Nonalcoholic steatohepatitis: the role of peroxisome proliferator-activated receptors

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TOC blurb

This Review describes the pathophysiological roles of peroxisome proliferator-activated receptors (PPARs) in nonalcoholic steatohepatitis (NASH) and related metabolic diseases, and summarizes the preclinical and clinical data on the use of PPAR agonists to treat NASH as part of a systemic metabolic disease.

Abstract

The increasing epidemic of obesity abound the world is linked to serious health effects, including increased prevalence of type 2 diabetes mellitus, cardiovascular disease and nonalcoholic fatty liver disease (NAFLD). NAFLD is the liver manifestation of the metabolic syndrome and includes the spectrum of liver steatosis (known as nonalcoholic fatty liver) and steatohepatitis (known as nonalcoholic steatohepatitis), which can evolve into progressive liver fibrosis and eventually cause cirrhosis. Although NAFLD is becoming the number one cause of chronic liver diseases, it is part of a systemic disease that affects many other parts

of the body, including adipose tissue, pancreatic β-cells and the cardiovascular system. The pathomechanism of NAFLD is multifactorial across a spectrum of metabolic derangements and changes in the host microbiome that trigger low-grade inflammation in the liver and other organs. Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear regulatory factors that provide fine tuning for key elements of glucose and fat metabolism, and regulate inflammatory cell activation and fibrotic processes. This Review summarizes and discusses the current literature on NAFLD as the liver manifestation of the systemic metabolic syndrome and focuses on the role of PPARs in the pathomechanisms, as well as in the potential targeting, of disease.

[H1] Introduction

With an estimated global prevalence of 25%[1], nonalcoholic fatty liver disease (NAFLD) is defined by evidence of steatosis in ≥5% of hepatocytes according to histological analysis or imaging in the absence of secondary causes of hepatic fat accumulation such as clinically significant alcohol consumption[2,3]. NAFLD can be subdivided into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NASH is characterized by hepatic steatosis accompanied by lobular inflammation and ballooned hepatocytes (as a marker of hepatocyte injury), with or without hepatic fibrosis, whereas NAFL is defined as hepatic fat content of ≥5% of liver weight or ≥5% fat-loaded hepatocytes with no evidence of hepatocellular injury (namely, ballooning) or fibrosis.[2,3] An estimated 10–25% of patients with NAFLD have NASH, and up to 25% of patients with NASH progress to cirrhosis, liver failure and, more rarely, hepatocellular carcinoma (HCC)[3,4]. By the end of 2020, NASH is expected to be the leading cause of liver transplantation in the United States[5], and NAFLD-related mortality and morbidity in terms of advanced liver disease will more than double from 2016 to 2030[6].

NAFLD is part of a multisystem disease with ramifications that extend beyond the liver[7]. Morbidity and mortality are also related to the effect of the diseased liver on the cardiovascular system[8] and on organs involved in glycaemic control. Patients with NAFLD are two to five times more likely to develop type 2 diabetes mellitus (T2DM) after adjustment for multiple confounders than those without NAFLD[9,10], and NAFLD can add to coexisting metabolic risk factors[11]. Conversely, patients with T2DM are also at a high risk of NAFLD: the global prevalence of NAFLD among patients with T2DM exceeds 55%[12].

The liver is involved in the pathogenesis of the metabolic syndrome (Table 1), or components of the metabolic syndrome, which frequently occurs in >50% of patients with NAFLD[13,14]. The liver is a key organ that is affected by nutritional overload (so-called substrate-overload liver injury), adipose tissue dysfunction, insulin resistance and gut dysbiosis[15]. The role of liver disease in the pathogenesis of T2DM and cardiovascular disease (CVD) shows that it is a driving force of a vicious circle[16,17]. For example, NAFLD increases the risk of T2DM[18] and CVD[19]. With the development of T2DM, there is a further increase in risk of CVD[20,21], a worsening of liver disease (to fibrosis and cirrhosis)[22-24] and an increased risk of HCC[25]. The development of advanced liver fibrosis with NAFLD also increases the risk of CVD[26]. Figure 1 illustrates the relationships between NAFLD, T2DM and the metabolic syndrome, CVD and HCC.

Lifestyle interventions that lead to weight loss are recommended for the treatment of NASH[2,3], but these interventions are difficult to sustain and are not even always sufficient (Box 1). Currently, the only commercially available pharmacological options for NASH supported by clinical trials with histological endpoints are vitamin E and pioglitazone (and to a lesser extent liraglutide), but these are not approved by the Food and Drug Administration (FDA) for the treatment of NAFLD[27-29]. Therefore, there is a search for treatments across a

broad spectrum of new pharmacological agents that have a plethora of mode of actions and that target a variety of pathways (previously reviewed[30,31]). Of particular interest are peroxisome proliferator-activated receptors (PPARs) because they are key regulators of lipid and glucose metabolism, as well as of inflammation in different tissues. Their differential expression and role in the liver as well as in the adipose tissue, skeletal muscle, vessel wall and pancreas make them highly relevant to NAFLD when it is considered part of a systemic metabolic disease[32]. Thus, PPARs represent interesting therapeutic targets both in a liver-centred approach as well as in a systemic approach to NAFLD, in terms of improving liver function and liver, cardiovascular and diabetes-related outcomes. This Review aims to comprehensively summarize the current pre-clinical and clinical knowledge on the varied roles of PPARs in the pathophysiology of NASH when viewed as an integrated part of metabolic and cardiovascular derangements, a framework that must serve as a rationale for their use in the treatment of this condition. Thus, this Review examines the pivotal elements in the pathogenesis of NASH (taking into account the systemic disease approach), the role of PPARs as key regulators of fatty acid metabolism, inflammation and fibrosis in the liver and in extrahepatic tissues, and the potential therapeutic advantages of the wide-ranging actions of PPAR agonists in the systemic ramifications of NAFLD.

[H1] Clinical implications of NAFLD

NAFLD is one part of the consequences of caloric overload and sedentarism and is therefore commonly referred to as the hepatic manifestation of the metabolic syndrome, a cluster of cardiovascular risk factors that include central obesity, increased plasma glucose and triglyceride concentrations, decreased high-density lipoprotein cholesterol (HDL-C) concentration, increased blood pressure and central obesity, which is therefore a very common condition in adults with NAFLD, with a prevalence of >50% in patients with NASH and significant fibrosis[1,7,11,33]. The relationships are multidirectional, with NAFLD being in part both the consequence and cause of metabolic and cardiovascular derangements. Adipose tissue dysfunction has been identified as an important driver of disease in a multisystem ‘metabolic’ disorder in which the liver, pancreas, muscle, gut and the cardiovascular system are implicated in a complex crosstalk[15,34]. Established T2DM in adults with NAFLD is a strong clinical risk factor for the more progressive forms of NAFLD, such as NASH with fibrosis [7,11,33]. Insulin resistance occurs in almost all adults [7,11,33] and children [35,36] with NAFLD. T2DM occurs in approximately 70% of patients with NAFLD[33], and a 2016 study reported that the prevalence of prediabetes and T2DM was 23.4% and 6.5%, respectively, in children and adolescents with obesity and biopsy-confirmed NASH[37]. This close entanglement between fatty liver disease and metabolic derangements have led to a proposal by a group of experts to redefine and rename NAFLD as metabolic (dysfunction)-associated fatty liver disease (MAFLD), and although this terminology has not yet been widely approved, it reflects the growing understanding of NAFLD as being part of this multisystem disorder of largely overlapping ‘metabolic’ conditions[38].

