Review

Roles of PPARα and PPARγ As Regulator of Free Fatty Acids in Nonalcoholic Steatohepatitis

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Roles of PPARα and PPARγ as Regulator of Free Fatty Acids in Nonalcoholic Steatohepatitis

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Abstract: In recent years, nonalcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) has become a leading worldwide disease, and its therapies are facing many complexities. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor (NR) superfamily and have three subtypes: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3). They have been identified to be essential in the regulation of lipid metabolism by controlling the transcription of genes related to fatty acids, bile acids, and cholesterol metabolism. Many PPAR agonists have been reported, such as natural agonists (fatty acids, eicosanoids, and phospholipids) and synthetic ligands (fibrates, thiazolidinediones, glitazars, and elafibranor). PPAR-based chemicals have a breakthrough in the innovation of therapy for fatty liver disease. This review will provide an overview of how PPARα and PPARγ play roles in lipid homeostasis, discussing the consideration of their agonists as potential therapeutic agents for NAFLD/NASH.

Keywords: PPAR; NAFLD; NASH

1. Introduction

Hepatic steatosis (fatty liver) is one of the most prevalent chronic liver diseases worldwide, affecting approximately one quarter of the global population, and is predicted to become the leading indication for liver transplantation by 2030, posing a significant burden on global health [1–5]. According to the history of alcohol intake, fatty liver is artificially categorized into two common forms: alcoholic liver disease (ALD) and nonalcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) [3–7]. While ALD is defined by the presence of hepatic steatosis associated with significant alcohol consumption, NAFLD is a generic term that includes a series of liver diseases with different injury severities and consequent fibrosis [3,5]. Among them, hepatic steatosis is referred to as NAFLD, which is defined as the composition of fat that takes up 5–10% of the liver weight. NASH is associated with inflammation and fibrosis, which may progress to cirrhosis and hepatocellular carcinoma (HCC) [8,9]. About 20% of patients with NAFLD develop NASH, and over 40% of patients with NASH progress to fibrosis [10]. It was proposed that the abnormality in lipid and lipoprotein metabolism accompanied by chronic inflammation and oxidative stress is the central pathway and/or the major risk factors involved in the pathogenesis of NAFLD/NASH. However, the pathogenesis of NAFLD/NASH is not fully understood. The "two-hit" hypothesis is now obsolete mainly because it is inadequate to explain some of the molecular and metabolic changes that occur in NAFLD/NASH. The "multiple hit" hypothesis is believed to describe more accurately the pathogenesis of NAFLD. The hits include insulin resistance (IR), hormones secreted by adipose tissue, nutritional factors, gut microbiota, and genetic and epigenetic factors. Thus, there are numerous factors involved in the pathogenesis of NAFLD/NASH [11].

Peroxisomes, originally called microbodies, were identified as intracellular organelles and named by de Dude et al. in 1960. Peroxisomes are present in most plant and animal cells and contain numerous enzymes, but they differ from other intracellular organelle membranes in their protein composition and are thin and fragile. The typical functions of peroxisomes are respiration through

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the H_2O_2 system and β-oxidation of fatty acids, and they are also involved in a wide range of other metabolic activities [12]. Proliferation of peroxisome is stimulated by compounds such as clofibrate and di[2'-ethylhexyl] phthalate. Peroxisome proliferator activated receptors (PPARs) have been identified as factors that mediate this proliferation, PPARs were first cloned in rodent hepatocytes in the 1990s and are ligand-activated transcription factors that belong to the nuclear receptor superfamily (NR). The PPARs are believed to control lipid metabolism via regulating the gene transcription involved in fatty acids (FAs), bile acid, and cholesterol metabolism. Up to now, three PPAR subtypes have been identified: PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3). PPARα is mainly expressed in tissues with high fatty acid metabolism, including the liver, kidney, and white and brown adipose tissue. PPARδ/β is ubiquitously and abundantly expressed in a broad range of tissues and regulates fatty acid oxidation [13]. PPARγ, consists of four isoforms in humans and rodents (PPARγ1-4), expressed in a variety of tissues such as liver, kidney heart. In contrast, PPARγ2 is restricted to adipose tissue. PPARγ3 is expressed in macrophage and colon and PPARγ4 is observed in endothelial cells [13,14]. PPARγ plays a variety of biological functions, including regulating fat and glucose metabolism, inducing tumor cell differentiation and apoptosis, promoting ovulation and anti-atherosclerotic activity, improving heart failure and ventricular remodeling, and inhibiting inflammatory reactions [13].

