



# Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus

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**Abstract** | Non-alcoholic fatty liver disease (NAFLD) has emerged as the most prevalent liver disease in the world, yet there are still no approved pharmacological therapies to prevent or treat this condition. NAFLD encompasses a spectrum of severity, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). Although NASH is linked to an increased risk of hepatocellular carcinoma and cirrhosis and has now become the leading cause of liver failure-related transplantation, the majority of patients with NASH will ultimately die as a result of complications of type 2 diabetes mellitus (T2DM) and cardiometabolic diseases. Importantly, NAFLD is closely linked to obesity and tightly interrelated with insulin resistance and T2DM. Thus, targeting these interconnected conditions and taking a holistic attitude to the treatment of metabolic disease could prove to be a very beneficial approach. This Review will explore the latest relevant literature and discuss the ongoing therapeutic options for NAFLD focused on targeting intermediary metabolism, insulin resistance and T2DM to remedy the global health burden of these diseases.

Non-alcoholic fatty liver disease (NAFLD) is defined as ectopic lipid accumulation in the liver in the absence of excessive alcohol intake or other attributable cause; 25% of the world's population might have NAFLD and direct costs are estimated at US\$100 billion annually in the USA alone<sup>1,2</sup>. NAFLD is tightly linked to obesity and, while diet and exercise have proven to be effective in the treatment of NAFLD<sup>3</sup>, the long-term sustainability of these interventions is poor. Furthermore, there is no licensed drug therapy for non-alcoholic steatohepatitis (NASH) and there is a critical need for the development of effective treatments for this global health problem.

NAFLD encompasses a spectrum of disease severity (FIG. 1) ranging from simple steatosis to more advanced disease characterized by hepatocyte death, inflammation and the development of fibrotic lesions (NASH). Simple steatosis is characterized by lipid accumulation in hepatocytes with minimal hepatocellular injury but is associated with an increased risk of other metabolic disorders, including insulin resistance, type 2 diabetes mellitus (T2DM), dyslipidaemia and hypertension<sup>4,5</sup>. Epidemiological studies show that NAFLD is more prevalent in men than in premenopausal women<sup>6</sup> and premenopausal women are less likely to develop fibrosis than men<sup>7,8</sup>. By contrast, the protective effect of the female sex is not observed after menopause. It is estimated that NASH is present in ~60% of patients with NAFLD who

undergo a liver biopsy and 41% of patients with NASH exhibit considerable fibrosis<sup>1</sup>. In patients with advanced fibrosis, ~22% go on to develop cirrhosis and ~2% of patients with cirrhosis develop hepatocellular carcinoma (HCC) within 3 years<sup>1,9</sup> (FIG. 1). HCC and cirrhotic liver failure can only be cured with an extreme treatment — liver transplantation. Liver disease secondary to NASH has become a leading cause for liver transplantation in the USA due to increasing rates of obesity-related NASH and the increasing availability of alternative curative therapies for other diseases, such as hepatitis C virus infection<sup>10,11</sup>. Unfortunately, most patients with liver failure will ultimately not receive a transplant and will die while waiting for a donor organ<sup>12</sup>. Further, the majority of patients with NASH will die from cardiometabolic disease rather than from a liver-related cause due to the tight relationship between NAFLD and T2DM. Thus, therapeutics that target NAFLD, T2DM and cardiometabolic risk factors might be the most effective treatment.

## Pathophysiology of NAFLD

The liver serves many functions, particularly through the regulation, mobilization and storage of nutrients. Hepatocytes have important roles in gluconeogenesis, ketogenesis, amino acid metabolism, and triglyceride and lipoprotein metabolism. While the liver is not a primary organ for lipid storage, ectopic lipid accumulation

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**Key points**

- Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease globally, yet there are currently no approved therapies.
- While NAFLD progression to non-alcoholic steatohepatitis is becoming the leading cause of end-stage liver failure, the leading causes of death in patients with NAFLD are complications of cardiometabolic disease.
- A tight relationship exists between NAFLD, insulin resistance and type 2 diabetes mellitus.
- It is likely that developing therapeutics that target both NAFLD and cardiometabolic risk factors might be extremely beneficial.

in the liver can occur under a variety of conditions (FIG. 2). In patients with NAFLD, it has been determined that several sources contribute to intrahepatic triglyceride accumulation, including hepatic uptake from the plasma of non-esterified fatty acids (NEFAs; 59%) and dietary fat (15%) as well as de novo lipogenesis (DNL; 26%)<sup>13</sup>. Insulin resistance promotes adipose tissue lipolysis leading to increased NEFA levels in the blood, which are taken up by the liver in a concentration-dependent manner<sup>13–15</sup>. Other metabolic perturbations common in insulin resistance and T2DM, such as hyperinsulinaemia and hyperglycaemia, drive the increased hepatic conversion of carbohydrates into fatty acids through DNL<sup>16</sup> and rates of hepatic DNL are markedly elevated in patients with NAFLD<sup>17</sup>. The expression and/or activity of several enzymes involved in DNL or the storage of fatty acids in triglycerides are known to be increased in patients with NAFLD<sup>18</sup> and targeting these enzymes, including ketohexokinase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase and diacylglycerol acyltransferase, has been therapeutically explored (FIG. 3). Given the contributing effects of insulin resistance on adipose tissue lipolysis and hepatic DNL, agents that improve systemic insulin sensitivity are being tested for their efficacy in patients with NAFLD.

Although impairments in the mitochondrial metabolism of fatty acids could also contribute to lipid accumulation, mitochondrial dysfunction seems to happen only in the later stages of NASH progression<sup>19</sup>. Nevertheless, compounds that stimulate mitochondrial oxidative metabolism, including thyroid hormone receptor agonists<sup>20</sup>, ligands for nuclear receptors that control the expression of mitochondrial genes<sup>21</sup> and inhibitors of mitochondrial pyruvate import<sup>22</sup>, have shown efficacy in the treatment of NASH.

Triglycerides comprise the primary form of storage lipid and are generally considered inert. However, the accumulation of toxic lipids, such as NEFAs, ceramides and diacylglycerol, is positively correlated with insulin resistance<sup>23,24</sup>. These lipids might also provoke endoplasmic reticulum stress, oxidative stress and mitochondrial dysfunction, which promotes inflammation and hepatocyte degeneration (ballooning)<sup>25,26</sup>. The ‘fatty’ liver phenotype is really an amalgam of these various lipids and is generally quantified by histology-based or MRI-based approaches that do not distinguish between triglycerides or other forms of neutral lipids. Therefore, we will refer to the therapeutic effects as reducing liver ‘steatosis’ or ‘lipid’ content as an endpoint that might represent the reduced accumulation of some or all of these species.