Consequently, current European Association for the Study of the Liver (EASL)[2] and American Association for the Study of Liver Diseases (AASLD)[3] guidelines recommend that patients with NAFLD be thoroughly investigated for these comorbid conditions. Components of the metabolic syndrome and cardiovascular risk factors have to be checked and treated according to their proper guidelines (Table 1).

The concept of NAFLD being part of a metabolic multisystem disease implies that patients with NAFLD need not only a liver-centred approach, but also in parallel a multidisciplinary approach. When it comes to specific therapies, these aspects also come into play. Evidently, therapies need to be safe from a metabolic and cardiovascular point of view[39]. The effect on these different co-morbid conditions of different pharmacological approaches (which are to

some extent artificially split into anti-metabolic, anti-inflammatory and anti-fibrotic strategies[31]) will potentially become increasingly important, not only in terms of safety but also in terms of efficacy. Compounds that target not only the intrahepatic processes of damage, inflammation and fibrogenesis, but that also have an effect on systemic inflammation, metabolic factors and the cardiovascular system might result in a greater overall improvement in outcomes compared with compounds with a more liver-restricted mode of action. Another consideration that emerges from this systemic approach is the growing awareness that part of the hepatic improvement observed with pharmacological treatment is an indirect result of extrahepatic inflammatory and metabolic improvements that subsequently benefit the liver[30,31]. Thus, this holistic, systemic approach towards NAFLD is relevant to the identification of suitable targets for pharmacological treatment of NAFLD and NASH.

[H1] Key mechanisms of NASH pathophysiology

In this section, we describe the key mechanisms involved in the development of insulin resistance and hepatic lipid accumulation, dyslipidaemia and inflammation that are part of the pathogenesis of NASH.

[H2] Insulin resistance

Insulin resistance can result from certain inherited factors but mainly from acquired factors such as obesity and specifically ectopic fat accumulation in visceral organs such as liver[15]. Insulin resistance is primarily characterized by increased lipolysis in dysfunctional adipose tissue and reduced glucose uptake in skeletal muscle[9,40]. Adipose tissue dysfunction comprises local inflammation and impaired anti-lipolytic action of insulin, resulting in an increased release of free fatty acids (FFA) and glycerol[40,41]. Higher hepatic uptake of FFA contributes via fatty acid esterification, while glucose uptake contributes to a lesser extent via de novo lipogenesis, to accumulation of triglycerides in liver and VLDL release, and thereby paves the way to steatosis (NAFL) and dyslipidaemia[42]. Increases in hepatic lipid metabolites, such as diacylglycerols and ceramides, interfere with insulin signalling and thereby lead to hepatic insulin resistance[9,40,43]. Insulin resistance is strongly associated with NAFLD, but its correlation with NASH severity is still unclear[44]. Three-quarters of people with obesity and T2DM have NAFLD[45], but it is unclear whether liver fat accumulation is a consequence or cause of insulin resistance. Nevertheless, increased delivery of plasma FFA from adipose tissue to the liver accounts for ~60% of all fatty acid incorporation into liver triglyceride[41,42], underlining the important role of adipose tissue in hepatic lipid accumulation, insulin resistance and increased VLDL secretion, therefore linking the pathogenesis of NASH to a key aspect of the atherogenic dyslipidaemia of metabolic syndrome (see later).

The development of hepatic damage in people with NASH requires both extrahepatic and intrahepatic factors, which highlights the involvement of multiorgan systems in the disease. Chronic adipose dysfunction creates an imbalance between the release of anti-inflammatory insulin-sensitizing cytokines (such as adiponectin) and pro-inflammatory cytokines (such as interleukin-6 (IL-6) and tumour necrosis factor (TNF)) that activate inflammatory pathways and contribute to insulin resistance in the liver[46].

[H2] Lipid metabolism and lipotoxicity in NASH

Lipotoxicity caused by lipid metabolites (such as diacylglycerols, ceramides and acylcarnitines) is generally thought to have a central role in the pathogenesis of NASH[41,47-49]. According to the substrate-overload liver injury model of NASH pathogenesis, the liver's capacity to handle the primary metabolic energy substrates, carbohydrates and fatty acids, is overwhelmed in NASH, leading to an accumulation of toxic lipid species[50]. In hepatocytes, fatty acids are metabolized via mitochondrial β-oxidation, or undergo re-esterification into

triglycerides that are then exported from the liver as VLDL or stored in hepatic lipid droplets. The latter undergo lipolysis and release fatty acids back into the hepatocyte FFA pool[15,41,42]. Excess accumulation of fatty acids leads to the formation of lipotoxic species, resulting in endoplasmic reticulum stress, oxidant stress and inflammasome activation.[9,40,51]

NAFLD is defined by an accumulation of triglycerides in the liver. This accumulation of liver triglycerides can occur for several different reasons, and the sources of fatty acids that are required for the generation of hepatic triglycerides are principally derived from adipose tissue[52,53]. For example, it has been estimated that of the triglycerides accounted for in the human liver, 59.0% ± 9.9% arise from adipose tissue-generated FFAs, 26.1% ± 6.7%, from hepatic de novo lipogenesis, and 14.9% ± 7.0% from the diet[54]. Consequently, with increasing adipose tissue mass and specifically with the accumulation of dysfunctional and insulin-resistant adipocytes, release of FFA to the liver provides the substrate for and stimulus to hepatic lipid accumulation, leading to insulin resistance, atherogenic dyslipidaemia, T2DM, atherosclerosis and increased CVD risk [47,55,56]. In addition, activated adipose tissue macrophages are also important in promoting adipose tissue insulin resistance, excess lipolysis and FFA release from adipocytes, and subsequent liver fat deposition.[57]

[H2] Inflammation in NASH

NASH is characterized by inflammation superimposed on hepatic steatosis, but inflammation is also present in other organs, which once again indicates systemic involvement [58]. Inflammation is the innate immune system's response to invading pathogens and to sterile tissue injury. In normal homeostasis, anti-inflammatory cytokines and natural tissue repair mechanisms resolve inflammation. In NASH, however, the sustained presence of metabolic danger signals maintains inflammation[59-61]. Multiple sequential and parallel triggers contribute to inflammation and create self-perpetuating low-grade hepatic and systemic inflammation[58].