NAFLD/NASH progresses to lipid accumulation (hepatocyte), inflammation (inflammatory cells such as macrophage), and fibrosis (hepatic stellate cell). It is important for understanding pathogenesis of NAFLD/NASH to unveil molecular changes occurring in these liver cells. We will mainly outline the roles of PPARα and PPARγ in the NAFLD/NASH and discuss the potential of PPARs for the NAFLD/NASH therapy.

2. Structure of PPAR proteins

PPARs show four distinguishable functional domains [an N-terminal region (A/B domain), a central DNA-binding domain (DBD, C domain), a flexible hinge region (D domain), and a C-terminal ligand-binding domain (LBD, E domain)], as well as other NRs (Figure 1). The A/B domain, which is the least conserved domain among PPARs, harbors a ligand-independent activation function-1 (AF-1) region. The DBD, which is highly conserved and rich in cysteine and basic amino acids, consists of two zinc-finger binding motifs [15]. The domain is responsible for physical interaction with DNA. PPARs form a heterodimer with retinoid X receptors (RXRs). The PPAR-RXR heterodimer recognizes and binds to PPAR response elements (PPREs), which are localized in gene regulatory regions and organized as a direct repeat type 1 (DR-1). DR-1 consists of two copies of the hexameric nucleotide recognition motif 5′-AGGTCA-3′ separated by a single nucleotide [16]. The PPAR-RXR heterodimer can form independently of the PPAR ligand. The unliganded heterodimers recruit the corepressor protein complex and inhibit target gene transcription. Upon ligand binding, the corepressor complex is released from the heterodimer and then the coactivator complexes will be recruited to the promoter region of target genes, thereby initiating transcription [17]. The PPAR-LBD harbor a ligand-dependent transactivation function (AF-2) and has a structure consisting of an α-helical sandwich and a four-stranded β-sheet [15]. The LBDs differ among PPARs and the LBD of PPARα is more lipophilic than other PPARs, which potentially explains the greater affinity of PPARα to bind the more saturated FAs [18]. Following ligand binding, the AF-2 domain undergoes conformational changes, which allows for the interaction with various coactivators carrying LXXLL motifs (L, leucine; X, any amino acid) [19], such as cAMP response element binding protein (CBP)/p300 and steroid receptor coactivator-1 (SRC-1/NCoA-1) [20].