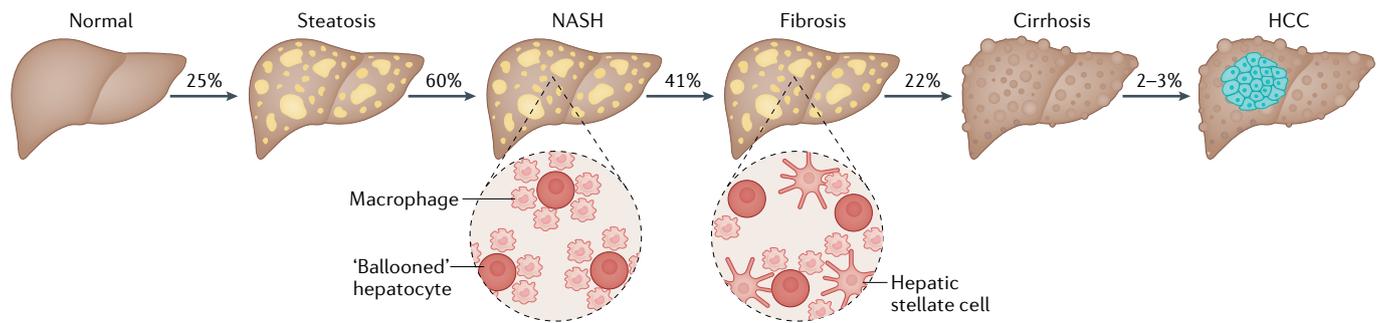
Various immune cell types are present in the liver, including macrophages, neutrophils and T cells<sup>27</sup>. These cells perform a variety of functions that can be protective (for example, remodelling damaged tissue) or deleterious (exacerbating hepatic inflammation). Inflammation and hepatocyte damage lead to the activation of hepatic stellate cells, which migrate to sites of liver injury and differentiate into myofibroblasts<sup>28,29</sup>. Stellate cells produce collagen and other components of fibrotic lesions and the development of fibrosis is the best predictor of future liver failure, need for transplantation and mortality<sup>30,31</sup>.

The current standard for a diagnosis of NASH requires a liver biopsy and histological confirmation of hepatocyte ballooning, inflammatory infiltrates and fibrosis<sup>4</sup>. To be judged as beneficial in phase III clinical trials, NAFLD therapeutics must lead to a resolution of steatohepatitis without worsening fibrosis or reduce fibrosis with no worsening of steatohepatitis<sup>32</sup>. However, liver biopsies are invasive, can cause internal bleeding, and considerable variability exists in histological evaluations between observers or upon repeat assessments<sup>33</sup>. The development of less invasive and more consistent standards could speed up the approval of effective therapeutics.

**NAFLD and T2DM: pathophysiological links**

NAFLD progression is closely associated with insulin resistance and T2DM<sup>34</sup> (FIG. 2). Available evidence also suggests that a bidirectional relationship exists between NAFLD (or NASH) and T2DM (TABLE 1). Both T2DM and obesity have been independently associated with an increased risk of developing NAFLD<sup>5,35</sup> and improvements in insulin sensitivity have been positively associated with histological improvements in NASH and fibrosis regression<sup>36</sup>. A baseline diagnosis of T2DM was the strongest independent risk factor for patients with NASH going on to develop cirrhosis and HCC<sup>37,38</sup> and was positively associated with overall mortality and liver-related outcomes in patients with NAFLD<sup>30</sup>.

Hepatic lipid accumulation is associated with impaired insulin sensitivity, which is exacerbated in patients with NASH and might be a predictor of fibrosis (TABLE 1)<sup>15,39,40</sup>. The accumulation of specific lipids, such as diacylglycerol or ceramides, can impair hepatic insulin signalling, which leads to a pathological increase in hepatic glucose production<sup>23</sup>. Elevations in circulating levels of transaminases (a surrogate marker of liver injury and possible NAFLD) that are not explained by other diagnoses are positively linked to an increased future risk of developing T2DM<sup>41</sup> and patients with NAFLD have a greater than two-fold increased risk of developing T2DM<sup>42</sup>. Similarly, the global prevalence of NAFLD in patients with T2DM is estimated to be more than 55%, with NASH expected to occur in 37% of patients with T2DM<sup>43</sup>. Due to this complex interrelationship, it has been suggested that NAFLD and NASH should be considered in the treatment of T2DM<sup>44</sup> and a study published in 2020 has highlighted that the non-invasive screening for NAFLD is cost-effective in patients with T2DM in the USA<sup>45</sup>.



**Fig. 1 | The pathological spectrum of NAFLD.** The excess accumulation of liver lipids, mostly in the form of triglycerides, is referred to as steatosis, which is defined as the presence of >5% lipid accumulation within hepatocytes<sup>4</sup>. It is estimated that approximately 25% of the global population has some form of non-alcoholic fatty liver disease (NAFLD)<sup>1</sup>. Eventually, excess lipid accumulation might overwhelm the liver’s capacity for the proper storage or disposal of fatty acids, leading to the generation of toxic lipid species. Lipotoxicity might produce endoplasmic reticulum stress, oxidative stress and mitochondrial dysfunction, resulting in inflammation and hepatocyte degeneration (ballooning), which are the defining characteristics of non-alcoholic steatohepatitis (NASH). NASH is

estimated to be present in up to 60% of patients with NAFLD who have undergone a liver biopsy<sup>1</sup>. Over time, hepatocyte death, inflammation and immune cell activation might promote hepatic stellate cell activation. Stellate cells differentiate into fibrogenic myofibroblasts that migrate to sites of hepatic injury and are the major drivers of fibrosis<sup>28</sup>. Detectable fibrosis is present in approximately 41% of patients with NASH<sup>1</sup> and 22% of individuals with advanced fibrosis go on to develop hepatic scarring, referred to as cirrhosis<sup>9</sup>. Lastly, ~2% of patients with cirrhosis will probably develop hepatocellular carcinoma (HCC) within 3 years<sup>1</sup>. Percentages in the figure are representative of the proportion of the subpopulation that will probably present or progress to more advanced liver disease.

Cardiovascular disease (CVD) is the primary cause of mortality in patients with T2DM, accounting for up to two-thirds of all deaths<sup>46</sup>, and increased HbA<sub>1c</sub> levels are strongly correlated to an increased risk of heart disease and overall mortality<sup>47</sup>. Accordingly, a meta-analysis published in 2020 demonstrated that several glucose-lowering strategies, particularly those that reduced body weight, decrease the risk of major adverse cardiovascular events, which demonstrates the usefulness of diabetic therapies in providing beneficial cardiovascular effects<sup>48</sup>. Not only would a reduction in CVD — the leading cause of mortality in patients with NAFLD — be valuable but, as stated previously, a loss of body weight is also effective in resolving the features of NASH<sup>3</sup>.

In addition to CVD-related macrovascular complications, patients with NAFLD are at increased risk of developing microvascular disease, particularly chronic kidney disease, which is also highly associated with CVD and T2DM. An increased histological severity of NAFLD correlates with progressively lower renal function, which is independent of other comorbidities, including T2DM, hypertension, dyslipidaemia and adiposity<sup>49</sup>. Patients with NASH who had improvements in fibrosis and/or a resolution of NASH due to lifestyle interventions also had a statistically significant increase in renal function<sup>50</sup>.

