby, augment ASCVD progression. While several publications have chronicled the many agents that are currently in development for NASH, this review focuses on the effects of leading anti-NASH drug therapies on lipids and lipoproteins in NASH patients and provides mechanistic insight on lipoprotein metabolism. Moreover evidence will be presented that these drug-induced alterations may not necessarily lead to increased CVD risk in NASH patients, if current best practices in management of CVD risk are followed.

2 | DYSLIPIDAEMIA IN PATIENTS WITH NAFLD AND/OR TYPE 2 DIABETES

2.1 | What is atherogenic dyslipidaemia?

It is well known that a lipid profile with low high density lipoprotein cholesterol (HDL-C) (<40 mg/dL) and high triglycerides (TG) (>200 mg/dL) is associated with increased risk of T2D, CVD and CVD-related events. These particular changes are referred to as diabetic or atherogenic dyslipidaemia and are most notable in patients with T2D, insulin resistance and/or NAFLD. LDL particles contribute to CVD risk, and in clinical practice monitoring LDL-C in the general population is sufficient for assessing a patient’s CVD risk. However, atherogenic dyslipidaemia is often associated with LDL-C levels within the normal or near normal range making it hard to assess a patient’s CVD risk using LDL-C alone (Figure 1). This occurs because the majority of the LDL particles in patients with atherogenic dyslipidaemia are small and dense and carry more TG and less cholesteryl esters and free cholesterol than large LDL particles (Figure 1). Small-dense LDL are highly atherogenic due to several factors including their propensity to traverse the endothelium and accumulate in atherosclerotic lesions as well as their longer plasma half-lives accounting for accumulation of oxidation products.

Therefore, an increase in small-dense LDL particles is indicative of metabolic dysregulation of lipoprotein metabolism and can contribute to the higher CVD risk observed in patients with T2D, hepatic steatosis, insulin resistance and/or NASH. Consequently, having LDL-C levels within the normal range, or somewhat raised, on a background of atherogenic dyslipidaemia, obesity, NAFLD and/or T2D, where the LDL particles are smaller, more numerous and highly atherogenic, is undesirable. Hence, measurement of total LDL and small-dense LDL particle concentrations along with a standard lipid panel [total cholesterol (TC), TG, LDL-C and HDL-C] may provide a clearer picture of CVD risk in patients with metabolic disease.

2.2 | Mechanism behind atherogenic dyslipidaemia

Insulin resistance is central to atherogenic dyslipidaemia and leads to an increase in fatty acid flux from adipose tissue to the liver and upregulation of hepatic synthesis of TG, free cholesterol and cholesteryl esters, which are secreted from the liver in large VLDL particles (Figure 1). These large TG-enriched VLDL are metabolised slowly in the periphery due to a reduction in lipoprotein lipase activity and contribute to the formation of TG-enriched LDL, which in turn leads to generation of small-dense LDL by hepatic lipase. In contrast, metabolism of cholesteryl ester-enriched small VLDL results in the formation of large-buoyant LDL, both being associated with a less-atherogenic lipoprotein profile. Additionally, TG in VLDL particles can exchange with cholesteryl ester in LDL and HDL via the activity of cholesteryl ester transfer protein (CETP), culminating in altered metabolism and further generation of the atherogenic lipoprotein profile (Figure 1). Overall, the changes in lipoprotein class and subclass levels (lipoprotein profile) that occur with insulin resistance and metabolic diseases such as NASH and T2D are: (a) increased particle concentrations for large VLDL and small LDL, (b) increased mean VLDL size, (c) decreased large HDL particles and (d) decrease in mean LDL and HDL size (Figure 1).

2.3 | Reversal of dyslipidaemia

Some therapies for T2D, and potentially for NASH, lead to improvements in insulin sensitivity and to a reversal in the atherogenic
lipoprotein phenotype. Because atherogenic dyslipidaemia results in an increase in small-dense LDL and a decrease in larger more buoyant LDL particles that carry more cholesteryl ester and free cholesterol, the reversal of this lipoprotein phenotype may lead to increased LDL-C, but not necessarily to an increase in the total number of LDL particles. The American Association of Clinical Endocrinologists is aware of this phenomenon and has recommended the evaluation of drug treatments on circulating lipoprotein and lipid levels.44 This evaluation would include a standard lipid panel along with apoB or a more in-depth analysis using a comprehensive lipoprotein profile (total VLDL, LDL, and HDL particle concentrations and mean sizes as well as concentrations of the small, medium and large sub-fractions).45 Understanding whether alterations in the lipoprotein particle profile are due to direct (mechanism-of-action of the drug) or indirect (consequence of improving insulin resistance and reversing atherogenic dyslipidaemia) drug actions is key to understanding the drug’s potential to elicit an increase or decrease in CVD risk with long-term treatment.

3 | LIPID AND LIPOPROTEIN EFFECTS OF POTENTIAL NASH THERAPIES

3.1 | Diet and exercise

Because NAFLD is considered the hepatic manifestation of metabolic disease, the same risk factors for obesity and T2D, such as sedentary lifestyle, excess dietary fat and carbohydrates, including excess fructose and glucose consumption, hold true for NAFLD as well.46 At present, there are no drug therapies that have been approved for the treatment of NAFLD or NASH, therefore lifestyle recommendations remain the standard of care.47–49 Improvements in nutrition with or without weight loss can have a positive impact on fatty liver.49 For example, a short-term isocaloric diet that was low in carbohydrates and high in protein in subjects with NAFLD led to a dramatic reduction in liver fat content, decreased hepatic de novo lipogenesis, and increased fatty acid oxidation reflected by increased circulating β-hydroxybutyrate concentrations.50 In another

![Comparison of aspects of normal lipoprotein metabolism and atherogenic dyslipidaemia. HDL, high-density lipoprotein particles; HDL-C, high-density lipoprotein cholesterol; IDL, intermediate density lipoprotein particles; LDL, low-density lipoprotein particles; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; VLDL, very low density lipoprotein.](image)
study, a low carbohydrate high protein diet resulted in improvements in HbA1c and liver fat content even though the subjects did not lose weight. Hypocaloric diets, especially those that elicit weight loss, have shown beneficial effects on hepatic and metabolic end points in subjects with NAFLD/NASH. Hypocaloric very low carbohydrate diets in particular tend to increase ketone bodies (ketogenic diets) and decrease liver fat content, insulin resistance and features of metabolic syndrome. In a recent study of 262 subjects who were placed on a diet that restricted carbohydrates and allowed for the consumption of protein and fat to satiety, subjects had reduced liver fat content and exhibited improvements in markers of CVD risk. While they experienced an increase in LDL-C, the increase appeared to be limited to the large LDL sub-fraction. LDL size increased, accompanied by a decrease in small LDL, while total LDL particle concentration and apoB remained the same. Additionally, HDL-C and apoA-I levels increased, while blood pressure, TG and inflammation decreased. These lipoprotein changes have been noted previously and are thought to be due to the reduction in carbohydrate and enhancement in fat utilisation leading to an increase in fatty acid oxidation. Taken together, these studies suggest that ketogenic diets may be good for reducing the accumulation of liver fat potentially leading to a reduction in progression to NASH or liver cirrhosis. Caution needs to be taken, however, because human observational data has associated hepatic steatosis with high fat intake (as employed in some ketogenic diets), and rodent studies of high-fat diets often show increased liver fat, insulin resistance and oxidative stress.