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Figure 1

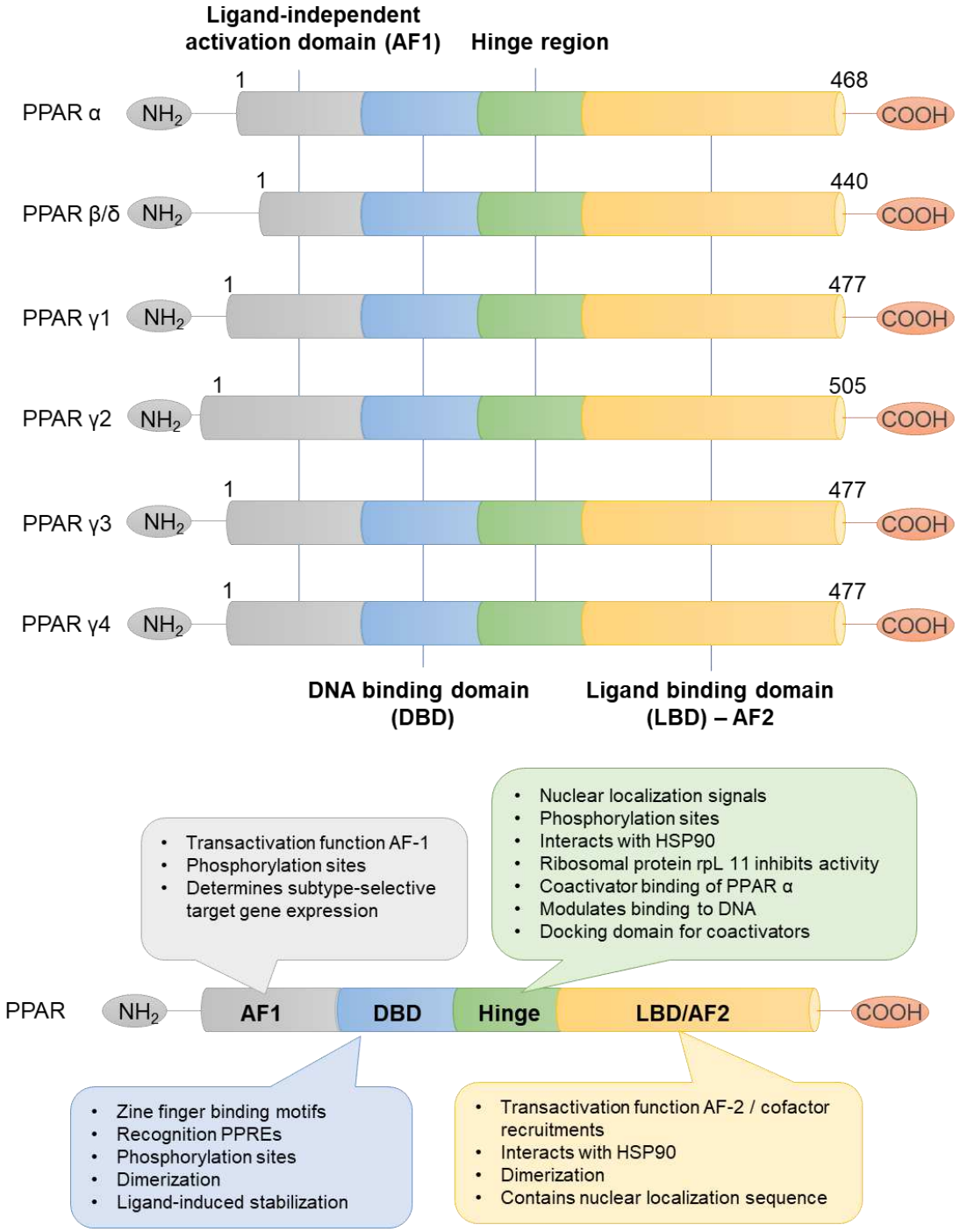

Figure 1. PPARs structure and functions. PPARs has four functional domains: a ligand-independent activation domain, a DNA binding domain, a hinge domain, and a ligand-binding domain.

3. Lipid homeostasis in the liver

Hepatic lipid homeostasis is regulated mainly by uptake, synthesis, degradation, and excretion of fatty acid (FAs) in hepatocytes [21]. Upon eating, glucose and insulin promote FA synthesis (lipogenesis) through synergistic effects between sterol regulatory element binding protein 1C (SREBP1c) and carbohydrate response element binding protein (ChREBP) [22] [23]. Insulin induces SREBP1c gene expression via AKT signaling and enhances SREBP1c activity. Hepatic SREBP-1c

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activation leads to the regulation of lipogenic genes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in the diet-related condition [24,25]. Elevated glucose levels induce phosphorylation-dependent nuclear relocalization of ChREBP and the ChREBP induces FAS and ACC gene expression [26]. In addition to the de novo synthesis, hepatic lipid stores consist of FAs derived from breakdown of triglycerides (lipolysis) in adipose tissue and dietary intake [27]. FAs are transported in the hepatocytes through a fatty acid transport protein (FATP) and a fatty acid translocase (FAT), CD36, which facilitate the uptake of long-chain fatty acids. Once inside the hepatocytes, FAs bind to fatty acid binding protein-1 (FABP1) and change their fate depending on the condition of hepatocytes. Generally, FAs undergo the peroxisomal β-oxidation by acyl-CoA oxidase 1 (ACOX1) or are transported into mitochondria by the shuttle of carnitine-palmitoyl transferase/carnitine-acyl carnitine carrier (CPT/CAC) going through β-oxidation. In mitochondria, FA β-oxidation is mediated mainly by medium-chain acyl-CoA dehydrogenase (MCAD) or long-chain acyl-CoA dehydrogenase (LCAD) [28]. When these β-oxidations can no longer process FAs, FAs are esterified to cholesteryl esters by acyl CoA cholesterol acyltransferases (ACAT1 and 2) [29], and to triglycerides (TGs) by diacylglycerol-O-acyltransferases (DGAT 1and 2) in the endoplasmic reticulum [30,31] and become contents of VLDL (secreted from hepatocytes via the Golgi apparatus) and lipid droplet (accumulated in hepatocytes). DGAT1 mainly localizes to the endoplasmic reticulum and catalyzes TG formation using FAs supplied from foods [30,31]. On the other hand, DGAT2 can translocate from the endoplasmic reticulum to lipid droplets [30,31] and promote TG formation using *de novo* synthesized FAs [31]. Hepatocytes perform lipolysis and autophagy to recruit FAs to mitochondrial/peroxisomal β-oxidation or the VLDL secretion. FAs for VLDL assembly derive from re-esterification of triglycerides stored in lipid droplets [32]. In many kinds of cells, adipose triglyceride lipase (ATGL) and its cofactor CGI-58 work with hormone sensitive lipase (HSL) and monoacylglycerol lipase (MGL) to cleave fatty acids from the TG [33,34]. However, in hepatocyte, contribution of these lipases to lipolysis remains unclear. HSL is very low expressed in human liver. Moreover, overexpression or knockdown of ATGL, HSL and MGL did not substantially alter hepatic VLDL secretion [35]. Thus, other lipases such as TG hydrolase existing endoplasmic reticulum are thought to contribute to hepatic VLDL formation. On the other hand, Liver-specific deletion of either ATGL or CGI-58 results in steatosis and fibrosis in mice following enhanced hepatic β-oxidation [33,35]. The ATGL and CGI-58 may mobilize lipids for β-oxidation.