In the past few years, the term ‘metabolic-associated fatty liver disease’ has been proposed to more accurately describe patients who have liver disease coincident with metabolic dysfunction. For example, a patient with hepatic steatosis in combination with obesity and/or T2DM or an individual with a normal BMI who has at least two metabolic risk factors<sup>51</sup>. Although not yet universally accepted, this new terminology seeks to include a larger population of patients than the term NAFLD, who probably share many underlying and possibly undiagnosed comorbidities, to help expand the collaboration between hepatologists and diabetologists to advance growth in our understanding and treatment

of this population of patients<sup>51-53</sup>. Due to the interplay between NAFLD, NASH and T2DM, it is likely that targeting insulin sensitivity and hyperglycaemia and adopting a holistic attitude towards the treatment of metabolic disease in patients with NAFLD might prove advantageous.

#### Effects of anti-diabetic agents on NAFLD

Given the tight link between T2DM and NAFLD, numerous anti-diabetic drugs have been tested as NASH therapeutics in experimental models and we will highlight the available evidence for therapeutic agents that have advanced to clinical trials. The first-line T2DM therapy, metformin, has not been shown to have beneficial effects on NASH in people and will not be discussed. However, there are a number of clinical trials for modulators of glucagon-like peptide 1 (GLP1) activity, insulin-sensitizing thiazolidinediones and inhibitors of the sodium–glucose cotransporter 2 (SGLT2), which will be discussed in this section.

#### GLP1 modulators

GLP1 is an endogenous intestinal hormone, acting through the G protein-coupled GLP1 receptor (GLP1R), that directly stimulates the production and release of insulin and indirectly inhibits glucagon secretion and reduces food intake<sup>54</sup>. The circulating half-life of GLP1 is only 1–2 minutes due to the action of dipeptidyl peptidase 4 (DPP4), which cleaves and inactivates GLP1. Given the positive effects of GLP1 on glucose homeostasis, engaging its receptor or optimizing its activity is a logical therapeutic target for the treatment of hyperglycaemia. Multiple GLP1R agonists (exenatide, liraglutide, dulaglutide and semaglutide) are approved for the treatment of T2DM<sup>54</sup>.

GLP1R agonists reduce hepatic steatosis and markers of liver damage in genetically induced and diet-induced diabetic mice, rats and rhesus macaques<sup>55,56</sup>. The treatment of patients with T2DM with either exenatide,

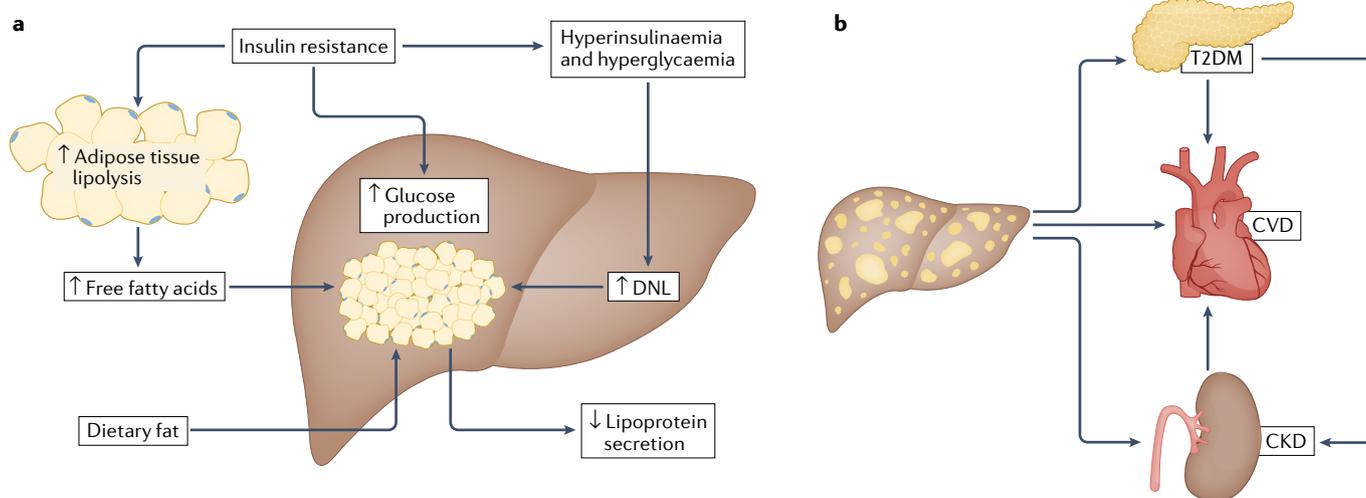
liraglutide or semaglutide has demonstrated efficacy in reducing hepatic lipid content as well as levels of liver enzymes and inflammatory markers, an effect associated with improved HbA<sub>1c</sub> levels and body-weight loss (TABLE 2)<sup>57–60</sup>. Furthermore, compared with control individuals given placebo, a greater percentage of patients with NASH who were treated with semaglutide achieved resolution of NASH with no worsening of fibrosis<sup>61</sup>. Additionally, semaglutide is being tested in patients with NASH and compensated cirrhosis (NCT03987451) to determine its efficacy in the reduction of fibrosis score without a worsening of NASH. Many of the beneficial aspects of GLP1R agonism on NAFLD endpoints are closely associated with weight loss and improved metabolic indexes. GLP1R is not expressed in the liver at appreciable levels<sup>62,63</sup> and, thus, the effects of activating this receptor on NASH endpoints are probably not due to direct hepatic actions but rather due to systemic improvements in metabolism.

The inhibition of DPP4 has also been targeted therapeutically to enhance GLP1 activity. Patients with NAFLD, T2DM and obesity have increased DPP4 levels, which is positively correlated with hepatocyte apoptosis and fibrosis<sup>64,65</sup>. Currently, four DPP4 inhibitors (alogliptin, linagliptin, saxagliptin and sitagliptin) are approved for treatment in T2DM. DPP4 inhibitors reduced hepatic inflammation, fibrosis and liver tumour development in mouse models of NASH<sup>64,66</sup>. By contrast, sitagliptin treatment failed to provide any improvement in hepatic lipid content, despite a statistically significant reduction in HbA<sub>1c</sub> (REFS<sup>67,68</sup>). Therefore, these inhibitors might not be an effective strategy for the treatment of patients with NAFLD.