Well-characterized triggers of liver inflammation include sterile danger signals such as saturated fatty acids and oxidized cholesterol, which cause lipotoxicity in hepatocytes, apoptosis and other types of hepatocellular death[62]. Alteration of intracellular signalling pathways during inflammation affects nuclear receptors such as PPARs, nuclear factor-κB (NF-κB) activation and microRNAs[63]. Damaged hepatocytes in turn release other sterile metabolic danger signals, including uric acid, high mobility group box 1 (HMGB1) and mitochondrial DNA, which activate a variety of pattern recognition receptors and trigger downstream signalling of the central NF-κB-mediated mechanisms for pro-inflammatory cytokine and chemokine production, and macrophage activation[59-62,64,65].

Abnormalities in the gut–liver axis have also been linked to NAFLD and NASH. For example, the importance of an altered gut microbiota has been extensively studied in NASH[66,67]. Increased gut permeability due to inflammation in the intestinal wall results in increased levels of pathogen-associated molecular patterns in the systemic circulation and liver[68].

[H2] Immune cells in the development of NASH

Activation of Kupffer cells, the liver's resident macrophages, in combination with recruitment of pro-inflammatory, monocyte-derived macrophages and neutrophil leukocytes from the circulation, characterize NASH-related changes in the immune cell populations of the liver[59,65]. Parallel to the strong accumulation of macrophages, their polarization appears 'pro-inflammatory' (often termed M1) at the expense of anti-inflammatory and repair macrophages (often termed M2), likely due to the high abundance of pro-inflammatory cytokines such as interferon-γ, TNF and lipopolysaccharide (LPS) in NASH[62] However, the classical M1/M2 paradigm does not fully capture hepatic macrophage populations in NAFLD, as the resident

and recruited macrophages rapidly and precisely adapt to the hepatic environment both in mice and humans[69,70]. Single-cell RNA sequencing analyses of mouse models of NASH have indeed revealed a common and unique inflammatory 'NAFLD phenotype' that is consistent across myeloid cells (monocytes, macrophages and dendritic cells) in the liver as well as in the bone marrow[71]. These inflammatory myeloid cells perpetuate hepatic inflammation, leading to hepatocyte death, activation of hepatic stellate cells (HSCs) and myofibroblasts that drive fibrogenesis, progressive liver damage, inflammation and fibrosis in NASH[61,65]. Importantly, PPARγ and PPARβ/δ activation (these isotypes are described in the next section) is a trigger for anti-inflammatory or repair-promoting macrophage polarization[72]. Pharmacological PPARβ/δ agonism reduced NAFLD-associated inflammatory macrophage activation in mouse models as well as in circulating monocytes from patients with NASH[73].

[H1] PPARs: metabolic pathway regulators

PPARs were first described as members of the steroid hormone receptor superfamily of ligand-activated transcription factors that cause proliferation of peroxisomes[74,75]. Peroxisomes have an important role in fatty acid catabolism and in the pentose phosphate pathway, and hence in energy metabolism. They also have a role in the reduction of ROS[76]. However, extensive research has subsequently revealed that PPAR signalling pathways involve several other cell organelles, most notably mitochondria, and that PPARs have pleiotropic actions, which ultimately makes them critical regulators not only of fatty acid metabolism[74], but also of glucose metabolism, inflammation and fibrogenesis[74].

Three PPAR isotypes have been identified—α, β/δ and γ (with two subtypes: γ1 and γ2)[77,78]—the expression and actions of which differ according to isotype, organ and intra-organ cell type, resulting in a complex system of nuclear receptor-mediated inter-organ crosstalk[79] (Figure 2). Furthermore, substantial inter-species differences exist and need to be taken into account to translate findings from pre-clinical studies to patients[76]. The importance of inter-species differences has best been documented for PPARα. Although latest reports indicate that human and mice liver PPARα expression is comparable (in contrast to previous reports suggesting a lower PPARα expression in human liver), PPARα activity has been repeatedly shown to be lower in human livers compared with rats and mice[80-83]. Also, differences between murine and human, but also guinea pig livers, have been found at the level of PPARα expression, ligand activation and biological response[84]. This interspecies difference is particularly relevant when it comes to the role of peroxisome proliferation and tumorigenesis (and hence safety of PPARα-targeting drugs, which has been demonstrated in rats and mice, whereas PPARα activation failed to induce peroxisome-proliferator genes in human hepatocytes)[85-87]. PPARγ is more conserved across species than PPARα, although some differences, for example in brown adipose tissue, have been documented[88,89]. Liver PPARγ expression, which is low compared with adipose tissue expression, is induced by obesity in mice (specifically, the γ2 subtype[90]), but no increased expression has been observed in human liver in relation to NASH[91].

[H2] Steatosis and beyond

The main ligands for PPARs are fatty acids and their metabolites. Endogenous ligands can result from lipogenesis, lipolysis and fatty acid catabolism, which explains the reciprocal effects between, for example, PPARα pathways and acyl-CoA oxidase 1 (implicated in peroxisomal β-oxidation), fatty acid synthase (FAS, implicated in de novo lipogenesis) or hepatic adipose triglyceride lipase (implicated in triglyceride hydrolysis)[92-94].

PPARα, which is encoded by *NR1C1* on human chromosome 22, binds to a wide range of saturated and unsaturated fatty acids, whereas the other PPAR isotypes have a lower

affinity that is mainly restricted to polyunsaturated fatty acids[95]. PPARα is predominantly expressed in tissues with a high rate of fatty acid oxidation, such as skeletal muscle, liver, heart, kidney and brown adipose tissue[79,96]. Within the liver, it is expressed mainly in hepatocytes[79,91,97]. In the vasculature, it is also expressed in various cell types within atherosclerotic plaques[98]. Notably, within the liver, expression has also been documented in sinusoidal endothelial cells and, at a lower level of expression, also in mice and human HSCs, which are maintained in the quiescent state by PPARα[99-102].

As a key regulator of fatty acid metabolism and ketogenesis, PPARα regulates fatty acid transport, peroxisomal and mitochondrial β-oxidation and lipolysis, and also influences the production of apolipoproteins[103]. The net overall result of PPARα-mediated lipid handling leads to a reduction of triglyceride-rich lipoproteins and triglyceride accumulation in the liver, whereas plasma HDL cholesterol (HDL-C) is increased[103]. In the fasting state, increased fatty acid oxidation produces acetyl-CoA, which is then converted into ketone bodies in a process involving mitochondrial hydroxymethylglutaryl-CoA synthase (HMGCS), which is upregulated by PPARα[104]. PPARα-deficient mice fed ad libitum have a mild phenotype, but the phenotype becomes more pronounced during fasting and is characterized by impaired fatty acid oxidation, lipid accumulation in the liver as well as an inability to augment ketone body synthesis, which indicates that PPARα is critically involved in the fasting state[105]. Transcriptomic studies in PPARα-deficient mice in a fasting or fed condition have confirmed that major changes in expression of its main target genes involved in fatty acid transport and catabolism, including peroxisomal and mitochondrial β-oxidation, in the liver occur in the fasting state [106]. Furthermore, PPARα seems to be implicated in the circadian clock[107-111]. It also regulates expression of a plethora of target genes by transactivation or transrepression (transrepression refers to the process of interaction with another nuclear factor, the activity of which is inhibited, resulting in reduced expression of its target genes; mainly inflammatory genes are transrepressed by PPARα), with approximately 50% conservation between mice and humans in terms of gene ontology categories[80]. This conservation across species is particularly relevant when it comes to drug development and translation of pre-clinical data into clinical studies.