4. The pathogenesis of NAFLD/NASH

It is generally believed that the occurrence of fatty liver is not only related to insulin resistance and disorder of fat metabolism, but also related to biological processes, such as glucometabolic disorder, oxidative stress, and intracellular inflammatory response. Moreover, these processes are correlated and/or coordinated with each other and accelerate the progress of NAFLD [36].

Increased fatty acid levels can trigger enhancement of lipid peroxidation in the liver. The enhanced lipid peroxidation results in persistent reactive oxygen species (ROS) production [37]. In addition to the pre-existing factors related to the elevated ROS levels, other new or additional factors can enhance lipid peroxidation in the liver, such as inflammatory cytokines, adipokines, endotoxins, and mitochondrial inactivation [11]. The oxidative stress following accumulation of fatty acids will eventually lead to NAFLD progression that promotes cell death and inflammation. Especially, the inflammation is positively correlated with liver injury, negatively correlated with hepatic lipolysis, and induces further lipid peroxidation aggravating the pathogenesis of NAFLD/NASH [38]. Additionally, endoplasmic reticulum (ER) stress is another important factor in the pathogenesis of NAFLD/NASH [39,40]. Metabolic disorders, such as obesity and diabetes, can cause ER stress, leading to the accumulation of unfolded protein response and affecting the normal physiological functions of hepatocytes [41]. The ER stress can activate sterol-regulatory element binding protein (SREBP), promoting the transcription of FAS and ACC, resulting in enhancement of fatty acid synthesis and lipid droplet formation in the liver [42]. Considering that the lipid peroxidation can also induce the ER stress, the fatty acid accumulation and lipid peroxidation in hepatocytes is an important factor for aggravating the NAFLD/NASH [43]. We summarized the relationships between

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fatty acid homeostasis in the liver and PPARα/ γ in (Figure 2). The homeostasis of fatty acids in the liver and the functions of PPARα/γ are described below.