Combining weight loss with an exercise regimen is the most beneficial in terms of improving the histological features of NASH. A systematic review and a recent meta-analysis of a number of interventions suggested that weight loss of 5% in NAFDL or 7%-10% in NASH was beneficial and could be achieved by a combination of a hypocaloric diet and 30-60 minutes of moderate intensity exercise for 3-5 days per week. Combined diet and exercise interventions tended to show improvements in serum levels of liver enzymes as well as liver histology. Dietary modifications such as reduction in carbohydrate intake as well as increased physical activity may lead to improvements in liver fat content, however, lifestyle changes including both diet and exercise are necessary to show improvement in hepatic inflammation and fibrosis. Most physicians realize, however, that achieving these results in real-life settings can be challenging.

With or without weight loss, the majority of studies have shown that lifestyle changes can be accompanied by alterations in CVD risk factors, such as circulating lipid levels and insulin resistance. Low fat diets tend to reduce TC and LDL-C levels while very low carbohydrate diets tend to increase LDL-C, which can be a result of a potential increase in the percentage of fat in the diet and/or due to the reversal of insulin resistance. It appears that regardless of the diet (low fat or low carbohydrate) or exercise regimen (high vs moderate intensity), reduced caloric intake accompanied by increased physical activity is likely to lead to weight loss as well as improvements in hepatic fat content, insulin resistance and serum lipid levels, all of which are thought to be anti-atherogenic.

### 3.2 Statins

The safety of statins in patients with NAFLD/NASH has achieved increasing consensus. Initially regulatory agencies such as the US Food and Drug Administration (FDA) recommended monitoring hepatic transaminases, which are commonly elevated in NAFLD/NASH, and stopping statins for elevations ≥3 times the upper limit of normal (ULN). The 2006 National Lipid Association (NLA) Statin Safety Task Force Report suggested that baseline liver function tests should be obtained prior to starting statin therapy, but periodic monitoring was not recommended unless clinically indicated. In 2012, the FDA decided to change statin labelling to reflect the 2006 NLA statement. The 2014 NLA Statin Safety Task Force Report commended this decision and encouraged the use of statins in NAFLD/NASH patients. Furthermore, a recent study reported that statin therapy was safely tolerated in patients with decompensated cirrhosis, thereby, adding further support for its safety in patients with chronic and advanced liver disease.

Over the years, studies have shown that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels may normalise on statin treatment, but randomised trials have not specifically addressed whether statins lead to improved biochemical and histological outcomes. Since patients with NAFLD/NASH are at higher risk of ASCVD, most experts assume that statins should improve ASCVD outcomes. Tikkanen et al conducted a post-hoc subgroup analysis of a large CV outcomes trial (IDEAL) and found that treatment with atorvastatin 80 mg or simvastatin 20-40 mg daily in patients with baseline normal vs moderately high ALT (>1 and <3 × ULN) was safe and resulted in similar lipoprotein changes irrespective of ALT elevation. Moreover patients with high ALT had significantly better prevention of major coronary events. The GREACE study aimed at demonstrating ASCVD risk reduction among 1600 participants randomized to receive statin therapy in a university clinic vs usual care elsewhere. A post-hoc subgroup analysis among 437 patients with moderately abnormal liver function tests, mostly attributable to NAFLD, showed that 227 receiving statins had favourable lipoprotein changes as well as 35% mean reduction in ALT and 47% reduction in AST. Interestingly, 89% of participants in the statin group with abnormal liver tests at baseline had ALT and AST concentrations within the normal range by the end of the study. No patient had more than minor increases in bilirubin or alkaline phosphatase. More importantly, patients treated with a statin had a significantly reduced risk of ASCVD events. Similar outcomes in ultrasound features, ALT and AST changes, and lipoprotein changes were seen in other post-hoc analyses (IDEAL and ATTEMPT).

Besides studies reporting changes in liver enzymes with statin treatment, others have looked at potential changes in liver fat
content and liver histology. For example, Athyros et. al. assessed changes in liver fat content by ultrasound in patients with metabolic syndrome, LDL-C >130 mg/dL, hepatic steatosis and elevated AST and/or ALT. The authors showed that intensive lifestyle modifications, in addition to treatment of hypertension, impaired fasting glucose, obesity and dyslipidaemia, led to reduced liver fat when assessed via ultrasound. In fact, 67% of the participants taking atorvastatin no longer had evidence of fatty liver. A recent

| Name of medication or drug candidate | Drug class/mechanism | Significant changes in lipids and lipoproteins | Potential mitigation | Shown to have CV benefit or no harm | References |
|--------------------------------------|----------------------|-----------------------------------------------|---------------------|------------------------------------|------------|
| Vitamin E                            | Nutritional Supp.    | No significant changes in lipids or lipoproteins observed. | —                   | —                                  | 72, 74-76  |
| Pioglitazone                         | PPARγ agonist        | ↓TG, ↑HDL-C, ↑LDL-C, ↑large LDL-P, ↓small LDL-P | Statin treatment    | Yes                                | 72, 83-89  |
| Elafibranor                          | PPARα/δ agonist      | Potential changes have not been reported to date. | —                   | —                                  | 94         |
| Seladelpar (MBX-8025)                | PPARδ agonist        | ↓TG, ↑HDL-C, ↓LDL-C, ↓small LDL-P | Statin treatment    | —                                  | 95, 96     |
| Saroglitazar                         | PPARα/γ agonist      | ↓TG, ↓TC, ↑HDL-C, ↓nonHDL-C                   | —                   | —                                  | 97, 98     |
| Liraglutide, exenatide               | GLP-1 agonists       | When given alone, no significant changes observed. | —                   | Yes                                | 99-106     |
| Canagliflozin                        | SGLT2 inhibitor      | ↓TG, ↑HDL-C, ↑LDL-C                           | Statin treatment    | Yes                                | 107, 112, 117-119 |
| Dapagliflozin                        | SGLT2 inhibitor      | ↓TG, ↑HDL-C, ↑LDL-P, ↓large LDL-P, ↑large HDL-P | Statin treatment    | Yes                                | 109, 110, 113, 116 |
| Empagliflozin                        | SGLT2 inhibitor      | ↔TG, ↑HDL-C, ↑LDL-C                           | Statin treatment    | Yes                                | 108, 111, 115, 120-122 |
| Obeticholic acid (OCA)               | FXR agonist          | ↑TC, ↑LDL-C, ↓HDL-C, ↓large VLDL-P, ↑small VLDL-P, ↑large and small LDL-P, ↓total HDL-P | Statin treatment    | —                                  | 128-137    |
| NGM282                               | FGF19 analogue       | ↑TC, ↑LDL-C, ↑HDL-C, ↓large LDL-P, ↑total, large and medium HDL-P | Statin treatment    | —                                  | 138-141    |
| Pegbelfermin                         | FGF21 analogue       | ↓TG, ↑HDL-C                                  | —                   | —                                  | 142, 143   |
| GS-0976                              | ACC1/2 inhibitor     | ↑TG, ↑VLDL-P                                 | Fish oil            | —                                  | 144-146    |
| MK-4702                              | ACC1/2 inhibitor     | ↑TG, ↑VLDL-P                                 | —                   | —                                  | 147        |
| Cenicriviroc                         | CCR2/5 antagonist    | Has not yet been studied or reported.         | —                   | —                                  | 149, 150   |
| Selonsertib (GS-4997)                | ASK1 inhibitor       | Has not yet been studied or reported.         | —                   | —                                  | 151, 152   |
| Emricasan (IDN-6556)                 | Caspase inhibitor    | Has not yet been studied or reported.         | —                   | —                                  | 153-156    |
| Resmetirom (MGL-3196)                | THRβ agonist         | ↓TG, trend ↓HDL-C                             | —                   | —                                  | 157-159    |
| Aramchol                             | SCD1 inhibitor       | Has not yet been studied or reported.         | —                   | —                                  | 160        |
| MSDC-0602K                           | MPC activator        | Has not yet been studied or reported.         | —                   | —                                  | 161-163    |