These aspects of PPARα physiology are all relevant to NASH pathogenesis, and pre-clinical data point to an important role for alterations in PPARα, deficiency of which leads to more-severe NASH lesions[112], which PPARα agonists prevent or improve[97,113]. Mice with a PPARα mutant that only has transrepressive activity are protected against the development of NASH but not steatosis, whereas mice with wild-type PPARα are protected from both NASH and steatosis[104]. Clinical data are in line with these experimental findings, showing that liver PPARα expression inversely correlates with NASH severity and that improvement of liver histology positively correlates with increased hepatic PPARα expression[91].

PPARβ/δ also has an important role in liver metabolism. Encoded by *NR1C2* on human chromosome 6, it is expressed in hepatocytes, sinusoidal endothelial cells, HSCs and Kupffer cells[79]. PPARβ/δ activates pathways of glucose utilization and de novo lipogenesis in the liver. In PPARβ/δ-null mice, transcriptional profiling has revealed a downregulation of genes associated with lipoprotein metabolism and glucose utilization pathways, indicating that these genes are positively regulated by PPARβ/δ[106]. In addition, PPARβ/δ increases the production of monounsaturated fatty acids and protects against lipotoxicity and saturated fatty acid cytotoxicity in an *in vitro* setting[114]. Although PPARβ/δ and PPARα are both implicated in the fasting and fed state, PPARα seems to be predominantly important in the fasting state whereas PPARβ/δ is more equally involved in both[115]. Synthetic PPARβ/δ ligands can mimic natural activation of PPARβ/δ pathways, although a differential response might be seen in response to different ligands[116], as has been reported for other PPAR ligands, which has

been attributed to the different capacities of these ligands to recruit various coactivators or corepressors[117].

[H2] Key inflammatory regulators

All three PPARs participate in the regulation of the inflammatory process, a key component in the development of NASH (Fig. 3). PPARγ, which is encoded by *NR1C3* on human chromosome 3, binds to the p65 component of the NF-κB complex and induces inhibitory iκB, which attenuates NF-κB-driven inflammatory cytokine and chemokine production both in the liver and elsewhere[118]. Upon ligand stimulation, PPARγ promotes the M2 anti-inflammatory macrophage phenotype by upregulating CD206 and CD163[119]. In addition, PPARγ improves endothelial cell function by lowering inflammation in patients with diabetes and atherosclerosis[120], has a substantial role in controlling vascular homeostasis and decreases blood pressure in patients with T2DM, thereby decreasing risk of CVD[121,122].

PPARα also has anti-inflammatory properties, mainly through transrepression of pro-inflammatory target genes[123]. In humans, this transrepression occurs not only in the liver but also in isolated vascular endothelial cells, linking PPARα to systemic inflammation and atherosclerosis[118,124].

PPARβ/δ is highly expressed in hepatocytes, liver sinusoidal endothelial cells and hepatic macrophages including Kupffer cells both in rodents and humans[73,125-127]. It modulates expression of key genes involved in innate immunity and inflammation[106], although its role in inflammation is incompletely understood. Unligated PPARβ/δ has pro-inflammatory effects mainly in atherosclerotic models, and its ligand engagement disrupts PPARβ/δ-corepressor complexes and has anti-inflammatory effects, including suppression of pro-inflammatory adhesion molecules on vascular endothelial cells[128-130]. Ligand binding to PPARβ/δ drives Kupffer cells towards a more anti-inflammatory phenotype[131]. This contributes to hepatic insulin resistance and NASH in mice[132]. Conversely, increasing PPARβ/δ leads to alternatively activated Kupffer cells that have anti-inflammatory properties and results in less severe metabolic and hepatic derangements[131] (Figure 3).

[H2] Fibrosis regulation

Fibrosis is the strongest predictor of adverse clinical outcomes in NASH, including liver-related death and overall mortality[133,134]. Fibrogenesis in HSCs is inhibited by PPARs[135]. PPARγ normally maintains HSCs in a quiescent state, and its overexpression decreases their myofibroblastic character, resulting in reduced collagen production. Reduced expression of PPARγ results in progression of liver fibrosis and increased collagen production[99]. PPARγ and PPARβ/δ are expressed in a stimulus-dependent and tissue-dependent manner in macrophages[136], which are a key factor for fibrosis, as inflammatory macrophages activate (whereas restorative macrophages deactivate) HSCs[137]. PPARβ/δ activation in fibroblasts with increased α-smooth muscle actin production and myofibroblast differentiation improves wound healing in skin diseases and myocardial infarction through regulation of IL-1 signalling[138,139].

Human liver PPARα gene expression negatively correlates with NASH severity, visceral adiposity and insulin resistance[91]. Interestingly, transcriptomic analysis of liver biopsy samples from patients with obesity and NASH before and after bariatric surgery combined with transcriptomic datasets from mice models of NASH and fibrosis identified common clusters of genes with specific functions in inflammation and extracellular matrix homeostasis and in particular a role for PPARα-regulated dermatopontin in NASH-related fibrogenesis[140].

Dermatopontin is a protein involved in fibrogenesis and collagen deposition and its expression is lowered by PPARα activation[140,141].

[H2] Non-hepatic tissue in NASH

PPARs, in particular PPARγ, have a key role in adipocyte biology and adaptation to nutrient supply[142,143]. PPARγ is highly expressed in adipose tissue, where it has an essential role in the regulation of adipocyte differentiation, adipogenesis and lipid metabolism[56]. As with the other PPARs, its distribution and actions are complex, and differ also for its two subtypes. PPARγ1 is widely distributed in skeletal and cardiac muscle and the vascular bed, as well as in macrophages, colon epithelium and adipose tissue, whereas PPARγ2 is predominantly expressed in adipose tissue[78].

In central obesity in humans, there is a switch in gene expression within adipocytes to a pattern that more closely resembles that of macrophages[47]. Thus, excess lipid storage associated with obesity promotes adipose tissue inflammation. In obesity, there is also an increased flux of free fatty acids from adipose tissue to the liver and to other organs, as well as an increase in secretion of proinflammatory adipokines[144]. The combination of altered adipokine secretion and increased flux of free fatty acids promotes the development of ectopic triglyceride accumulation and an increase in the synthesis of toxic lipid mediators in tissues other than adipose, such as liver, muscle and possibly pancreas[145].