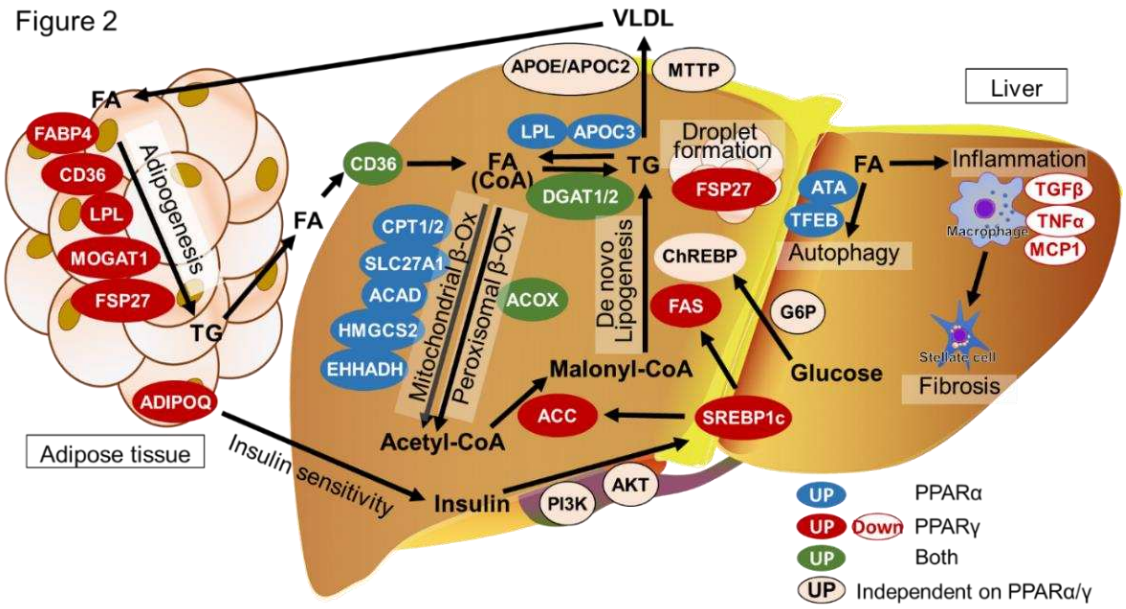

Figure 2. PPAR-targets and contributing factors in NAFLD/NASH. Lipid homeostasis is an essential process in the liver by keeping the balance between lipid acquisition and disposal. In lipid metabolism, insulin resistance plays a crucial role in the pathology of liver disease. Insulin level is regulated by ADIPOQ which is secreted by PPARγ from the adipose tissue. In the liver, insulin via PI3K and AKT signaling pathway regulates sterol regulatory element-binding protein 1c (SREBP-1c) by activation of PPARα. SREBP-1c regulates the essential glycolytic enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) via PPARα, which play a crucial role in the synthesis of triglycerides (TG) from carbohydrates. This process is known as de novo lipogenesis. Carbohydrate response element binding protein (ChREBP), which expression level is controlled by glucose from the bloodstream via PPARα activation through glucose-6-phosphate (G6P) signaling, is also involved in triglyceride synthesis. After, lipoprotein lipase (LPL) and apolipoprotein C3 (APOC3), secreted by PPARα, promote TG hydrolyzation into fatty acids for further homeostasis processes, called mitochondrial β-oxidation of fatty acids to acetyl-CoA. In mitochondrial β-oxidation CPT1/2, SLC27A1, ACAD, HMGCS2, and EHHADH are involved via regulating of PPARα. In further, with the presence of ACC that is regulated by SREBP-1c via PPARα activation, acetyl-CoA is converted to malonyl-CoA which will be involved in the de novo lipogenesis to TG, completing a circle of lipid homeostasis. When fatty acids are over-elevated, mitochondrial β-oxidation can be overburdened leading to hepatic accumulation and steatosis. As a compensatory effect, the peroxisomal β-oxidation is activated via ACOX signaling that causes oxidative stress, and a part of the fatty acid is converted back into TG via DGAT1/2, and both processes are controlled through PPARγ activation. Excess TG further will be transported to adipose tissue by very-low-density lipoprotein (VLDL) synthesis, via expression of APOE/APOC2 and MTTP, and transport by apolipoprotein. In adipose tissue fatty acids are converted into TGs in storage form by the adipogenesis process via the regulation of FABP4, CD36, LPL, MOGAT1 and FSP27 through PPARγ activation. Impaired VLDL synthesis and transport by apolipoprotein expression in the liver, which is essential for TG conversion into VLDL, may also contribute to hepatic fat accumulation by droplet formation via FSP27 which is also regulated by PPARγ. Overburden of fatty acids in the liver causes hepatic cell autophagy by ATA and TFEB regulation via PPARα activation. The progression of NAFLD to non-alcoholic steatohepatitis (NASH) is mainly caused by hepatic inflammation, which is triggered by downregulation of TGFβ, TNFα and MCP1 through PPARγ activation of macrophage that further activate the hepatic stellate cell, leading to fibrosis.

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5. PPARα and PPARγ functions in hepatocytes

PPARα serves as an intracellular lipid sensor and is activated by a wide range of endogenous or naturally occurring biological molecules encompassing a variety of FAs and FA derivatives, including acyl-CoAs, oxidized FAs, eicosanoids, endocannabinoids, phytanic acids [19]. PPARα promotes the transport and oxidation of fatty acid and ketogenesis, pointing out the role of PPARα as a master regulator of the hepatic lipid metabolism in fasting conditions. PPARα positively regulates CD36 (uptake into hepatocyte) and FABP1 (transfer to mitochondria) gene expressions [44]. In addition, PPARα induces expressions of mitochondrial FA β-oxidation-related genes, such as MCAD and LCAD [19]. Moreover, PPARα upregulates the gene expressions related with biosynthesis of ketone bodies, such as mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (a key ketogenic enzyme which catalyzes the condensation of acetyl-CoA and acetoacetyl-CoA to generate 3-hydroxy-3-methylglutaryl-CoA, the first ketone body) [44]. Therefore, PPARα contributes to decrease in hepatic FA levels in the liver and reduces lipid peroxidation and ROS production via enhancement of mitochondrial β-oxidation and ketone body generation, resulting in protection from hepatic lipotoxicity. Notably, PPARα are activated under condition of a carbohydrate-rich diet and can stimulate lipogenesis via promoting SREBP1C gene transcription [28]. The SREBP1C induces lipogenic genes such as FAS and ACC [42]. In addition, PPARα can indirectly control the SREBP1C activity by inducing expression of the liver X receptor that regulates the SREBP1C gene transcription [45]. PPARα induces expression of lipoprotein lipase (LPL) that catalyzes the hydrolysis of plasma triglycerides contained in lipoproteins, while inducing gene expression of apolipoproteins A-I (APOAI) and A-II (APOAII), the major proteins of HDL [44,46]. Thus, PPARα also influences lipoprotein metabolism by reduction in hepatic VLDL synthesis, leading to increase of high-density lipoproteins (HDL). In hepatocytes, PPARα works as a metabolic switch between fasting and postprandial states and as a part of the triglyceride/fatty acid cycle [44].