Abbreviations: ACC1/2, acetyl-CoA carboxylase 1 and 2; ASK1, apoptosis signal-regulating kinase 1; CCR2/5, chemokine receptor type 2/5; CV, cardiovascular; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide 1; HDL-C, high-density lipoprotein cholesterol; LDL-P, high-density lipoprotein particles; LDL-C, low density lipoprotein cholesterol; LDL-P, low density lipoprotein particles; MPC, mitochondrial pyruvate carrier; NASH, non-alcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SCD1, stearoyl-CoA desaturase-1; SGLT2, sodium glucose co-transporter 2; Supp., supplement; T2D, type 2 diabetes; TC, total cholesterol; TG, triglycerides; THRβ, thyroid hormone receptor β; VLDL-P, very large density lipoprotein particles.
Cochrane review demonstrated no significant effects of statins on ALT, AST, or liver fat by ultrasonography and showed mixed outcomes with regard to histological improvement in NASH parameters on liver biopsy. Therefore, the therapeutic benefits of statins in NAFLD and NASH patients, besides the known reductions in LDL-C, remain elusive. However, despite the cardiovascular benefit of statin therapy, in patients with NAFLD or suspected NASH, statin therapy is often stopped.

In summary, evidence suggests that statins are safe and effective for LDL lowering in patients with NAFLD/NASH, at least when baseline transaminase elevations are less than 3 times ULN. They should be considered in patients meeting criteria per the ASCVD risk calculation. However, improvements in overall NAFLD/NASH parameters and hepatic steatosis by ultrasound have been inconsistent. Further, we lack evidence to suggest improvements in outcomes such as biopsy-proven histological and fibrotic scores with statin treatment.

### 3.3 Vitamin E

Prior to 2000, the recognition of vitamin E's antioxidant role and new evidence for lipoprotein oxidation as a proatherogenic mechanism generated enthusiasm for vitamin E as a natural antiatherogenic strategy. This led to large cardiovascular outcome trials, which demonstrated neither beneficial nor harmful effects of vitamin E in doses averaging 300-400 IU per day on cardiovascular events. Likewise, in the Pioglitazone vs Vitamin E vs Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, only treatment with vitamin E (800 IU daily) met the primary therapeutic endpoint. Based on these results, guidelines suggest the use of vitamin E in nondiabetic subjects with biopsy-proven NASH.

Besides PIVENS, a number of clinical studies have explored the use of vitamin E in adult or paediatric subjects with NAFLD, NASH and/or T2D. Many of these studies reported reductions in ALT and/or improvements in hepatic steatosis or NASH (alone or in combination therapy) which have been confirmed in a meta-analysis. To date, however, no significant changes in lipids or lipoprotein levels have been noted with vitamin E treatment (Table 1).

The story with vitamin E treatment in patients with NASH and/or T2D, however, is not finished. Recently it was shown that the haptoglobin 2 allele is associated with a histologic response to vitamin E in subjects with NASH, suggesting that genotyping of the haptoglobin allele may be a way of identifying patients who are more likely to respond to vitamin E treatment. Moreover, the haptoglobin 2 allele was shown to be associated with a reduction in CVD in subjects with T2D treated with vitamin E. This was shown in the Heart Outcome Prevention Evaluation (HOPE) study, the Women’s Health Study and the Israel Cardiovascular Vitamin E (ICARE) study. The authors suggest that the protection afforded by the haptoglobin 2 allele may be related to oxidative stress and improvements in HDL functionality. Additional large prospective studies are needed to confirm these observations, however, it is intriguing to think that there may be a way to identify NASH and/or T2D patients who would realize both hepatic and CVD benefits with vitamin E treatment.

### 3.4 Peroxisome proliferator-activated receptor agonists

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptors that are expressed in liver, adipose tissue, heart, skeletal muscle and kidney and are responsible for the transcriptional regulation of processes such as fatty acid oxidation, lipid transport and gluconeogenesis. There are three PPARs (α, β/δ and γ) that bind the same target DNA sequence but differ in tissue distribution. A few PPAR agonists are on the market for treatment of T2D (pioglitazone) and hypertriglyceridaemia (gemfibrozil, fenofibrate). PPARα agonists or fibrates reduce TG but have not been shown to provide beneficial effects on liver histological endpoints in patients with NAFLD. Several PPAR single or dual receptor agonists have been evaluated or are currently being tested in clinical trials in NAFLD/NASH patients.

Pioglitazone is a PPARγ agonist that has been shown to be efficacious in dyslipidaemia and T2D. In the PIVENS trial, while it did not meet the primary liver endpoint, pioglitazone treatment did lead to a significant reduction in ALT, AST, hepatic steatosis, and lobular inflammation in NASH patients. A meta-analysis of four clinical studies confirmed these results. In a longer term study in patients with biopsy-proven NASH and either prediabetes or T2D, pioglitazone treatment, compared to placebo, led to improvements in steatosis, inflammation and ballooning with 58% of the patients achieving the primary outcome after 18 months. Moreover there was a reduction in the mean fibrosis score but not the number of patients with improvement in fibrosis stage ≥1 in the pioglitazone treated group compared to the placebo treated group.