### Thiazolidinedione insulin sensitizers

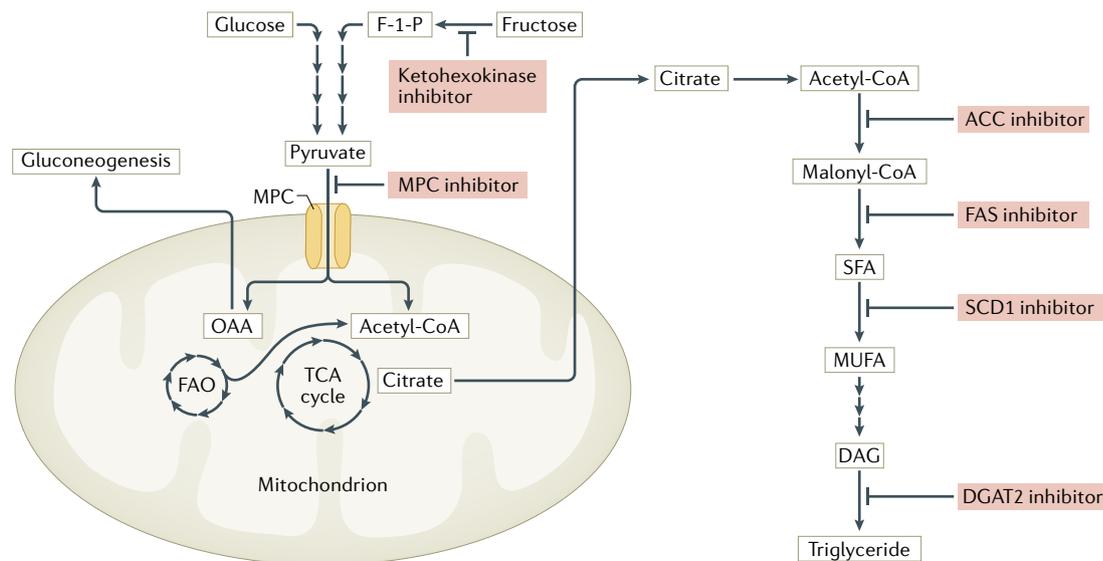
Pioglitazone and rosiglitazone treat T2DM by sensitizing the body to the effects of insulin and are identifiable by their thiazolidinedione ring. Pioglitazone and rosiglitazone are potent activators of the nuclear receptor PPAR $\gamma$ , which is most highly expressed in adipose tissue and has a critical role in adipocyte differentiation and lipid and glucose metabolism as well as in suppressing inflammation<sup>69</sup>. Thiazolidinediones probably improve peripheral insulin sensitivity by stimulating the release of adipokines, promoting triglyceride storage in adipose tissue and enhancing the suppressive action of insulin on lipolysis, resulting in reduced plasma levels of free fatty acids and decreased hepatic lipid accretion<sup>70</sup>. Additionally, thiazolidinedione treatment directly reduces hepatic stellate cell activation and fibrosis in rats<sup>71</sup>. Thus, thiazolidinediones might improve hepatic and peripheral insulin sensitivity to attenuate NASH and might also have direct effects on hepatic pathophysiology. However, the use of thiazolidinediones in people is now limited by PPAR $\gamma$ -driven adverse effects, including weight gain, oedema and the risk of bone fracture<sup>72</sup>. In addition, a meta-analysis published in 2007 found that rosiglitazone use was associated with an increased risk of myocardial infarction and cardiovascular death compared with placebo<sup>73</sup>, resulting in a black-box warning and restriction of rosiglitazone use by the FDA. However, the warning and restriction were later removed after additional studies failed to replicate the findings regarding cardiovascular risks<sup>74</sup>. Nonetheless, at present, rosiglitazone is used very rarely to treat patients with T2DM.

Of the two approved thiazolidinediones, rosiglitazone is the most potent activator of PPAR $\gamma$ <sup>75</sup>. Studies have



**Fig. 2 | Role of insulin resistance in NAFLD. a** | Insulin resistance occurs when tissues inadequately respond to the hormone insulin<sup>23</sup>. In adipose tissue, the normal ability of insulin to suppress lipolysis is impaired, resulting in the excess release of free fatty acids, which are subsequently taken up by the liver in addition to fat obtained from the diet<sup>13–15</sup>. The inability of tissues to properly respond to insulin results in abnormally elevated glucose levels (hyperglycaemia), which leads to the production and secretion of surplus insulin levels to those needed to maintain blood levels of glucose (hyperinsulinaemia)<sup>23</sup>. Both hyperglycaemia and hyperlipidaemia stimulate de novo lipogenesis (DNL), which further elevates hepatic lipid

accumulation<sup>16,17</sup>. Normally, appropriate hepatic levels of lipids would be maintained by their increased export through lipoprotein secretion; however, lipoprotein secretion is not adequately elevated in non-alcoholic fatty liver disease (NAFLD)<sup>167</sup>. **b** | NAFLD is also linked to several extrahepatic comorbidities. Patients with NAFLD have an increased risk of developing type 2 diabetes mellitus (T2DM)<sup>42</sup> and cardiovascular disease (CVD)<sup>168</sup>. In addition, T2DM is an independent risk factor for NAFLD<sup>5,35</sup> and is also correlated with an increased risk of heart disease<sup>47</sup>. Lastly, NAFLD is associated with an elevated risk of developing chronic kidney disease (CKD), which is also highly correlated to T2DM and CVD<sup>49</sup>.



**Fig. 3 | Targeting of intermediary metabolism for NAFLD therapy.** Mechanistic illustration of investigational drugs currently in clinical trials. Inhibitors of ketohexokinase block the rate-limiting step of fructose metabolism, an inducer of de novo lipogenesis<sup>149</sup>. Mitochondrial pyruvate carrier (MPC) inhibitors act downstream to prevent the import of pyruvate into the mitochondria, where it can be metabolized to intermediates that are used for gluconeogenesis (OAA) or fatty acid synthesis (citrate)<sup>85,152</sup>. Lastly, several inhibitors are being investigated that target various enzymes (such as ACC, FAS, SCD1 and DGAT2) involved in the synthesis of triglycerides<sup>18</sup>. ACC, acetyl-CoA carboxylase; DAG, diacylglycerol; DGAT2, diacylglycerol acyltransferase 2; F-1-P, fructose-1-phosphate; FAO, fatty acid oxidation; FAS, fatty acid synthase; MUFA, monounsaturated fatty acid; OAA, oxaloacetate; SCD1, stearoyl-CoA desaturase 1; SFA, saturated fatty acid; TCA, tricarboxylic acid cycle.