Hepatic PPARγ expression is low under normal physiological conditions but induced during the development of steatosis. The PPARγ promotes accumulation of lipid droplets in hepatocyte via inducing hepatic fat-specific protein 27 (Fsp27) expression that is involved in formation of lipid droplet [47]. In addition, Hepatocyte-specific PPARγ deletion improves hepatic steatosis via attenuating expressions of de novo lipogenesis genes (FAS, ACC, SCD1). PPARα activation contributes to decrease in hepatic lipid accumulation under the condition of NAFLD/NASH, although PPARα has the ability to induce the de novo lipogenesis genes. Thus, hepatocyte PPARγ is thought to play an important factor in the development of hepatic steatosis, rather than PPARα [48]. On the other hand, PPARγ is a ligand-activated nuclear receptor with potent anti-inflammatory properties [49]. A PPARγ agonist exerted an anti-inflammatory effect by inhibiting NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in lipid-laden hepatocytes [50]. Furthermore, PPARγ deficiency enhanced ROS generation, NLRP3 inflammasome activation, and IL1β secretion in hepatocytes after treatment with FA in in vitro study, and modulation of PPARγ activity attenuated high fat diet-induced NAFLD by regulating lipid metabolism and oxidative stress in hepatocytes via nuclear factor erythroid 2-related factor 2 activation [51]. Interestingly, enhancement of PPARγ activity in lipid-laden hepatocytes reversed macrophage M1 polarization and reduced the toll-like receptor 4/nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) pathway activation, and the hepatocyte-specific PPARγ deletion accelerated the macrophage M1 polarization and toll-like receptor 4/NF-κB pathway activation. Hepatocyte-specific PPARγ deletion highly has been to augment TNFα-induced production of C-X-C Motif Chemokine Ligand 1 (CXCL1) [52]. Thus, hepatic PPARγ can work as a potent regulator for hepatic inflammation.

6. PPARγ function in liver macrophages and hepatic stellate cells

PPARγ mainly works as suppressor of inflammation in livers of NASH. Macrophage-specific PPARγ deficient (PPARγΔLyz2) showed that upregulated differentially expressed genes were enriched in the NOD-like receptor signaling pathway, NF-κB signaling pathway, chemokine-signaling pathway and cytokine–cytokine receptor interaction, suggesting an enhancement of NLRP3 and NF-κB-driven inflammatory cytokine and chemokine production [53]. While downregulated

differentially expressed genes were enriched in the PPAR signaling pathway and insulin resistance, suggesting severe metabolic disorder. In addition, Macrophage-specific PPARγ deficient showed significantly increased expression of liver fibrosis-related genes, such as TGFβ1, ACTA2, COL1A1 and TIMP metallopeptidase inhibitor 1, in the NASH livers. The PPARγ deletion in macrophages can affect macrophage phenotypes and hence influence hepatic stellate cell (HSC) activation and NASH development in mice [52] [13]. Activation of HSCs results in excess accumulation of extracellular matrix such as type I collagen (COL1A) in the liver (called liver fibrosis).

7. PPARα and PPARγ functions in adipose tissue

The role of PPARα is not fully understood in adipose tissue because its role overlaps with that of PPARγ, which has potent activity, although some studies indicated that the role of PPARα in brown adipose tissue thermogenesis and white adipose tissue browning [54]. The lipolysis of white adipose tissue results in increased FFA levels and enhanced TAG synthesis in the liver. Among the several factors involved in adipose tissue regulation, PPARγ is considered the "master regulator" of adipogenesis [55,56]. The PPARγ is a well-characterized regulator of energy metabolism mainly by improving uptake and expenditure of fatty acid in adipocyte and is clearly involved in the pathophysiology of obesity and related complications. Adipocyte PPARγ deletion has been linked to insulin resistance in mice [54]. Furthermore, PPARγ activation has been demonstrated to enhance expression of adiponectin in adipocytes. In addition, PPARγ is known to induce adipocyte expression of Fsp27 which is a regulator of lipid droplet formation. Adipocyte-specific PPARγ deletion leads to severe lipoatrophy, highlighting the role of PPARγ in mouse adipocyte development. Adipocyte-specific Fsp27 deletion also inhibits lipid accumulation in mouse adipose tissue as well as the PPARγ deletion. Furthermore, the adipocyte-specific Fsp27 deletion exacerbates hepatic steatosis after a challenge of high-fat diet. The adipocyte PPARγ-FSP27 cascade may be a key regulator for hepatic fatty homeostasis and suppress the procession of NAFLD/NASH [57–59].