Analysis of lipoprotein sub-fraction concentrations in the PIVENS trial revealed that NASH resolution was associated with favourable changes in a subset of lipoproteins, suggesting a reduction in the atherogenic profile, which may translate to a reduction in CVD risk (Table 1). These positive effects of pioglitazone on the lipoprotein profile had been previously noted. While small LDL particles were reduced, pioglitazone treatment elicited an increase in large LDL particles and a small but significant increase in LDL-C levels (as the label for pioglitazone reflects). These lipid changes may be related to the drug’s ability to reduce insulin resistance and reverse atherogenic dyslipidaemia (Figure 1).

Regardless of the mechanism for the lipoprotein changes observed with pioglitazone treatment, a prospective 3-year study, called the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive), revealed that pioglitazone reduced the composite of all-cause mortality, non-fatal myocardial infarction and stroke in T2D patients at high CVD risk. Although there was no statistically significant difference between pioglitazone and placebo treatment in the incidence of a first event in the composite endpoint, meaning that pioglitazone did not have a mortality or CVD
risk benefit, there was no increase in mortality or in total CVD events.\textsuperscript{88} A 10-year follow up confirmed that there were no increases in all-cause mortality or CVD events between pioglitazone and placebo treatment.\textsuperscript{89} It should be noted, however, that fluid retention and weight gain are common side effects of pioglitazone which may limit its use. In addition, pioglitazone is not widely recommended for routine T2D treatment because of concerns about reduced bone density and questionable risk of bladder cancer. Nevertheless, the overall results illustrate that a small increase in LDL-C accompanied by favourable effects on TG and HDL-C may not lead to an increase in CVD events, at least in patients with T2D. This remains to be investigated in patients with NASH. Of course, due to the pleiotropic effects of therapeutic agents, each drug that raises LDL-C, or has what appears to be a non-beneficial effect on CVD risk factors, needs to be studied mechanically so that a full picture of the relative benefit-risk profile can be understood and monitored over time.

The PPAR\textgamma{} agonist rosiglitazone that was approved for the treatment of T2D has been shown to increase LDL-C, non-HDL-C and triglyceride levels.\textsuperscript{86} As a result of a meta-analysis of forty-two clinical studies showing untoward effects of rosiglitazone on CVD outcomes and plausible concerns over a potential increase in ASCVD, rosiglitazone was removed from the market.\textsuperscript{90} After multiple studies revealed that there was no clear increase in CVD events with rosiglitazone treatment in T2D patients, the FDA allowed it to be marketed with a black box warning on the label.\textsuperscript{91-93} This warning includes language stating that while the meta-analysis showed rosiglitazone was associated with an increased risk of myocardial ischemic events, three large, long-term, prospective, randomised, controlled clinical trials have not confirmed or excluded this risk. It is because of lingering concerns over the relationship between LDL-C, oedema and the potential for increased CVD events, however, that rosiglitazone is not recommended in guidelines for use in patients with T2D, nor has it been tested in NASH trials.

Elafibranor is a dual PPAR\alpha/\gamma{} agonist that showed efficacy in treatment of NASH in the Phase 2b GOLDEN505 trial in patients with NASH without cirrhosis.\textsuperscript{94} Although it did not meet the primary endpoint, in the post-hoc analysis elafibranor resolved NASH in a larger proportion of patients with NAFLD activity score \textgreater{}4, than placebo. In addition, elafibranor treatment led to improvements in insulin sensitivity, glucose homeostasis, and lipid metabolism and reduced liver enzymes and systemic inflammation.\textsuperscript{94} However, results from the Phase 3 RESOLVE-IT trial (NCT02704403) were recently revealed and the data showed that elafibranor treatment did not meet its primary endpoint of NASH resolution without worsening of fibrosis. The response rate was 19.2\% for patients who received elafibranor 120 mg compared to 14.7\% for patients in the placebo arm. In addition, the other key secondary endpoint related to metabolic parameters did not achieve statistical significance. To date, no comprehensive lipoprotein profile results have been published with elafibranor (Table 1).

In an early study in patients with obesity and atherogenic dyslipidaemia, seladelpar (MBX-8025), a selective PPAR\textgamma{} agonist, was tested with and without atorvastatin.\textsuperscript{95} Results revealed that treatment with seladelpar led to a reduction in apoB, LDL-C, TG, non-HDL-C and high sensitivity C-reactive protein (hsCRP), and an increase in HDL-C.\textsuperscript{95} After seladelpar treatment, there was a lower percentage of study participants with a preponderance of small LDL particles. These results were extended by analysing concentrations of lipoprotein.\textsuperscript{96} In short, seladelpar treatment led to a decrease in the atherogenic lipoprotein phenotype and seladelpar combined with atorvastatin led to a further reduction across all apoB-containing lipoproteins (Table 1). These effects may have been due to the reduced waist circumference and improved insulin sensitivity that were observed in these subjects. Unfortunately, the Phase 2b proof-of-concept study in patients with biopsy-proven NASH (NCT03551522) was placed on hold after 52 weeks of treatment due to unexpected histological changes suggestive of immune mediated injury.

Lipaglyn (saroglitazar) is a PPAR\textalpha/\gamma{} agonist used in India for the treatment of dyslipidaemia in T2D patients. The results of EVIDENCES IV, a Phase 2 trial in 106 patients with NAFLD and/or NASH, revealed that saroglitazar treatment led to an improvement in ALT, liver fat content, insulin resistance and dyslipidaemia.\textsuperscript{97} Previously it was shown that saroglitazar treatment reduced TG, TC, non-HDL-C, LDL-C and HbA1c as well as increased HDL-C in patients with NAFLD and diabetic dyslipidaemia.\textsuperscript{98} However, overall safety of saroglitazar has yet to be studied in a large randomised controlled trial. IVA337 is a pan PPAR agonist that is in a Phase 2 trial in patients with NAFLD and/or NASH (NCT03008070). It will be interesting to see if or how the lipoprotein profiles differ in NAFLD/NASH patients treated with these varied PPAR agonists.

### 3.5 Glucagon-like peptide 1 agonists

Glucagon-like peptide 1 (GLP-1) is an incretin hormone that is secreted from the gut in response to food intake. GLP-1 peptidomimetic receptor agonists, or GLP-1 peptides that have been modified to increase half-life, have been shown to improve glucose control in T2D patients via several mechanisms including enhanced insulin secretion, delayed gastric emptying, preserved \( \beta \)-cell function, and inhibition of glucagon secretion.\textsuperscript{99} Clinically, treatment with GLP-1 agonists results in weight loss and improvement systolic blood pressure and HbA1c. Given the improvements in multiple CVD risk factors, it is not surprising that GLP-1 receptor agonists have shown beneficial effects, or at least no harm, in CVD outcomes trials to date.\textsuperscript{100-102} For example, results of CVD outcome trials in T2D patients with increased CVD risk demonstrated a significant reduction in time to first major adverse cardiovascular event (MACE) with liraglutide (LEADER), semaglutide (SUSTAIN-6) and dulaglutide (REWIND), while a CVD outcome trial examining lixisenatide (ELIXA) demonstrated no difference in CVD events.\textsuperscript{100-102} While GLP-1 receptor agonists have been shown to elicit weight loss, few studies have reported a significant improvement in insulin sensitivity.\textsuperscript{103} In addition, when given alone (eg without a concomitant diet regimen) GLP-1 receptor agonists did not lead to the types of changes in lipids and lipoprotein parameters that would suggest an improvement or
reversal of atherogenic dyslipidaemia (Table 1). Therefore, the mechanism for the CVD benefits with GLP-1 receptor agonism do not seem related to modifications in lipids, but may be related to amelioration of other CVD risk factors such as reducing blood pressure and weight.