demonstrated that patients with NASH receiving rosiglitazone had enhanced insulin sensitivity and reduced hepatic steatosis; however, considerable weight gain and oedema in the lower extremities were reported (TABLE 2)<sup>76,77</sup>. As the clinical use of rosiglitazone as an insulin sensitizer has largely been discontinued, there has been very little further evaluation of the drug for the treatment of NASH subsequent to the studies conducted almost 15 years ago. Pioglitazone is also an effective insulin sensitizer but is a less potent PPAR $\gamma$  agonist and is associated with fewer adverse effects than rosiglitazone<sup>75,78</sup>. Treatment with pioglitazone in patients with T2DM and NASH resulted in improved insulin sensitivity and reduced inflammation and hepatocyte degeneration as well as in reduced fibrosis relative to pretreatment measurements, yet there was no difference in the resolution of fibrosis compared with the placebo group<sup>79</sup>. Additionally, patients with NASH but no T2DM receiving pioglitazone had improved fasting glucose levels and a greater resolution of NASH, though with no improvement in fibrosis, and increased weight gain versus patients receiving placebo<sup>80,81</sup>. A meta-analysis of randomized controlled trials using the thiazolidinediones, either pioglitazone or rosiglitazone, demonstrated that thiazolidinedione treatment led to improved liver histology, including reduced steatosis, inflammation and degeneration<sup>82</sup>. However, a statistically significant decrease in fibrosis was only seen in studies using pioglitazone versus placebo, suggesting that pioglitazone might be more efficacious than rosiglitazone for the resolution of fibrosis. Further examination demonstrated that responders to pioglitazone had increased plasma concentrations of pioglitazone<sup>83</sup> and genetic

analysis identified a variant of *CYP2C8* (the gene that encodes the predominant pioglitazone-metabolizing enzyme) that was associated with fibrosis score<sup>84</sup>. Collectively, these studies indicate the usefulness of pioglitazone in the resolution of NASH and highlight the importance of pharmacogenetics, which can drastically alter drug metabolism and response to treatment.

Given the greater efficacy of pioglitazone than rosiglitazone, despite a markedly lower affinity for PPAR $\gamma$ , it seems reasonable to explore whether the therapeutic pharmacology of thiazolidinediones might not be entirely dependent on PPAR $\gamma$  activation. Indeed, other molecular targets of thiazolidinediones have been identified<sup>85</sup> and one will be discussed in greater detail in subsequent sections.

**SGLT2 inhibitors**

SGLT2 is a sodium-dependent glucose transporter primarily expressed in the proximal tubule epithelium of the kidney and is responsible for the majority (>90%) of filtered glucose reabsorption<sup>86</sup>. Inhibitors of SGLT2 (canagliflozin, dapagliflozin, empagliflozin and ertugliflozin) lead to the increased urinary excretion of glucose and are approved anti-hyperglycaemic agents for T2DM. In addition, SGLT2 inhibitors induce weight loss in many people and reduce the risk of major cardiovascular events<sup>86</sup>. In a mouse model of T2DM, SGLT2 inhibition significantly reduced levels of liver enzymes, steatosis, hepatocyte damage and fibrosis<sup>87</sup>.

Several trials have tested the efficacy of SGLT2 inhibitors in human NAFLD and T2DM. Patients treated with either canagliflozin, dapagliflozin or empagliflozin showed reduced hyperglycaemia and improvements in

hepatic lipid content, liver enzymes and liver stiffness (TABLE 2)<sup>88–92</sup>. Notably, as with GLP1R agonists, SGLT2 inhibitors caused weight loss, which strongly correlated with the decrease in hepatic lipid content<sup>88</sup>. However, a large retrospective study comparing canagliflozin, dapagliflozin, liraglutide or sitagliptin added to previous T2DM treatment (insulin, metformin or sulfonylurea), demonstrated that only the SGLT2 inhibitors canagliflozin and dapagliflozin resulted in significantly improved levels of liver enzymes independently of body-weight loss and HbA<sub>1c</sub> (REF.<sup>93</sup>). As with GLP1R, SGLT2 is not expressed in the liver<sup>86</sup> and treatment-induced weight loss or metabolic improvements might indirectly result in reduced liver lipid content. However, SGLT2 inhibitor use is associated with an increased risk of lower limb amputation and diabetic ketoacidosis compared with GLP1R agonist treatment<sup>94</sup>. Overall, SGLT2 inhibitors are effective in managing glycaemic control and lowering hepatic lipid content but histological analyses are needed to evaluate their usefulness in NASH.

### Targeting intermediary metabolism in NAFLD

While the previous discussion detailed the experimental use of approved diabetic drugs for the treatment of NASH, the following section will highlight the metabolic therapeutics in development that might improve NAFLD as well as address diabetic and cardiometabolic disease endpoints. Due to space limitations and the lack of positive data, we will not discuss clinical trials evaluating the use of statins<sup>4</sup> or ezetimibe<sup>95</sup> (an agent that lowers levels of LDL cholesterol by inhibiting intestinal cholesterol absorption) in patients with NASH. Furthermore, other drug classes targeting apoptosis, inflammation and fibrosis, which might reduce insulin resistance, hepatic inflammation and fibrosis in mice fed a high-fat diet<sup>96,97</sup>, have few reported outcomes on metabolic parameters in clinical trials and will not be discussed. Due to the number of agents tested only in preclinical models, the discussion will be centred on experimental agents that have progressed to clinical trials.

### Nuclear receptor modulators

**FXR agonists, bile acids and synthetic bile acids.** Bile acids are cholesterol metabolites, synthesized in the liver, that facilitate dietary lipid absorption. The synthesis of bile acids is elevated in T2DM<sup>98</sup> and altered bile acid concentrations correlate with histological features in patients with NASH<sup>99</sup>. The use of nor-ursodeoxycholic acid, a synthetic bile acid homologue, reduces inflammation and fibrosis in mice<sup>100</sup>. Additionally, nor-ursodeoxycholic acid demonstrated a dose-dependent decrease in liver enzymes in patients with NAFLD<sup>101</sup> and is currently being tested in patients with NASH.

In addition to playing a role in lipid absorption, bile acids act as lipid signalling mediators. The farnesoid X receptor (FXR) is a nuclear receptor that is activated by selected bile acids. FXR is highly expressed in the liver and intestine and regulates the expression of genes involved in cholesterol and bile acid synthesis<sup>98</sup>. In mice, FXR activation reduced blood levels of glucose and plasma levels of lipids, but the long-term administration of FXR agonists led to glucose intolerance<sup>102,103</sup>. The use of

obeticholic acid, a bile acid derivative that activates FXR, increased insulin sensitivity and reduced markers of liver fibrosis and inflammation in patients with NAFLD and T2DM<sup>104</sup>. More recent studies demonstrated improvements in histological markers of NASH and fibrosis with obeticholic acid treatment (TABLE 2)<sup>105,106</sup>. However, patients exhibited increased levels of LDL cholesterol, elevated insulin concentrations and pruritus as adverse effects<sup>105,106</sup>. The elevations in insulin and LDL cholesterol concentrations could lead to an increased risk of developing T2DM and CVD. While obeticholic acid might eventually be approved for the treatment of NASH, additional data on its safety and efficacy are necessary.

Interestingly, a secondary analysis of the FLINT trial demonstrated that those who responded to obeticholic acid treatment, defined as patients with a  $\geq 30\%$  reduction in liver lipid content, had substantially increased odds of improved steatosis and reduced cell death upon histological analysis<sup>107</sup>. Further examination in patients with NASH demonstrated that various clinical parameters could be integrated into a model to predict a patient's histological response to treatment<sup>108</sup>.