8. Clinical drugs and trial for NASH (The potency of PPAR ligands as clinic therapeutic agents.)

PPARs can be activated by various natural agonists, such as fatty acids (FAs), eicosanoids, and phospholipids derived from cellular FA metabolism or dietary lipids. Synthetic ligands, including fibrates, thiazolidinediones (TZDs), glitazars, elafibranor, and others, can also activate PPARs (Table 1). These PPAR agonists have been widely used in basic research and are progressing to clinical development as therapeutic agents for NAFLD/NASH [60].

8.1. PPARα agonists

Studies using mouse models have shown that PPARα agonists, such as Wy-14643, can prevent hepatic triglyceride accumulation induced by a methionine and choline-deficient (MCD) diet in wild-type mice but have no effect on the PPARα deficient mice [61,62]. The potential use of PPARα agonists, particularly fibrates, in the treatment of NASH has been of interest for over two decades [63,64]. Gemfibrozil can reduce levels of liver enzymes (aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase) and very low-density lipoprotein in NASH patients. Fenofibrate has been shown to decrease serum levels of the inflammatory marker RANTES in type 2 diabetes patients with hypertriglyceridemia, indicating potential anti-inflammatory properties related to NAFLD. However, a 48-week trial of fenofibrate in NASH patients with biopsy-proven NAFLD did not show any improvement in liver histology despite improving metabolic syndrome-related parameters. Clofibrate was also assessed for its potential anti-NASH properties but did not show histological improvements in the liver enzyme levels. Pemafibrate, by enhancing lipid metabolism and reducing inflammation, has shown potential to ameliorate liver dysfunction in patients with type 2 diabetes and to ameliorate NASH pathology in mouse and rat models [46,64–66].

8.2. PPARγ agonists

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Function of PPARγ agonists have been most investigated with TZDs. TZDs (pioglitazone and rosiglitazone) are a class of insulin-sensitizing drugs and currently used in clinical therapy [67]. These drugs enhance the uptake and storage of FAs in adipose tissue, thereby protecting the skeletal muscle and the liver. Rosiglitazone shown to prevent form development of NASH induced by MCD diet [64]. Pioglitazone exhibits anti-inflammatory and antifibrotic properties by suppressing the expression of platelet-derived growth factor and tissue inhibitor of metalloproteinase-2 in mouse model. [62]. However, rosiglitazone was withdrawn from the European market due to its association with an increased risk of myocardial infarction and heart failure. The PIVENS (Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis) trial evaluated the potential efficacy of the pioglitazone/vitamin E for NASH therapy. In the PIVENS trial, long-term pioglitazone treatment resulted in the resolution of NASH in more than half of the studied patients with prediabetes or type 2 diabetes [64]. However, we need to keep it in mind that pioglitazone is associated with an increased risk of bladder cancer. Consequently, the AASLD and EASL now recommend the use of pioglitazone/vitamin E for treating the biopsy-proven NASH but mentioned that pioglitazone is off-label in patients without type 2 diabetes and may cause weight gain. Lobeglitazone, another PPARγ agonist, has also been evaluated in type 2 diabetes patients with NAFLD, resulting in moderate weight gain and attenuated hepatic steatosis with improvement of glucose and lipid homeostasis. Further randomized controlled trials are needed to assess the effect of lobeglitazone [64].

8.3. PPAR-α/γ Agonists

In randomized trials for NAFLD or NASH treatment, pioglitazone (PPARγ ligand) and elafibranor (dual PPARα and β/δ ligand) were tested. These drug candidates have strong side effects [68,69]. Thus, it is required to minimize undesirable side effects. Saroglitazar (PPARα/γ-dual agonist) was designed to have both PPARα and PPARγ agonisms. The PPARα agonism enhances fatty acid oxidation in the liver, reduces the synthesis and secretion of triglycerides, and improves circulating lipoprotein profiles. The PPAR-γ agonism regulates insulin-responsive genes, increases insulin sensitivity, and reduces blood glucose and glycosylated hemoglobin A1c levels. A phase 3 clinical trial with saroglitazar treatment is currently underway in NAFLD/NASH patients. Lanifibranor (IVA-337) is a pan agonist that has shown promising results in improving NASH histology, reducing weight gain, and normalizing plasma glucose and insulin levels in mouse model. Additionally, IVA337 has been shown to be more effective at preventing and reversing liver fibrosis than any of the single agonists [70]. Phase 2 trials have been conducted to assess the efficacy of IVA337 in humans with intrahepatic triglycerides and histologically proven NASH (now phase 3 is going) [71]. These results may suggest that dual and pan agonists are available for the NAFLD/NASH treatment.