In LEAN, a Phase 2 study in patients with biopsy proven NASH, a larger percentage of subjects treated with liraglutide exhibited resolution of NASH compared to placebo. Larger clinical studies testing liraglutide (NCT02654665), semaglutide (NCT03884075, NCT03987451, NCT02970942, NCT03987074) or dulaglutide (NCT03648554, NCT03590626), in patients with NAFLD or NASH are currently in progress. These studies should reveal whether the same beneficial effects observed on blood pressure and CVD outcomes in T2D patients are recapitulated in larger studies in NASH patients.

### 3.6 Sodium glucose co-transporter 2 inhibitors

Several sodium glucose co-transporter 2 (SGLT2) inhibitors have been approved for treatment of T2D (canagliflozin, dapagliflozin, empagliflozin, ertugliflozin). SGLT2 inhibitors lower plasma glucose by inhibiting renal reabsorption of glucose thereby increasing urinary glucose excretion. SGLT2 inhibitors, such as canagliflozin, have been shown to enhance insulin sensitivity, probably due to a reduction in body weight, and also to reduce HbA1c, systolic and diastolic blood pressure, and liver enzymes. They also cause a decrease in circulating TG and small but statistically significant 2%-5% increases in LDL-C and HDL-C. Dapagliflozin was also shown to decrease small-dense LDL-C and increase large-buoyant LDL and HDL2 cholesterol, suggesting that the lipoprotein changes observed with SGLT2 inhibition may be related to decreased insulin resistance and a reversal of atherogenic dyslipidaemia (Table 1). Furthermore, the pattern of lipoprotein changes observed in clinical trials with various SGLT2 inhibitors is reminiscent of metabolic switching from glucose utilisation as a fuel source (glucose oxidation) to use of fat as a fuel source (fatty acid oxidation), which occurs largely in liver and muscle tissue (Figure 2). This is reflected by the increases in ketone bodies that have been observed in T2D subjects treated with SGLT2 inhibitors. These changes are thought to be due to a reduction in carbohydrate and enhancement in fat utilisation which then leads...
to an increase in fatty acid oxidation, similar to the effects of a ketogenic diet. In the case of SGLT2 inhibition, the increased urinary glucose excretion forces a switch in energy utilisation from glucose to fatty acids. The liver responds by secreting more VLDL-TG, which are catabolized by muscle to produce energy (lipoprotein lipase-mediated lipolysis and fatty acid oxidation), creating cholesteryl ester-enriched LDL and HDL particles (increased LDL-C and HDL-C). In addition, the reduced body weight is consistent with the loss of calories due to increased urinary glucose excretion and fatty acid oxidation in adipose tissue. Therefore, one possible interpretation of the clinical data with SGLT2 inhibition is that the overall metabolic changes observed (favourable changes in blood pressure, HDL-C, TG and weight, but unfavourable changes in LDL-C) are partly the result of a switch in fuel source from glucose to fatty acids and a reversal of the atherogenic dyslipidaemia (Figure 2).

Despite the increases in LDL-C that have been observed with SGLT2 inhibitors and are reflected in their product labels, this class of therapeutic agents has shown signs of providing benefits with respect to CVD outcomes (Table 1). The results of the EMPA-REG OUTCOME trial revealed that in patients with T2D and high CVD risk empagliflozin treatment led to a significantly lower rate of death from cardiovascular causes compared to placebo as well as a reduction in hospitalisation for heart failure and all-cause mortality.111 The CANVAS trials revealed that canagliflozin treatment in patients with T2D and high CVD risk led to a lower risk of CVD events.112 In addition, the DECLARE-TIMI trial reported that dapagliflozin treatment in a similar population of patients led to a lower rate of CVD death or hospitalisation for heart failure.113 Subsequently, multiple meta-analyses, post-registration investigations and independent clinical studies have corroborated these findings.100,114 While the exact mechanisms for the improvements in CVD outcomes have not yet been fully delineated, it appears that the pleiotropic effects of SGLT2 inhibition may work in concert to elicit both CVD and renal protection (Figure 2). It is therefore clear that one must examine the spectrum of changes in CVD risk factors (positive or negative) before hypothesising whether long-term treatment with a particular drug class might result in benefit or harm with respect to CVD outcomes (Figure 2).

SGLT2 inhibitors have shown benefits for reduction of hepatic steatosis and liver enzymes and show promise for treatment of NASH.116 Treatment with canagliflozin for 24 weeks in a small number of patients with NASH and T2D resulted in improvements in the histological features of NASH. In addition, canagliflozin treatment led to a reduction in hepatic steatosis and an improvement in insulin sensitivity and insulin secretion in patients with T2D and NAFLD.118-119 Analysis of multiple trials with empagliflozin showed improvements in liver enzymes that might be a reflection of weight loss and reduced liver fat.120,121 Additionally, empagliflozin elicited improvements in steatosis, ballooning and fibrosis in a small pilot study of subjects with NASH.122 Dapagliflozin, luseogliflozin and ipragliflozin treatment showed similar benefits in patients with T2D or NAFLD/NASH.123 It will be essential, however, to see if these effects are recapitulated by SGLT2 inhibition in large Phase 3 trials in patients with NASH, especially since these patients would benefit from the improvements in renal function and CVD outcomes.

3.7 Farnesoid X receptor agonists and fibroblast growth factor 19 peptidomimetics

Farnesoid X receptor (FXR) is an intracellular nuclear receptor that binds bile acids and elicits transcriptional signalling leading to the expression of a number of target genes relevant for bile acid, cholesterol, lipid and carbohydrate metabolism as well as inflammation, fibrosis and carcinogenesis. Specifically, FXR agonists decrease bile acid synthesis, bile acid uptake, lipogenesis, gluconeogenesis and liver inflammation, and increase hepatic bile acid export. Many of these effects are mediated by FGF19, a peptide hormone synthesised by gut epithelial cells and hepatocytes in response to FXR signalling. FXR activation reduces VLDL levels and circulating TG via repression of hepatic sterol responsive element binding protein 1c (SREBP1c), microsomal triglyceride transfer protein, and apoB gene expression. Moreover FXR amplifies lipoprotein lipase activity by increasing apoCII (activator of lipoprotein lipase) and reducing apoCIII expression (inhibitor of lipoprotein lipase), thereby promoting peripheral VLDL-TG lipolysis. Based on these effects, FXR agonism would appear to be an attractive therapeutic target for the treatment of liver disease. However, since FXR inhibits the synthesis of bile acids from cholesterol, thereby increasing an active pool of hepatic cholesterol, FXR agonism may lead to reduced LDL receptor cell surface expression. A reduction in LDL receptors, coupled with increased lipoprotein lipase activity, may result in reduced LDL clearance and elevated concentrations of circulating LDL particles and LDL-C (Figure 3). Additionally, FXR agonism has been shown to increase HDL-C clearance by increasing scavenger receptor-B1 (SR-B1) and to decrease HDL production by reducing apoA1. FXR agonists in development for treatment of NASH have exhibited both the positive and negative aspects of FXR agonism, and clinical studies have been undertaken to understand the effects on disease activity as well as CVD risk.