Other FXR agonists, including EDP-305 and tropifexor, have proven effective in resolving fibrosis in mouse models of NASH<sup>109,110</sup>. However, glucose homeostasis outcomes were not reported. The non-steroidal FXR agonist cilofexor caused a reduction in liver lipid content in patients with NASH without altering blood levels of lipids or indicators of insulin resistance<sup>111</sup>. The differences between obeticholic acid and cilofexor outcomes reported in trials are possibly due to the site-specific activation of FXR. While cilofexor primarily targets intestinal FXR, obeticholic acid is available systemically. The restriction of FXR activation to the intestine led to weight loss in mice by promoting the secretion of fibroblast growth factors (discussed below) to normalize glucose metabolism without increasing plasma levels of lipids<sup>112</sup>. Ultimately, intestine-specific FXR agonists might reduce adverse effects and ongoing trials (NCT03449446 and NCT03987074) in patients with NASH should be insightful with regards to their usefulness.

**Experimental PPAR agonists.** The PPAR family of nuclear receptor transcription factors (PPAR $\alpha$ , PPAR $\delta$  and PPAR $\gamma$ ) are characterized by large, promiscuous

Table 1 | Conditions associated with NAFLD

Condition	Prevalence in NAFLD (%)	Prevalence in NASH (%)	Ref.
Obesity	51	82	1
Type 2 diabetes mellitus	23	44	1
Dyslipidaemia	69	72	1
Hypertriglyceridaemia	41	83	1
Hypertension	39	68	1
Metabolic syndrome	43	71	1
CKD	20–55	NA	49

CKD, chronic kidney disease; NA, not available; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

ligand-binding pockets and are known to regulate numerous aspects of metabolism through gene transcription. Whereas PPAR $\gamma$  is adipocyte enriched and tends to regulate lipid storage, PPAR $\alpha$  and PPAR $\delta$  are expressed most highly in oxidative tissues and regulate genes encoding enzymes involved in mitochondrial biogenesis and metabolism, fatty acid oxidation, ketogenesis, fatty acid uptake, and triglyceride turnover<sup>21</sup>.

Numerous natural and synthetic PPAR agonists have been studied for therapeutic efficacy, including agonists that can activate two or three PPAR isoforms<sup>21</sup>.

Elafibranor (GFT505), a dual PPAR $\alpha$ -PPAR $\delta$  agonist, reduced hepatic steatosis, inflammation and fibrosis in both mouse and rat models of NAFLD<sup>113</sup>. Patients with obesity who were treated with elafibranor had reduced levels of liver enzymes and improved insulin sensitivity<sup>114</sup>.

Table 2 | Major clinical trial outcomes of anti-diabetic agents in NAFLD

Drug target	Drug name (duration)	Study population	Hepatic outcomes reported	Diabetic outcomes reported	Refs
GLP1R agonist	Exenatide (26 weeks)	Obesity, T2DM <sup>a</sup> , NAFLD (95%)	↓ Hepatic triglyceride	↓ Weight	57
	Liraglutide (meta-analysis)	Obesity, T2DM	↓ ALT	Not reported	166
	Liraglutide (12 weeks)	Obesity, NASH	↓ ALT, AST, DNL	↓ Weight and adipose mass, ↓ HbA <sub>1c</sub> and serum levels of glucose, ↓ LDL cholesterol, ↑ insulin sensitivity (clamp studies)	58
	Liraglutide (48 weeks)	Obesity, NASH	↑ NASH resolution, ↔ fibrosis worsening	↓ Weight, ↓ HbA <sub>1c</sub> and serum levels of glucose, ↑ HDL cholesterol	59
	Semaglutide (meta-analysis)	Obesity, with or without T2DM	↓ ALT	Not reported	60
	Semaglutide (72 weeks)	Obesity, NASH (F1–F3 fibrosis)	↑ NASH resolution, ↓ ALT, AST <sup>b</sup>	↓ Weight <sup>c</sup> , ↓ HbA <sub>1c</sub> <sup>c</sup>	61
DPP4 inhibitor	Sitagliptin (24 weeks)	Obesity, pre-diabetes, early T2DM	↔ No changes	↔ No changes	67
	Sitagliptin (24 weeks)	Obesity, T2DM, NASH	↔ No changes	↔ No changes	68
Thiazolidinedione	Rosiglitazone <sup>b</sup> (48 weeks; single arm)	Obesity, NASH	↓ ALT and AST, ↓ steatosis and inflammatory score	↓ HbA <sub>1c</sub> and fasting insulin, ↑ glucose tolerance, ↑ weight	77
	Rosiglitazone (52 weeks)	Obesity, NASH	↓ ALT, ↓ steatosis	↓ Fasting glucose and insulin, ↑ weight	76
	Pioglitazone (24 weeks) and a hypocaloric diet	Obesity, T2DM, NASH	↓ ALT and AST, ↓ steatosis and inflammatory score	↓ Fasting glucose and insulin, ↑ insulin sensitivity, ↑ HDL cholesterol, ↑ weight	79
	Pioglitazone (96 weeks)	Obesity, NASH	↓ ALT and AST, ↓ NAS, ↑ NASH resolution	↓ Fasting glucose, insulin and triglyceride, ↑ insulin sensitivity, ↑ HDL cholesterol, ↑ weight	80,81
SGLT2 inhibitor	Canagliflozin (24 weeks)	Obesity, T2DM	↔ No changes	↓ Weight, ↓ HbA <sub>1c</sub> , fasting glucose and insulin, ↑ hepatic insulin sensitivity	88
	Dapagliflozin (8 weeks)	Obesity, T2DM	↓ Liver lipids, ↓ liver stiffness	↓ Weight, ↓ HbA <sub>1c</sub> , fasting glucose	89
	Dapagliflozin (24 weeks)	T2DM, NAFLD	↓ ALT	↓ Weight, ↓ HOMA-IR	90
	Empagliflozin (24 weeks)	Obesity, T2DM	↓ Liver lipids	↓ Weight, ↓ glucose	91
	Empagliflozin (20 weeks)	T2DM, NAFLD	↓ ALT, ↓ liver lipids	↔ No differences relative to control	92
FXR	Obeticholic acid (FLINT) (72 weeks)	Obesity, T2DM, NASH	↓ ALT and AST, ↓ steatosis and NAS, ↓ fibrosis	↓ Weight, ↑ insulin and HOMA-IR, ↓ LDL cholesterol, ↓ HDL cholesterol	105
	Obeticholic acid (REGENERATE) (72 weeks)	NASH (F1–F3 fibrosis)	↓ ALT and AST, ↓ fibrosis	Not analysed	106
	Cilofexor (GS-9674) (24 weeks)	Obesity, NASH	↓ Liver lipids	No significant changes	111

Unless otherwise stated, reported outcomes are differences from the placebo or control group. For non-alcoholic steatohepatitis (NASH), patients were diagnosed with NASH via histological analysis. Non-alcoholic fatty liver disease (NAFLD) is considered to be the presence of steatosis (histological or imaging (that is, MRI)) and/or elevated alanine transaminase (ALT) or AST (aspartate transaminase) levels. Steatosis is based on liver histology. ↓, decrease; ↑, increase; ↔, no change; DNL, de novo lipogenesis; DPP4, dipeptidyl peptidase 4; FXR, farnesoid X receptor; GLP1R, G protein-coupled GLP1 receptor; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NAS, NAFLD activity score; SGLT2, sodium–glucose cotransporter 2; T2DM, type 2 diabetes mellitus. <sup>a</sup>Uncontrolled T2DM: patients with T2DM not effectively managed with oral anti-diabetic therapy. <sup>b</sup>Results are comparisons within treatment group, relative to baseline. <sup>c</sup>Results not statistically analysed. Liver lipids are based on MRI-PDFF (or ultrasonography).