9. Conclusion

PPAR is activated by ligand and has metabolic actions that regulate energy, glucose, and lipid homeostasis. PPARα enhances FA oxidation in the liver and brown adipose tissue, while PPARγ promotes lipid storage and adipogenesis in the liver and white adipose tissue. In the liver, these functions contribute to reduced free FA level and results in suppressing lipid peroxidation and ROS generation in the hepatocytes. The PPARα and PPARγ agonists show promise for the treatment of NAFLD/NASH, but will be required for a contrivance, such as liver-specific delivery system, to avoid the side effects**.**

Table 1. The potency of PPAR ligands as clinic therapeutic agents.

PPAR Ligands	Isotype	Status	Source	Reference
Arachidonic acid	α		Nature	[72]
Leukotriene B4	α		Nature	[73,74]
Phosphatidylcholine	α		Nature	[75,76]
Resveratrol	α		Nature	[77,78]

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Linoleic Acid	γ		Nature	[79]
Prostaglandin D2	γ		Nature	[80]
15-deoxy-delta 12,14-prostaglandin J2	γ		Nature	[81]
Lysophosphatidic acid	γ		Nature	[82]
Clofibrate	α	Clinical	Synthesis	[83]
Fenofibrate	α	Clinical	Synthesis	[84,85]
Bezafibrate	α	Clinical	Synthesis	[86]
Gemfibrozil	α	Clinical	Synthesis	[87]
Pemafibrate	α	Clinical	Synthesis	NCT03350165 [65,88–93]
WY14643	α	Basic	Synthesis	[94]
GW9578	α	Basic	Synthesis	[95]
GW7647	α	Basic	Synthesis	[96,97]
Pioglitazone	γ	Clinical	Synthesis	NCT00063622 NCT00062764 NCT00013598 NCT03646292 NCT04976283 NCT04501406
Ciglitazone	γ	Clinical	Synthesis	[98]
Troglitazone	γ	Clinical	Synthesis	[99,100]
Rosiglitazone	γ	Clinical	Synthesis	[101–103]
S26948	γ	Clinical	Synthesis	[104]
INT131	γ	Clinical	Synthesis	[105,106]
Saroglitazar	α/γ	Clinical	Synthesis	NCT05011305 NCT03639623 NCT03061721 NCT02265276 NCT03863574 NCT03617263 NCT03112681 NCT04193982
Lobeglitazone	α/γ	Clinical	Synthesis	[107,108]
Lanifibranor (IVA-337)	pan	Clinical	Synthesis	NCT03008070 NCT02503644 NCT05232071 NCT04849728

Abbreviations:

ACAD, Acyl-CoA dehydrogenase; ACC, Acetyl-CoA carboxylase; ACOX, Acyl-CoA oxidase; ADIPOQ, Adiponectin; AKT, Alpha serine/threonine-protein kinase; APOC2, Apolipoprotein C2; APOC3 Apolipoprotein C3; APOE; Apolipoprotein E, ATA, Ataxia-telangiectasia group A; CD36, Cluster of differentiation 36; ChREBP, Carbohydrate response element binding protein; CPT1/2, Carnitine palmitoyltransferase 1/2; DGAT1/2 Diacylglycerol O-acyltransferase 1/2; EHHADH, Enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase; FABP4, Fatty acid binding protein 4; FAS, Fatty acid synthase; FSP27, Fat-specific protein 27; G6P, Glucose-6-phosphate; HMGCS2, 3-Hydroxy-3-methylglutaryl-CoA synthase 2; LPL; Lipoprotein lipase; MCP1, Monocyte chemoattractant protein 1; MOGAT1, Monoacylglycerol O-acyltransferase 1; MTTP, Microsomal triglyceride transfer protein; PI3K, Phosphoinositide 3-kinase; SLC27A1, Solute carrier family 27 member 1; SREBP1C, Sterol regulatory element-binding protein; TFEB, Transcription factor EB; TGFβ, Transforming growth factor beta; TNFα, Tumor necrosis factor alpha.

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