Obeticholic acid (OCA) is a potent FXR agonist that was recently shown to improve disease-related factors in patients with NASH. In a small proof-of-concept study, OCA treatment for 6 weeks led to an increase in serum FGF19 and insulin sensitivity, and a decrease in bile acids, the bile acid precursor C4, ALT and γ-glutamyl transferase (GGT). In the Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) trial, OCA elicited improvements in the histological features of NASH. In both studies, OCA vs placebo treatment led to 16%-24% increases in LDL-C. To gain a better understanding of OCA’s effects, nuclear magnetic resonance analysis of lipoprotein particle concentrations was obtained before and after OCA treatment. OCA treatment elicited increases in large-buoyant and small-dense LDL particles. The increases trended higher at 12 weeks than 72 weeks, which may be attributed to increased use of statins in patients whose LDL-C levels were increased by OCA, and tended to be much higher for large than small LDL.
OCA treatment elicited a reduction in large VLDL and an increase in small VLDL without any change in total VLDL concentration. Thus, the net effect of FXR agonism is a shift to what some lipidologists believe is a less atherogenic lipoprotein profile. OCA vs placebo treatment also led to 2.6%-4.5% reductions in HDL-C. Total HDL particles were similarly reduced including both large and medium HDL particle concentrations, most likely due to increased clearance. This is likely due to an increase in scavenger receptor B1 (SR-B1) which would lead to an increase in the reverse cholesterol transport pathway and would be anti-atherogenic. However, kinetic studies are warranted to fully understand the mechanism. All of the lipoprotein changes reverted to baseline 12 weeks after OCA discontinuation.

A study in healthy volunteers also revealed an increase in LDL-C, confirming OCA’s direct effects on LDL-C, presumably via increased intrahepatic cholesterol content and decreased LDL receptor cell surface expression (Figure 3). In this study, HDL particles decreased largely due to a reduction in medium and small HDL, and total LDL particles increased along with an increase in large LDL and LDL-C. In order to evaluate whether OCA has long-term detrimental effects on CVD, it would be important to determine if concurrent statin treatment can reduce LDL-C in OCA-treated subjects. To this end, the recently conducted CONTROL Phase 2 study investigated the effects of atorvastatin treatment on OCA-induced changes in LDL-C in patients with NASH. Results revealed that OCA increased mean LDL-C, mostly attributable to an increase in large LDL. More importantly, the study showed that the lowest available dose of atorvastatin was able to reverse the OCA-mediated increase in LDL-C to below baseline levels. However, atorvastatin did not reverse the OCA-mediated decrease in HDL-C. More importantly, the study showed that the lowest available dose of atorvastatin was able to reverse the OCA-mediated increase in LDL-C to below baseline levels. However, atorvastatin did not reverse the OCA-mediated decrease in HDL-C. More importantly, the study showed that the lowest available dose of atorvastatin was able to reverse the OCA-mediated increase in LDL-C to below baseline levels. However, atorvastatin did not reverse the OCA-mediated decrease in HDL-C. While not meeting the endpoint of NASH resolution, the 18-month interim analysis of the Phase 3 REGENERATE trial (NCT02548351) revealed that OCA treatment met the primary endpoint of reduction in liver fibrosis (23% OCA vs 12% placebo) with no worsening of NASH. More will be understood about the effects of OCA on hepatic function and long-term liver-related outcomes, regardless of alterations in lipid and lipoprotein parameters, when the final results of the REGENERATE trial are reported. Furthermore, additional large clinical studies need to be conducted to monitor the safety of
OCA treatment with regard to long-term CVD outcomes. While the strategies employed in clinical trials of co-administration of statin therapy in patients with NASH lead to improvement in lipid profile, the "true-world" clinical practice remains anchored in multi-society guidelines that recommend monitoring lipid profiles and adjust statin therapy accordingly. Besides the indirect and direct effects on LDL-C, subjects treated with OCA also experienced pruritus which led to discontinuation during each of the NASH trials and may affect long term use.

Several selective, non-bile acid FXR agonists are currently in development. While their mechanism of action through FXR and fibroblast growth factor 19 (FGF19) may still raise LDL-C, they may not elicit other adverse effects of OCA such as pruritus. Recent results from the Phase 2 ATLAS trial with the FXR agonist cilogefoxor (GS-09674) revealed that neither monotherapy nor combined therapy reached the pre-specified endpoint of significant improvement in liver fibrosis without worsening of NASH, leaving questions regarding the potential efficacy of FXR agonists that are not bile acids. The field will watch carefully the results of ongoing Phase 2 NASH studies with the other FXR agonists, such as EDP-305, LNJ452 and LMB763, and lipid and lipoprotein levels are being closely monitored in these trials.

Aldafermin (NGM282) is an engineered analogue of the gut hormone FGF19 that potently inhibits de novo bile acid synthesis and reduces liver fat content, ALT and AST, while eliminating the FGF19 associated tumorigenicity.138,139 More importantly, NGM282 treatment has been shown to improve the histological features of NASH in only 12 weeks with significant reductions in the NAFLD activity score of two or more points without worsening in liver fibrosis.140 All components of NAFLD activity score (steatosis, hepatocellular ballooning and lobular inflammation) improved significantly from baseline with NGM282 treatment.140 A significant portion of the NASH patients treated with NGM282 (42%) exhibited a decrease in the fibrosis score from baseline of at least one stage in only 12 weeks. In addition, NGM282 treatment led to the reduction in the liver fibrosis marker N-terminal type III collagen propeptide (Pro-C3) and the Enhanced Liver Fibrosis (ELF) score.140 Along with the impressive efficacy, NGM282 suppresses hepatic bile acid synthesis from cholesterol which leads to an increase in serum TC and LDL-C (Figure 3).139 A comprehensive lipoprotein class/subclass analysis of a recent Phase 2 trial revealed that NGM282 treatment resulted in significant elevation in total LDL particle concentrations, driven mainly by an increase in large-buoyant LDL particles.141 Also consistent with its mechanism-of-action of reducing insulin resistance and hepatic lipogenesis, NGM282 elicited reductions in large VLDL, mean VLDL size and small HDL particles. Importantly, this study showed that initiation of a statin in NGM282-treated patients resulted in a rapid decline in plasma lipid levels (Figure 3), showing that the increase in LDL-C is related to the LDL receptor and that statins can reduce the increased LDL-C observed with NGM-282 treatment.141 At 12 weeks, there were significant reductions in TC, LDL-C, TG, total LDL and VLDL particle concentrations, and elevations in HDL-C.141 The reduction in LDL was driven mainly by a reduction in large LDL.141 It can be concluded that statin treatment leads to an increase in LDL receptor expression on the hepatocyte surface, thereby reducing LDL-C and LDL particle concentrations in NASH patients treated with NGM282 (Figure 3). The fact that NASH patients may receive a benefit from NGM282 treatment for their liver disease simultaneously with a statin-mediated reduction in their CVD risk factors suggests that Aldafermin (NGM282) coupled with a statin may be useful for the treatment of NASH despite initial concerns regarding increased LDL-C (Figure 3).