In a phase IIb clinical trial (GOLDEN-505), elafibranor promoted moderate resolution of NASH; however, some patients developed elevated serum levels of creatinine and renal impairment<sup>115</sup>. A larger phase III trial is under way but interim results showed no statistically significant improvement in histological NASH endpoints compared with placebo<sup>116</sup>. The use of lanifibranor (IVA337), a pan-PPAR agonist, improved glucose metabolism, steatosis and hepatocyte injury in multiple mouse models of NASH<sup>117</sup>. A phase II trial might demonstrate that lanifibranor was effective in promoting the resolution of NASH with improved fibrosis in patients with T2DM and NASH<sup>118</sup>; however, the peer-reviewed publication for this work is yet to be published. A dual PPAR $\alpha$ / $\gamma$  agonist, saroglitazar, reduced hepatic steatosis and inflammation and prevented fibrosis in mice<sup>119</sup> and improved insulin sensitivity in patients with T2DM<sup>120</sup>. Trials in patients with NASH are ongoing (NCT03061721 and NCT03863574). Collectively, experimental PPAR agonists demonstrate some efficacy in NAFLD but their use might be hobbled by adverse effects attributable to PPAR $\gamma$  and PPAR $\delta$  activation.

**THR $\beta$  agonists.** The thyroid hormone receptor (THR) is a nuclear receptor that mediates various functions important for growth and metabolism. Two THR isoforms, THR $\alpha$  and THR $\beta$ , are encoded by the genes *THRA* and *THRB*, respectively<sup>121</sup>. While excess THR $\alpha$  activation might lead to cardiac abnormalities<sup>122</sup>, specific targeting of THR $\beta$ , the primary liver isoform, increases hepatic fatty acid oxidation and reduces steatosis and hyperlipidaemia in rats and mice with genetically induced or diet-induced T2DM<sup>20</sup>. The liver-targeted THR $\beta$  agonist MGL-3196 produced statistically significant reductions in hepatic lipid content and levels of liver enzymes and plasma lipids in patients with NASH but no change was observed in glucose or insulin levels<sup>123</sup>. Another THR $\beta$  agonist, VK2809, reduced hepatic steatosis in mice<sup>124</sup> and led to a decreased liver lipid content in patients with NAFLD<sup>125</sup>. While no changes in glucose homeostasis were observed, the beneficial effects of THR $\beta$  on liver lipid content and enzymes, coupled with the potential to improve cardiometabolic outcomes, has warranted an ongoing clinical trial (NCT04173065) to determine its efficacy in the resolution of NASH.

#### **Inhibitors of DNL**

**ACC inhibitors.** Acetyl-CoA carboxylases (ACCs) catalyse the committed step in DNL through the conversion of acetyl-CoA to malonyl-CoA (FIG. 3). Furthermore, the product of this reaction, malonyl-CoA, is a signalling molecule that suppresses fatty acid oxidation through the inhibition of carnitine palmitoyltransferase I. Thus, ACC inhibition could reduce hepatic lipid accumulation by suppressing DNL and stimulating fatty acid oxidation. ACC inhibitors improve hepatic steatosis and insulin sensitivity but increase plasma concentrations of triglyceride in mice<sup>126</sup>, rats<sup>127</sup> and humans<sup>126</sup>. The hypertriglyceridaemia is probably secondary to reduced long-chain polyunsaturated fatty acid concentrations, which promotes VLDL secretion<sup>126</sup>. In patients with NAFLD, the ACC inhibitors firsocostat (GS-0976)

and PF-05221304 resulted in reduced liver lipid content, stiffness and enzyme levels, yet both resulted in hypertriglyceridaemia<sup>128–131</sup>. Although somewhat efficacious, ACC inhibitor use might be derailed by the secondary consequence of causing hypertriglyceridaemia, but ongoing trials (NCT04399538) using combination therapies are exploring whether these adverse effects can be circumvented.

**FAS inhibitors.** Fatty acid synthase (FAS) is a critical enzyme in the DNL pathway that uses malonyl-CoA, synthesized by ACC, to form saturated long-chain fatty acids (FIG. 3). The hepatic expression and activity of FAS are upregulated in NAFLD and preclinical studies in obese mice demonstrate that FAS inhibition lowers liver levels of triglycerides and improves insulin sensitivity<sup>132</sup>. A phase I trial showed that the FAS inhibitor TVB-2640 reduced DNL, hepatic accumulation of lipids and blood alanine transaminase levels, when compared with baseline values after 10 days of treatment<sup>133</sup>. Unlike ACC inhibition, targeting the downstream enzyme FAS did not induce increased plasma levels of triglycerides in this early-stage trial. Findings released in 2020 from a phase II trial indicate that TVB-2640 reduces liver lipid content in patients with NASH without treatment-related adverse effects<sup>134</sup>, but the full results have not yet been reported.

**SCD1 inhibitors.** Stearoyl-CoA desaturase 1 (SCD1) is a fatty acid desaturase, highly expressed in lipogenic tissues, such as adipose and liver, that converts saturated fatty acids, such as palmitate and stearate, to mono-unsaturated fatty acids<sup>135</sup> (FIG. 3). SCD1 activity was elevated in proportion to liver lipid content in patients with NAFLD<sup>136</sup> and the genetic knockout of hepatic SCD1 expression effectively reduced fatty liver and insulin resistance in high-fat diet-fed mice and rats<sup>137</sup>. The liver-targeted SCD1 inhibitor, aramchol, reduced hepatic oxidative stress, inflammation and fibrosis in a mouse model of NASH<sup>138</sup> and decreased levels of lipids in the liver, liver enzymes and HbA<sub>1c</sub> in patients with NAFLD<sup>139,140</sup>. A phase III trial is currently evaluating the efficacy of aramchol in patients with NASH and fibrosis.