Interventions that reduce fat mass or adipocyte hypertrophy (weight loss) or pharmacologically improve the insulin sensitivity of adipose tissue (such as thiazolidinediones (TZDs))[28,146-148] restore adipose tissue biology and are beneficial in NAFLD given the dynamic crosstalk between the liver and adipose tissue, which adapts to day-to-day changes in energy needs. In humans, at least two-thirds of fatty acids reaching the liver are released from subcutaneous fat[47]. In NAFLD, there is a strong linear relationship between the severity of adipose tissue insulin resistance and that of hepatic steatosis[44], and patients with steatohepatitis tend to have worse insulin resistance than those with isolated steatosis[149]. However, once patients have developed steatosis, this is closely associated with severe hepatic and muscle insulin resistance, atherogenic dyslipidaemia (elevated triglycerides and low HDL-C), and even hepatocyte necroinflammation[44].

[H1] PPAR-targeted treatment for NASH

[H2] PPAR agonist effects on liver

Owing to their key role in the transcriptional regulation of glucose and lipid metabolism, PPAR ligands hold promise as therapeutic agents for NAFLD[56]. Despite pre-clinical rationale[150], clinical data on PPARα single agonists are scarce. The PPARα agonist fenofibrate reduces lipid levels by activating PPARα, which is highly expressed in the liver, but has no effect on insulin sensitivity[151] or magnetic resonance imaging (MRI)-assessed hepatic steatosis[152]. Rodents and humans differ substantially in terms of the differential expression and roles of the different PPAR isotypes[76,85-87,153], which might in part explain why pre-clinical data on the efficacy of isolated PPARα agonism[150] have not to date been translated into histological improvement in patients with NASH. As receptor binding and subsequent effects might substantially differ between ligands (which is known as the selective PPAR modulator concept[154]), studies are ongoing with other compounds. In a controlled prospective study of 46 patients with NASH, the PPARα agonist gemfibrozil was shown to improve lipid profiles[155,56]. Pemafibrate, which also showed benefits in terms of liver enzymes and liver histology in pre-clinical NAFLD models and in patients with diabetes and dyslipidaemia, is currently under clinical investigation for NAFLD treatment[156-158] (Table 2).

PPARγ activation by TZDs in humans is associated with a broad spectrum of metabolic effects in great part derived from restoring adipose tissue biology[47,159] and with a decrease in chronic systemic inflammation[142,143], changes that are strongly associated with an improvement in liver histology in patients with NASH[160]. In patients with biopsy-proven NASH, rosiglitazone improves hepatic steatosis and serum alanine aminotransferase (ALT) levels, but not other histological features of NASH, including fibrosis after 1 year[161] or 2 years[162] of therapy. In rat models of fibrosis, pioglitazone prevented choline-deficient diet-induced fibrosis, but was ineffective once hepatic fibrosis was established[163]. In 55 patients with prediabetes or T2DM, pioglitazone 45 mg once daily for 6 months improved NASH. Mean fibrosis score decreased significantly in the pioglitazone group ($P = 0.002$) but, although this was not the case in the placebo-treated group, the difference in change from baseline between the placebo group and the pioglitazone group did not reach statistical significance ($P = 0.08$)[146]. This trial was followed by an 18-month randomized controlled trial (RCT) of 101 patients with biopsy-proven NASH, showing a significant treatment benefit in terms of reduction of the NAFLD activity score (NAS) of ≥2 points, resolution of NASH and reduction in mean fibrosis score, along with improvement in metabolic endpoints[147]. In an RCT of 105 patients with T2DM, pioglitazone plus vitamin E improved steatosis, hepatocyte ballooning and inflammation compared with vitamin E alone or placebo[164]. In patients with histologically proven NASH but without diabetes, pioglitazone 30 mg once daily for 12 months was reported to improve hepatic fibrosis[148], but this was not observed in another study, in which pioglitazone improved all other individual histological parameters except for fibrosis and induced resolution of NASH in 47% of patients (n = 80) compared with 21% in the placebo arm (n = 83)[28]. A meta-analysis indicated that pioglitazone, but not rosiglitazone, significantly reduces fibrosis in patients with NASH.[27]

Why pioglitazone and rosiglitazone have drastically different efficacy in reversing steatohepatitis remains unclear, but it is often attributed to pioglitazone also being a weak agonist of the PPARα isotype[165]. However, the action of PPARα agonists alone seems unlikely to explain the broad effects of pioglitazone on liver histology in NASH. Pioglitazone improves mitochondrial function, for example by downregulation of the tricarboxylic acid cycle flux[166], but there are many other potential mechanisms by which it might have beneficial effects on the liver (for example, by modulating branched chain amino acid metabolism and decreasing the accumulation of several ceramides)[142,143,167,168]. Evidently, each PPARγ agonist has a unique cardiometabolic signature and biology in the liver.

The PPARα/γ dual agonist saroglitazar has beneficial effects in experimental models of NASH[169]. A meta-analysis of the use of saroglitazar in patients with diabetic dyslipidaemia in 318 non-invasively diagnosed patients with NAFLD demonstrated that it produced a statistically significant decreases in ALT levels (and liver stiffness in some patients) and improved cardiometabolic profiles[170]; whereas positive results from India on liver histology in patients with biopsy-proven NASH have been announced but not fully released[171]. Preliminary results of a randomized, double-blind, phase II trial with non-invasive endpoints (EVIDENCES II; NCT03061721) have also been released, showing that it met its primary and secondary endpoints (reduced liver fat, liver enzymes and disease activity on liver histology), but final results have yet to be published[172,173]. The selective PPARβ/δ agonist seladelpar (MBX-8025) improves insulin sensitivity and steatohepatitis in mouse models of NAFLD[174]. In humans, its effect is more on atherogenic dyslipidaemia (for example, a reduction of apolipoprotein B-100 by 20–38% and LDL cholesterol by 18–43%)[175] and is rather modest on insulin sensitivity or steatosis compared with PPARβ/δ agonists. Preliminary results from 171 patients with NASH from a phase II, double-blind RCT [176], with change in liver fat measured by MRI proton density fat fraction (MRI-PDFF) as the primary endpoint, showed three doses of seladelpar (10 mg, 20 mg and 50 mg) to be worse than placebo (a 9.8%, 14.2% and 13% reduction versus baseline, respectively, compared with a 20.8% reduction from baseline with placebo). There was, however, a significant dose-response reduction in ALT and γ-glutamyl

transferase (GGT) levels[177]. The clinical development of seladelpar in liver diseases has, nevertheless, been halted because of atypical findings, including interface hepatitis, in 52-week end-of-treatment biopsies in this NASH trial [178].

In mouse and rat models of NASH and/or liver fibrosis, the dual PPARα/δ agonist elafibranor reduced liver fibrosis progression[179]. In a phase IIb study of 274 patients with biopsy-proven NASH but not cirrhosis, elafibranor 120 mg once daily was superior to placebo in achieving reversal of NASH (requiring one of the three components to have a score of 0) without worsening of fibrosis (20% versus 11%; *P*=0.018) in patients with higher baseline NAS (≥4)[180]. Furthermore, in a secondary post-hoc analysis based on a revised definition for the resolution of NASH requiring the disappearance of ballooning but allowing the persistence of a minor degree of lobular inflammation (a NAS of ≤1) without worsening in liver fibrosis (progression by ≥1 stage), this endpoint was met in 19% of patients receiving elafibranor 120 mg daily (*P*=0.045) compared to 12% of patients receiving placebo [180]. Furthermore, patients whose NASH improved also had improved fibrosis. Elafibranor has a positive effect on hepatic and muscle insulin sensitivity [181], and on steatohepatitis in patients with NASH[180]. Its efficacy and safety in patients with NASH and fibrosis but no cirrhosis have been evaluated in a phase III trial[182]. Interim results on 717 patients treated with 120 mg of elafibranor for 72 weeks versus 353 placebo-treated patients in intention-to-treat failed to reach the primary histological endpoint of NASH resolution without worsening of fibrosis, but detailed analyses have not been disclosed yet and the trial has been halted [183].