3.8 | Fibroblast growth factor 21 analogues

Pegbelfermin (BMS-986036) is a pegylated human fibroblast growth factor 21 (FGF21) analogue that was recently tested in patients with either obesity, T2D or NASH. FGF21 is a key regulator of energy metabolism that is produced by the liver, adipose tissue and pancreas. Pegbelfermin treatment for 12 weeks increased HDL-C, adiponectin levels and insulin sensitivity, and reduced weight, ALT, AST, TG and Pro-C3 in subjects who were obese with T2D.142 In patients with NASH, daily and weekly treatment for 16 weeks led to a significant decrease in hepatic fat fraction as well as an increase in adiponectin and HDL-C.143 There was also a reduction in LDL-C in the once daily arm of the study suggesting that pegbelfermin treatment may lead to beneficial changes in lipid levels.143 Future studies with larger numbers of subjects will need to be done to fully understand the effects of this FGF21 analogue on lipids and CVD risk. Phase 2b study (FALCON 1) is underway in patients with biopsy-proven NASH in order to evaluate potential changes in histological endpoints (NCT03486899). Another FGF21 “mimic,” a bispecific antibody agonist to the receptor complex for FGF21 (FGFR1c and βKlotho) called BFKB8488A, is also in clinical development for NASH (NCT0360538). Because these agonists may interact with the receptors in very different ways, it will be interesting to see if their effects on lipoproteins differ as well.

3.9 | Acetyl-CoA carboxylase inhibitors

Acetyl-CoA carboxylase (ACC) catalyses the first committed step of de novo fatty acid synthesis. Therefore, it has been hypothesised that ACC inhibition may be a target for reducing hepatic steatosis and treating patients with insulin resistance, T2D and NAFLD. Currently there are ACC inhibitors in clinical development that may provide answers to these questions. Firsocostat (GS-0976) is a liver-targeted, dual ACC1/ACC2 inhibitor, that was tested in a Phase 2 trial in patients with NASH. After 12 weeks administration of GS-0976, patients with NASH exhibited reduced hepatic de novo lipogenesis, hepatic steatosis and markers of liver injury.144,145 However, median increases of 11%-13% in TG were observed. Sixteen of 100 patients treated with GS-0976 developed substantial hypertriglyceridaemia (>500 mg/dL), which was associated with baseline TG of ≥250 mg/dL. Four of these subjects received fibrates or fish oil,
which reduced their TG levels to <500 mg/dL by week 12.\textsuperscript{145} In order to gain a more comprehensive view of the changes in lipid-related CVD risk factors, a complete lipoprotein class/subclass profile was obtained. Treatment with GS-0976 led to an increase in VLDL particles and VLDL-TG that peaked at 1 week.\textsuperscript{145} Additionally, there was no change in TC, HDL-C, LDL-C, total LDL or small LDL particle concentrations (Table 1).\textsuperscript{145} Therefore, GS-0976 treatment did not elicit alterations suggestive of an atherogenic lipoprotein phenotype. The mechanisms for the increased TG are thought to be due to a combination of increased VLDL synthesis and secretion\textsuperscript{146} and a reduction in TG clearance due to a decrease in peripheral lipoprotein lipase activity. Similar findings were observed with another ACC inhibitor, MK-4704.\textsuperscript{147} Because elevated serum TG may increase risk of CVD and, in extreme cases, pancreatitis, treatment with ACC1/ACC2 inhibitors would likely require lipid monitoring and sometimes addition of TG lowering therapy.\textsuperscript{145} PF-05221304 is a liver-targeted ACC1/ACC2 inhibitor that was evaluated in a Phase 1 clinical study in healthy subjects.\textsuperscript{148} PF-05221304 demonstrated robust inhibition of de novo lipogenesis in NASH patients, with minimal effects on platelet count or TG.\textsuperscript{148} Given the effects of previously tested ACC inhibitors, a comprehensive lipid and lipoprotein profile should be conducted in future studies in NASH patients.

### 3.10 | Anti-chemokine receptor therapeutic agents

The chemokine receptor type 2/5 (CCR2/5) antagonist, cenicriviroc, has been shown to have anti-inflammatory and anti-fibrotic properties in preclinical and clinical studies.\textsuperscript{149} By virtue of its antagonism of CCR2, cenicriviroc is expected to impair the recruitment, migration, and infiltration of pro-inflammatory monocytes and macrophages at the site of liver injury. In a recent Phase 2b study, cenicriviroc achieved the fibrosis endpoint (improvement in fibrosis ≥1 with no worsening of NASH) in a larger percentage of subjects than placebo.\textsuperscript{149,150} While biomarkers of systemic inflammation were reduced with cenicriviroc treatment, no changes in lipid or lipoprotein levels were reported.

### 3.11 | Apoptosis inhibitors

Selonsertib (GS-4997) is a selective inhibitor of apoptosis signal-regulating kinase (ASK1), a ubiquitously expressed serine/threonine kinase that is activated by oxidative stress to promote hepatocellular apoptosis, inflammation and fibrosis.\textsuperscript{151} Recently selonsertib was tested in a Phase 2 trial in NASH patients with or without addition of simtuzumab (GS-6624), a humanized monoclonal antibody to LOXL2 that failed to show efficacy for reducing fibrosis in a NASH trial.\textsuperscript{151,152} After 24 weeks of treatment in patients with biopsy-proven NASH, the proportion of patients with ≥1 stage reduction in fibrosis was: 43% with selonsertib + simtuzumab, 30% with selonsertib + simtuzumab and 20% with simtuzumab-alone. Improvements in fibrosis were associated with reductions in liver stiffness by magnetic resonance elastography, collagen content and lobular inflammation on liver biopsy.\textsuperscript{151} Unfortunately, two Phase 3 studies with selonsertib (STELLAR-3 in subjects with NASH and bridging fibrosis and STELLAR-4 in subjects with NASH and compensated cirrhosis) did not meet the primary endpoint of ≥1-stage histologic improvement in fibrosis without worsening of NASH, suggesting that a apoptosis signal-regulating kinase (ASK1) inhibition alone may not be sufficient to reduce fibrotic disease in the liver. No lipid or lipoprotein results were reported for any of these trials.