**DGAT inhibitors.** The final step in triglyceride synthesis, esterifying a fatty acyl-CoA moiety to diacylglycerol, is catalysed by diacylglycerol acyltransferase (DGAT) (FIG. 3). Two isoforms of this enzyme have been identified, DGAT1 and DGAT2, which have distinct patterns of expression and substrate specificity<sup>141</sup>. Early studies, using antisense oligonucleotides to reduce DGAT2 expression in diabetic db/db mice have shown reduced levels of steatosis and glucose but hepatocyte damage was exacerbated by lipotoxicity due to increased hepatic levels of free fatty acids<sup>142</sup>. By contrast, mice with liver-specific deletion of DGAT2 showed decreased liver levels of triglycerides without worsening hepatic inflammation<sup>143</sup>. Interestingly, the beneficial effects on hepatic steatosis were not associated with decreased plasma levels of glucose or insulin in this insulin-resistant mouse model. Clinically, an antisense oligonucleotide inhibitor of hepatic DGAT2 expression,

IONIS-DGAT2<sub>Rx</sub>, reduces liver levels of lipids in patients with T2DM and NAFLD but had no effects on the plasma levels of glucose or lipids<sup>144,145</sup>. A small-molecule DGAT2 inhibitor, PF-06865571, has also been shown to reduce levels of lipids in the liver in phase I trials<sup>146</sup>. Unfortunately, many patients receiving PF-06865571 experienced minor gastrointestinal events, such as diarrhoea, probably due to fat malabsorption. If this effect can be overcome, there seems to be great potential for DGAT2 inhibition as a treatment for NAFLD.

**Ketohexokinase inhibitors.** Ketohexokinase is an enzyme that catalyses the phosphorylation of fructose to fructose-1-phosphate, an important step in fructose metabolism (FIG. 3). Fructose overconsumption leads to elevated ketohexokinase expression, impaired fatty acid oxidation, increased DNL, hepatic steatosis and impaired insulin signalling, but this effect can be mitigated by the liver-specific deletion of ketohexokinase in high fructose-fed mice<sup>147,148</sup>. In early stage clinical trials, patients receiving the ketohexokinase inhibitor PF-06835919 had reduced lipid content in the liver but no effects on markers of insulin resistance were seen<sup>149,150</sup>. Longer phase II trials are under way to evaluate its efficacy in patients with NAFLD and T2DM (NCT03969719).

**MPC inhibitors.** In the past decade, the mitochondrial pyruvate carrier (MPC) was identified as a heterodimeric complex of two proteins, MPC1 and MPC2, which functions to transport pyruvate from the cytosol across the inner mitochondrial membrane<sup>151</sup> (FIG. 3). This is a required step in the conversion of carbohydrate to fatty acids via DNL. Unexpectedly, the MPC has been identified as a target of the potent insulin-sensitizing thiazolidinediones<sup>85</sup> and it has been postulated that thiazolidinedione-mediated inhibition of the MPC might mediate some of the beneficial pharmacology of these agents. The hepatocyte-specific deletion of MPC2 in mice, which also leads to the destabilization and degradation of MPC1, protects against hyperglycaemia by reducing gluconeogenesis and ameliorates fibrosis in a mouse model of NASH<sup>22,152</sup>. MSDC-0602K, a PPAR $\gamma$ -sparing thiazolidinedione with inhibitory effects on the MPC<sup>85</sup>, reduces the severity of liver injury and fibrosis in mice<sup>22</sup>. In a clinical trial, MSDC-0602K did not achieve primary histological endpoints but resulted in reduced hepatic steatosis, circulating levels of liver enzymes and other NASH biomarkers, with a markedly improved insulin sensitivity compared with placebo control in patients with biopsy-confirmed NASH<sup>153</sup>. Although the efficacy of this approach for reversing NASH is still unclear, this PPAR $\gamma$ -sparing thiazolidinedione might have beneficial effects on a variety of cardiometabolic risk factors with fewer adverse effects than traditional PPAR $\gamma$ -activating thiazolidinediones.

#### **Fibroblast growth factors**

**FGF19.** Fibroblast growth factor 19 (FGF19; mouse orthologue is FGF15) is a gastrointestinal hormone that regulates bile acid synthesis, glucose metabolism and hepatic fatty acid oxidation, and is known to be a target of FXR<sup>154</sup>. The overexpression or administration of

FGF19 in mice reduces adiposity and hepatic steatosis and improves glucose homeostasis by reducing lipogenic gene expression and increasing energy expenditure<sup>155</sup>. Levels of FGF19 are reduced in patients with NASH and the administration of an FGF19 analogue, NGM282, effectively decreased levels of hepatic lipid content and liver enzymes with no effect on blood levels of glucose<sup>156</sup>. However, NGM282 elevated levels of LDL cholesterol<sup>156</sup> and could potentially enhance the risk of CVD in these patients. Elevated LDL cholesterol levels are possibly a result of FGF19-mediated suppression of cholesterol 7 $\alpha$ -hydroxylase (CYP7A1)<sup>157</sup>, the rate-limiting enzyme that promotes cholesterol disposal through its conversion to bile acids. This adverse effect is similar to that seen with FXR agonists but the dual use of NGM282 with a statin produced adequate management of cholesterol levels in patients with NASH in a clinical trial<sup>158</sup>. Though histological improvements have been observed in patients in a small study<sup>159</sup>, the long-term safety and effectiveness of NGM282 remains to be determined.

**FGF21.** FGF21 acts as a hormone to regulate food preference, intermediary metabolism and energy expenditure<sup>154</sup>. The expression of FGF21 is activated by the PPARs but is also induced in response to a variety of stressors, including high levels of fructose, liver injury or long-term fasting<sup>154</sup>. Interestingly, patients with NAFLD have elevated FGF21 levels that are inversely correlated with impaired insulin sensitivity<sup>154,160</sup>, suggesting an FGF21-resistant state<sup>161</sup>. However, high doses of recombinant FGF21 effectively reduced lipid content in the liver, plasma concentrations of lipid and body weight, and improved glucose and insulin intolerance in obese mice and rhesus macaques<sup>162,163</sup>. These effects were largely driven by the enhanced energy expenditure and diminished hepatic expression of lipogenic enzymes. In patients with NASH, treatment with recombinant PEGylated FGF21 (BMS-986036) improved plasma levels of glucose and lipids, decreased steatosis scoring and lowered markers of liver injury compared with placebo control<sup>164,165</sup>. The added benefit of reduced cardiometabolic parameters with improvements in liver steatosis and injury make this FGF21 analogue an attractive option and it is currently being investigated in patients with NASH and advanced fibrosis (NCT03486912 and NCT03486899).

#### **Conclusions**

NAFLD is the most common form of liver disease yet there are no drugs licensed for its treatment. Due to the close association between NAFLD and T2DM, many agents that are currently prescribed for hyperglycaemia have yielded positive results on NASH biomarkers. Experimental agents that target aspects of intermediary metabolism have also proven beneficial to varying degrees but adverse effects might limit their use. The overall benefit of metabolic-targeted therapies for NAFLD and NASH is attractive as these drugs might also reduce other cardiometabolic risk factors that contribute to CVD and complications of T2DM, which are the leading cause of mortality in patients with NAFLD and NASH.

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#### Author contributions

The authors contributed equally to all aspects of the article.

#### Competing interests

B.N.F. is a stockholder and member of the scientific advisory board of Cirius Therapeutics Inc., which is developing MSDC-0602 for the treatment of NASH. D.F. declares no competing interests.

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