Activation of PPARβ/δ results in modulation of lipid and glucose homeostasis, skeletal muscle function and brown adipose tissue activity, and PPARβ/δ agonists have been used to successfully treat fibrosis in preclinical animal studies[184].

[H2] Cardiovascular effects of PPAR agonists

PPARα, PPARβ/δ and PPARγ agonists improve endothelial dysfunction and regulate multiple pathways involved in subclinical inflammation and atherosclerosis[121,122]. PPARγ is also highly expressed in atherosclerotic lesions, and its activation reduces inflammatory pathways in cardiomyocytes and in the vascular bed in animal models[185-187].

The TZDs rosiglitazone and pioglitazone prevent the progression of prediabetes, which affects many patients with NAFLD[188], to T2DM[189,190]. TZDs also exert longer-lasting glycaemic control than metformin or glibenclamide[191]. Rosiglitazone and pioglitazone increase HDL-C, and rosiglitazone (but not pioglitazone) increases LDL-C and has no effect on plasma triglycerides, which are reduced with pioglitazone treatment[192,193]. This observation might account for the reduction of atherosclerosis progression[189,194,195] and reduction of CVD risk[196-198] observed with pioglitazone in patients with T2DM. Moreover, in patients with prediabetes and good adherence to treatment (intake of ≥80% of prescribed dosage; *n* = 644), pioglitazone reduces stroke by 36%, acute coronary syndromes by 53%, and the combined endpoint of stroke or myocardial infarction or hospitalization for heart failure by 39%[199]. There is still a misperception that rosiglitazone increases the risk of death from CVD because of a controversial meta-analysis[200,201]. A large RCT found no such increase in the overall risk of cardiovascular morbidity or mortality in people with T2DM treated with rosiglitazone during a mean of 5.5 years of follow up [202], a conclusion shared in 2013 by the FDA, which led to the removal of regulatory restrictions on rosiglitazone [203].

Use of the selective PPARβ/δ agonist seladelpar improved the lipid profile of 166 overweight or obese patients with dyslipidaemia and increased risk of CVD [204], but fibrates (PPARα agonists) have been more broadly tested in large RCTs and are often associated with reduction in CVD[205,206]. Also, dual PPARα/γ agonism by saroglitazar improves cardiovascular risk profile[170,207]. The dual PPARα/δ agonist elafibranor improved glycaemic control and lipid profile in patients with NASH[180].

[H2] Pan-PPAR agonists

Taken together, the concept of combining PPARα, PPARβ/δ and PPARγ activation might represent a novel and potentially more efficacious therapeutic approach by targeting the large array of pathways that contribute to the development and progression of NASH[208]. Lanifibranor (IVA337) is an indole sulfonamide PPAR agonist that activates all three subtypes, α, β/δ and γ, giving it the potential to address all the key features of NASH, namely inflammation, steatosis, ballooning and fibrosis[209]. In *in vitro* and *in vivo* preclinical studies, lanifibranor prevented and induced the regression of pre-existing fibrotic damage in the liver and other organs, for example skin and lung [210,211], without the classic effects on body weight, fluid retention and increase in heart weight that are reported with TZDs[212]. Lanifibranor also improved insulin sensitivity, diet-induced weight gain, adiposity index and lipid profile — all metabolic features relevant to NASH in diet-induced and genetic models[208]. The effects of lanifibranor on liver histology, proinflammatory and profibrotic gene expression and macrophage accumulation and activation has been shown to be significantly superior to single and dual PPAR agonists in several models of NASH and fibrosis [73,213] and is being investigated in a double-blind, randomized, placebo-controlled phase IIb trial[214], which is evaluating the efficacy and safety of 24-week lanifibranor treatment compared with placebo in 247 adult patients with NASH with liver steatosis and moderate-to-severe necroinflammation without cirrhosis. Highly significant positive results have been reported for reduction in the activity of steatohepatitis, resolution of NASH, regression of fibrosis and a combination of the latter two (in 31% of patients on the high dose of 1200 mg versus patients receiving placebo; $P<0.001$), along with improvement in glycaemic control and lipid profile and a good safety and tolerability profile [215]. The main clinical outcomes of PPAR agonists are summarized in Table 2.

[H1] Safety profile of PPARs

In a phase II study of patients with NASH, elafibranor treatment was associated with a slight rise in creatinine[180]. This increase is believed to be due to a rise in renal tubular reabsorption of creatinine, which is related to PPARα agonism and has been observed with other PPARα agonists[216]. It is not deleterious to renal function, as demonstrated with fenofibrate, which reduced the progression of chronic kidney disease in patients with T2DM in the large phase III FIELD trial[217].

In practice, pioglitazone is the only TZD in use clinically today for the treatment of T2DM. There is increasing recognition of its cardiometabolic benefits[199,218,219], but it might alter bone metabolism and promote an increase in fractures with long-term use[198], although the risk remains low and can be monitored and minimized with vitamin D and calcium supplementation[220]. Haematuria should be checked before and during treatment, although most studies have shown no increased risk of bladder cancer[221].

A gain of 2–4% of body weight has been reported after 6–36 months of therapy with pioglitazone in NASH trials[28,146-148] and in studies of longer duration in patients with T2DM[189,196,198]. These side effects might be treatment-limiting but are reversible upon treatment discontinuation. Furthermore, this weight gain is associated with improved insulin sensitivity by shifting fat from ectopic tissues to subcutaneous and less metabolically deleterious depots, which is consistent with the observed reduction in CVD in RCTs[196-198]. In most patients, weight gain on pioglitazone treatment is exclusively due to an increase in subcutaneous fat and not in visceral fat[15,222], but peripheral oedema occurs in approximately 5% of patients, who might require treatment discontinuation.