Emricasan (IDN-6556) is an irreversible pan-caspase inhibitor that has been tested recently in patients with various liver diseases.\textsuperscript{153-155} Emricasan treatment lowered portal pressure in patients with compensated cirrhosis and severe portal hypertension\textsuperscript{154} and improved hepatic function in patients with cirrhosis.\textsuperscript{155} In a pilot study in patients with NAFLD, emricasan treatment led to a reduction in ALT and cytokeratin 18 (CK18), suggesting that emricasan should be tested in larger trials in patients with NASH.\textsuperscript{153} Unfortunatley in a recent Phase 2b trial, emricasan treatment did not improve histological endpoints in patients with NASH and liver fibrosis and may have even worsened fibrosis and ballooning.\textsuperscript{156} To date, no effects on lipoproteins, lipids or other CVD risk factors have been reported.

### 3.12 | Thyroid hormone receptor β agonist

Resmetirom (MGL-3196) is a highly selective thyroid hormone receptor β (THRβ) agonist.\textsuperscript{157} THRβ-selective agonism is hypothesised to elicit the beneficial effects on dyslipidaemia without eliciting the adverse effects of thyroid hormone receptor α agonism in heart and bone. In a Phase 1 study, MGL-3196 dosed at 50 mg per day or higher for 2 weeks significantly reduced LDL-C by 30% and showed a trend for decreased TG.\textsuperscript{158} A Phase 2 trial in patients with biopsy-proven NASH revealed that resmetirom treatment resulted in a significant reduction in hepatic fat content as well as in multiple pro-atherogenic proteins and lipids including LDL-C, apoB, TG, apolipoprotein CIII, lipoprotein (a), small dense LDL particles and large VLDL.\textsuperscript{159} Notably, resmetirom is one of the few potential therapeutics for NASH that has been shown to reduce lipoprotein (a), a lipoprotein closely associated with atherogenic risk and CVD.

### 3.13 | Stearoyl-CoA desaturase-1 inhibitor

Aramchol is a synthetic lipid inhibitor of stearoyl-CoA desaturase-1 (SCD1) that was tested for efficacy in patients with biopsy-proven NAFLD.\textsuperscript{160} Aramchol treatment (300 mg) elicited a reduction in liver fat content and is currently being evaluated in a Phase 2b trial in NASH patients (NCT02279524). Because SCD1 is an enzyme that catalyses the synthesis of monounsaturated FA from saturated FA, there may be an effect of SCD1 inhibition on the lipid species carried by lipoprotein particles. However, effects of SCD1 inhibition on the overall lipoprotein profile are not known at this time. Therefore, it is
difficult to predict if SCD1 inhibition will affect CVD risk with long-term treatment.

3.14 | Mitochondrial pyruvate carrier modulator

MSDC-0602K is a next-generation insulin sensitizer that modulates the mitochondrial pyruvate carrier (MPC), but avoids direct PPARγ activation. In mice fed a diet rich in trans-fatty acid, fructose and cholesterol, MSDC-0602 prevented and reversed liver fibrosis and reduced hepatic markers of stellate cell activation presumably by modulating pyruvate metabolism since reduction of MPC expression in hepatocytes led to similar effects. In the one-year Phase 2 trial in NASH subjects with and without type 2 diabetes (EMMINENCE), MSDC-0602K treatment produced dose-dependent reductions in glycaemia and fasting insulin, and while there no changes in overall lipids, there were shifts in particles that could be consistent with a reduction in atherogenic dyslipidaemia secondary to increased insulin sensitivity. Treatment led to an increase in VLDL particles, largely due to an increase in small VLDL that was not accompanied by a significant increase in TG. MSDC-0602K treatment also led to an increase in large LDL and a reduction in small dense LDL particles. In addition, there was an increase in medium HDL and a decrease in small HDL, which led to an overall increase in total HDL particles.

4 | CONCLUSIONS

Recently physicians have begun to appreciate the fact that many therapeutics on the market, or currently in development, elicit effects on lipoprotein metabolism leading to direct or indirect effects on circulating lipid and lipoprotein levels. Many of these medications have overall positive effects lipid profiles, however, there are a number of drug classes that negatively impact circulating lipids and lipoproteins, leading to concern that these agents may augment CVD progression. This is especially true for anti-diabetic agents, some of which are in development for treatment of NAFLD/NASH. Some of these agents have direct effects (increased hepatic cholesterol content leading to increased serum LDL-C), indirect effects (enhanced insulin sensitivity and reversal of atherogenic dyslipidaemia) or both. Moreover the mechanisms for the alterations in the lipoprotein profile often differ. Evidence to date suggests that these drug-induced alterations may not necessarily lead to increased CVD risk in NASH patients. Co-administration of lipid modifying therapies that reduce CVD risk factors such as statins may help reduce CVD risk, however, larger clinical studies are needed in order to prove this to be the case for each of the potential therapeutics in question. It is clear, however, that in order to fully understand the overall impact of these agents on both hepatic and CVD outcomes, it may be necessary to consider all of the benefits and risks that are associated with each of these drugs or drug candidates.

ACKNOWLEDGEMENTS

The authors thank Drs. Damiano and Garcia for their careful review of the manuscript and valuable input as well as Sarah Perry for her assistance with Figure 1.

Declaration of personal interest: Dr Connelly is an employee of and owns stock in LabCorp. Dr Velez Rivera has no conflicts to disclose. Dr Guyton has received research support from Sanofi, Regeneron, Amgen and Amarin. Dr Siddiqui is a member of an advisory board at Pfizer and receives research grant support from Zydus. Dr Sanyal is the President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Direx Inversago and Galmed. Has served as a consultant to Astra Zeneca, Nitto Denko, Conatus, Nimbus, Salix, Tobira, Takeda, Janssen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Bristol Myers Squibb, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Zydus, Nordic Bioscience, Albireo, Prosciento, Surrozen. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, Astra Zeneca, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate. Virginia Commonwealth University, where he is a full-time employee, also has ownership interests in Sanyal Biotechnology. Dr Sanyal also acknowledges funding from NIH project R01 DK 105961.

AUTHORSHIP

Guarantor of the article: None.

Authorship contributions: MAC wrote the first draft of the article. JVR, MSS, JRG and AS critically reviewed and revised the manuscript. All authors approved the final version for publication.

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How to cite this article: Connelly MA, Velez Rivera J, Guyton JR, Siddiqui MS, Sanyal AJ. Review article: the impact of liver-directed therapies on the atherogenic risk profile in non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2020;52:619–636. https://doi.org/10.1111/apt.15935