Pioglitazone reduces the risk of cardiovascular events, but substantial confusion remains regarding its effects on cardiac function. In a phase III trial in 5,238 patients with T2DM patients at high risk of cardiovascular events, that is, who had evidence of macrovascular

disease at baseline, heart failure was precipitated in 11% of patients on pioglitazone compared with 8% of patients receiving placebo, with 1% experiencing fatal heart failure in both arms. In that same trial, pioglitazone produced a statistically significant reduction in overall mortality and non-fatal cardiovascular events (composite endpoint, 301 out of 2633 patients on pioglitazone versus 358 out of 2633 on placebo, average time of observation 34.5 months, P=0.027)[196]. The increase in the occurrence of heart failure has not been observed in other placebo-controlled studies[28,147,189,194,195,198]. In a large RCT of 3,851 insulin-resistant patients without T2DM, the 5-year heart failure risk did not differ according to treatment (4.1% pioglitazone and 4.2% placebo)[223]. While pioglitazone improves whole-body and myocardial insulin sensitivity and left ventricular diastolic and systolic function in healthy patients with T2DM[224,225], undiagnosed 'diastolic dysfunction' (that is, heart failure with preserved left ventricular function) can occur in ≥10% of patients with longstanding obesity, T2DM and/or NASH[226]. If fluid retention occurs during pioglitazone therapy in such patients, it might seem to be causing heart failure rather than revealing established but subclinical heart disease. Therefore, in patients with established heart failure or with increased risk of heart failure, pioglitazone is contraindicated. In general, 15 mg per day of pioglitazone is not associated with weight gain (~1%), oedema or other side effects and can be the recommended dose for initiation in most patients. Uptitration to 30 mg per day might offer safe and maximal, or near-maximal, cardiometabolic [227] and liver histological [28,148] benefits for patients with NASH. Large RCTs with 15 mg per day of pioglitazone are needed to assess its long-term cardiovascular and histological benefit in NASH.

The dual PPARα/γ agonist saroglitazar has not been associated with weight gain and edema, which have been reported with PPARγ agonists. Indeed, no major serious adverse events have been reported. Long-term cardiovascular safety has not yet been established, but as mentioned, the overall cardiovascular risk factor profile of patients with diabetic dyslipidaemia improves[170,228]. The PPARβ/δ receptor agonist seladelpar, when investigated in a randomized phase II dose-finding study for patients with primary biliary cholangitis, was not associated with drug-induced transaminitis or pruritus[229], but as mentioned previously, the observation of atypical histological lesions suggestive of interface hepatitis in patients with NASH halted its further development in liver disease.

[H1] Conclusions

NAFLD is a multisystem disease with extra-hepatic disease implications that include development of T2DM and CVD. Patients with NAFLD often present with many of the features of the metabolic syndrome (for example, central obesity, atherogenic dyslipidaemia, hypertension or abnormal glucose tolerance and insulin resistance), and in progression of liver disease to NASH there is development of hepatic inflammation and often fibrosis. PPARs are key regulators of many of the adversely affected mechanistic pathways involved, which makes PPARs attractive therapeutic targets in the treatment of NASH, not only to benefit the liver but also to ameliorate features of the metabolic syndrome and to attenuate the risk of related extra-hepatic diseases such as T2DM and CVD. Although previous studies have shown limited efficacy of activation of individual PPARs (PPARα and PPARγ), ongoing clinical trials suggest that dual and pan-PPAR agonists might have broader and more efficacious therapeutic potential to affect the multisystem disease of NASH by targeting different interrelated mechanisms in the pathophysiology of NASH.

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Author contributions

G.S. and S.V. researched data for the article, made a substantial contribution to discussion of content, wrote the article, and reviewed/edited the manuscript before submission. M.F.A., C.D.B., K.C., J.-F.D., M.R., F.M.S. and F.T. made a substantial contribution to discussion of content and reviewed/edited the manuscript before submission.

Competing interests

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Key points

- Nonalcoholic steatohepatitis (NASH) is the fastest growing liver disease worldwide; however, it is often not recognized until advanced disease stages.
- NASH, the liver manifestation of the metabolic syndrome, requires a holistic approach for management and treatment.
- Peroxisome proliferator-activated receptors (PPARs) regulate metabolism, inflammation and fibrosis, all of which determine NASH progression.
- There is an urgent need for medical therapy for NASH patients.
- Both PPARα-β/δ dual agonism as well as PPARγ agonism have shown beneficial effects on liver histology in phase IIb clinical trials for NASH.
- Single, dual and pan-PPAR agonists are under development for the pharmacological treatment of NASH.

Table 1. Criteria for diagnosing metabolic syndrome

Criteria	**WHO (1999)[230]**	**NCEP (2001)[231]**	**IDF (2005)[232]**	**Joint Societies (2009)[233]**
Required for diagnosis	Impaired glucose tolerance or diabetes and/or insulin resistance	None	Central obesity as defined below	None
Number of features	Two other factors	≥3 of the below	≥2 of the below	≥3 of the below
Central obesity	Waist–hip ratio of >0.9 in men, >0.85 in women or BMI ≥30 kg/m^2	Waist circumference ≥102 cm in men, ≥88 cm in women	Waist circumference ≥ 94 cm European men; ≥ 90 cm South Asian or Chinese men; ≥ 80 cm women	Waist circumference – population-specific definitions
Triglycerides	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L)	≥150 mg/dL (1.7 mmol/L) or treatment for	≥150 mg/dL (1.7 mmol/L) or treatment for high triglycerides

			high triglycerides	
HDL-cholesterol	<40 mg/dL (1 mmol/L) in men, <50 mg/dL (1.3 mmol/L) in women	<40 mg/dL (1 mmol/L) in men, <50 mg/dL (1.3 mmol/L) in women	<40 mg/dL (1 mmol/L) in men, <50 mg/dL (1.3 mmol/L) in women	<40 mg/dL (1 mmol/L) in men, <50 mg/dL (1.3 mmol/L) in women
Hypertension	≥140/90 mmHg	≥135/85 mmHg or treated hypertension	≥135/85 mmHg or treated hypertension	≥135/85 mmHg or treated hypertension
Glucose	NA	110 mg/dL (6.1 mmol/L)	≥100 mg/dL (5.6 mmol/L) or diagnosed with type 2 diabetes mellitus	≥100 mg/dL (5.6 mmol/L), or drug treatment for diabetes
Microalbuminuria	Albumin–creatinine ratio >30 mg/g; albumin excretion rate >20 μg/min	NA	NA	NA

NA, not applicable; NCEP, National Cholesterol Education Program; IDF, International Diabetes Federation.

Table 2: Main clinical outcomes of PPAR agonists

PPAR target	PPAR agonist	Action	Effect on liver	Clinical status	Safety profile
Single PPAR agonists					
PPARα	Fibrates	Enhanced free fatty acid (FFA) metabolism; many antiatherogenic effects on lipoprotein metabolism: ↓ plasma triglycerides, ↑ HDL-C	No effect on hepatic steatosis or nonalcoholic steatohepatitis (NASH)[151,152]	Pemafibrate[157,158] in phase II (MRI-based endpoint) NCT03350165[234]	Toxic liver injury, impaired renal function (less with pemafibrate)
PPARγ	Rosiglitazone	Improved glucose and FFA metabolism; ↑ LDL-C and HDL-C	Reduction of hepatic steatosis; no effect on resolution of NASH[161,162]	Phase II trials have been conducted[161,162]	Weight gain, fluid retention and cardiac decompensation, bone fractures
PPARβ/δ	Seladelpar	Improved FFA/lipid (LDL-C, TG, HDL-C) and glucose metabolism[235]	No effect on hepatic steatosis[177]*	Phase II (MRI-based endpoint at 12 weeks, histological secondary endpoints at 52 weeks)	Gastrointestinal side effects, headache

				(NCT03551522, suspended[176])	
Dual PPAR agonists					
PPARα/γ	Pioglitazone	Improved glucose and FFA metabolism; ↓ plasma triglycerides, ↑ HDL-C, neutral effect on LDL-C	Induces resolution of NASH[55]**;	Pioglitazone: five phase II trials involving 498 patients with NASH have been conducted [28,147,148,236,237]	Weight gain, fluid retention and cardiac decompensation in patients with pre-existing reduced cardiac function (pioglitazone improves overall cardiovascular outcomes), bone fractures
PPARα/γ	Saroglitazar	Improved glucose and FFA metabolism; ↓ plasma triglycerides; ↑ HDL-C; neutral effect on LDL-C	Improves ALT and steatosis[170,238]	Phase II with non-invasive endpoints and histology EVIDENCES II (NCT03061721, [173])	Body weight neutral, gastritis and dyspepsia
PPARα/δ	Elafibranor	Improvement of atherogenic profile and FFA/glucose metabolism	Might induce resolution of NASH[180]; negative interim results, but full disclosure of the results is pending. [183]	Phase III (with histological endpoint at interim analysis for conditional approval) (NCT02704403)[182] Trial has been discontinued.	Body weight neutral; headache; increase in serum creatinine but no other markers of impaired renal function
Pan PPAR agonists					
PPAR α/δ/γ	Lanifibranor	Improved glucose and FFA metabolism; ↓ plasma triglycerides; ↑ HDL-C; neutral effect on LDL-C	Lowering of ALT[208]; positive results on histology with significant benefit over placebo for resolution of steatohepatitis, regression of fibrosis and the combination of both[215]	Phase II (histological endpoint) NCT03459079[239]	Headache, dizziness

*No liver biopsy data available for seladelpar in NASH (ongoing studies).
**Liver biopsy data only available for pioglitazone, not saroglitazar (ongoing studies).

Figure 1. Relationships between NAFLD, T2DM and metabolic syndrome, CVD and HCC. This figure schematically describes the relationships between nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM) and metabolic syndrome, cardiovascular disease (CVD) and hepatocellular carcinoma (HCC). NAFLD is associated with features of the metabolic syndrome (such as central obesity, atherogenic dyslipidaemia, hyperglycaemia and hypertension). NAFLD increases the risk of T2DM[18] and CVD[19]. With the development of T2DM there is a further increase in risk of CVD[20,21], a worsening of liver disease (fibrosis and cirrhosis)[22-24] and increased risk of HCC[25]. Development of advanced liver fibrosis with NAFLD also increases risk of CVD[26].

Figure 2. The role of PPARs in NASH and fibrosis development. The development of nonalcoholic steatohepatitis (NASH), starting from isolated steatosis to steatohepatitis accompanied by necroinflammation and then leading to the development of fibrosis, cirrhosis and vascular injury, is an interplay between all the different cells present within the liver (such as hepatocytes, infiltrating macrophages, Kupffer cells, hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells) and surrounding organs such as the adipose tissue, intestine and skeletal muscle. Peroxisome proliferator-activated receptors (PPARs), composed of three different isotypes (α, β/δ and γ) are implicated in regulating lipids and carbohydrate metabolism. In NASH, PPARα could improve lipid metabolism by controlling lipid flux and regulating fatty acid transport as well as β-oxidation. It also reduces inflammation through its action on hepatocytes as well as reducing splanchnic inflammation and intestinal permeability. PPARα is also involved in decreasing portal pressure in the context of cirrhosis. PPARβ/δ is also involved in glucose and lipoprotein metabolism and reduces insulin resistance in skeletal muscle. Furthermore, PPARβ/δ inhibits inflammatory macrophage phenotypes and favours the alternatively activated phenotype. PPARγ regulates insulin sensitivity within the adipose tissue and is a master regulator of HSC fate. PPARγ prevents HSC activation, which is a key event in fibrogenesis. Moreover, in the context of cirrhosis, PPARγ reduces portal pressure, splanchnic inflammation, angiogenesis and porto-systemic shunts. Together, the three PPAR isotypes act in different cells and organs and therefore influence different pathways and mechanisms involved in NASH and fibrosis progression. This figure is based on publications presenting animal models and human data. Thus, they should be interpreted with caution from a translational perspective[32,208,240]. FFAs, free fatty acids; HCC, hepatocellular carcinoma; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TG, triglyceride; TGF, transforming growth factor; TNF, tumour necrosis factor.

Figure 3. PPARs and inflammation. a | Peroxisome proliferator-activated receptors (PPARs) as nuclear regulators. PPARs form a complex with the retinoid X receptor (RXR) that activates expression of the PPAR response element (PPRE), which promotes trans-activating effects (lipid metabolism, glucose homeostasis and cell differentiation). Alternatively, PPARs can also function as trans-repressors through inhibition of nuclear factor-κB (NF-κB), activator protein 1 (AP-1), signal transducer and activator of transcription (STAT) or nuclear factor of activated T cells (NFAT) to induce anti-inflammatory effects. **b** | PPAR-mediated regulation of inflammation. PPARα regulates inflammation via increasing expression of LTB4-catabolizing enzymes that inhibit extracellular leukotriene B4 (LTB4)-mediated inflammation, preventing NF-κB-mediated increases in IL-6 and IL-12 expression via inhibition of NF-κB directly or inhibition of AP-1 and/or increasing expression of IκBα.

Additionally, PPARα and PPARβ/δ inhibit vascular cell adhesion molecule 1 (VCAM1) expression via inhibition of tumour necrosis factor receptor (TNFR)-mediated activation of NF-κB. Boxes are colour-coded to indicate the effects of specific PPARs. Green, yellow and orange boxes indicate effects of PPARα, PPARβ/δ and PPARγ, respectively. PPARγ inhibits NF-κB-mediated macrophage survival and increased expression of iNOS, pro-inflammatory chemokines and cytokines via direct inhibition of TLR-mediated or TNFR-mediated activation of NF-κB and inhibition of TLR-mediated activation of AP-1. iNOS, inducible NO synthase; IκB, inhibitory subunit of NFκB; LPS, lipopolysaccharide; TLR, Toll-like receptors.

Box 1 | Suggested lifestyle interventions to improve liver histology in NASH

[H1] Energy restriction
- 500–1000 kcal energy deficit
- 7–10% total weight loss target
- Long-term maintenance approach

[H1] Nutrition
- Mediterranean diet
- Avoid fructose
- Low-to-moderate fat
- Moderate-to-high carbohydrate
- Low-carbohydrate ketogenic diets or high protein

[H1] Alcohol intake
- Strictly keep alcohol below the risk threshold (30 g/day for men; 20 g/day for women)

[H1] Exercise and physical activity
- Moderate intensity aerobic physical activities (150–200 min/week)
- 3–5 sessions
- Resistance training

Diet and lifestyle changes are mandatory in all patients[2,3].