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REVIEW

Transcriptional Regulation of Metabolic Pathways via Lipid-Sensing Nuclear Receptors PPARs, FXR, and LXR in NASH

Marica Cariello,[1] Elena Piccinin,[2] and Antonio Moschetta[1,3,4]

[1]Department of Interdisciplinary Medicine, University of Bari "Aldo Moro," Bari, Italy; [2]Department of Basic Medical Sciences, Neurosciences and Sense Organs, University of Bari "Aldo Moro," Bari, Italy; [3]National Institute for Biostructures and Biosystems (INBB), Rome, Italy; [4]Scientific Institute for Research, Hospitalization and Healthcare (IRCCS) Istituto Tumori Giovanni Paolo II, Bari, Italy

SUMMARY

This review analyzes the impact of the lipid-sensing nuclear receptors peroxisome proliferator activated receptors, farnesoid X receptor, and liver X receptors in the pathogenesis and treatment of nonalcoholic steatohepatitis. Agonists of peroxisome proliferator-activated receptors and farnesoid X receptor have been studied extensively in mouse models, and phase II and III clinical trials currently are ongoing to test the safety and efficacy of these nuclear-receptor–based drugs for treating nonalcoholic steatohepatitis.

Nonalcoholic fatty liver disease comprises a wide spectrum of liver injuries from simple steatosis to steatohepatitis and cirrhosis. Nonalcoholic steatohepatitis (NASH) is defined when liver steatosis is associated with inflammation, hepatocyte damage, and fibrosis. A genetic predisposition and environmental insults (ie, dietary habits, obesity) are putatively responsible for NASH progression. Here, we present the impact of the lipid-sensing nuclear receptors in the pathogenesis and treatment of NASH. In detail, we discuss the pros and cons of the putative transcriptional action of the fatty acid sensors (peroxisome proliferator-activated receptors), the bile acid sensor (farnesoid X receptor), and the oxysterol sensor (liver X receptors) in the pathogenesis and bona fide treatment of NASH. *(Cell Mol Gastroenterol Hepatol 2021;11:1519–1539; https://doi.org/10.1016/j.jcmgh.2021.01.012)*

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In the world, with approximately 2 billion adults being overweight or obese, obesity currently is considered a pandemic of the 21st century.[1] In particular, obesity represents a risk factor for different clinical conditions, including cardiovascular and gastrointestinal diseases. Among the latter, nonalcoholic fatty liver disease (NAFLD) has become one of the most studied hepatic dysfunctions in the past years, also because of its continuous increasing prevalence worldwide ($\sim$25%).[2] NAFLD encompasses a wide spectrum of liver injuries, ranging from simple steatosis to steatohepatitis, and eventually fibrosis and cirrhosis.

NAFLD-related cirrhosis represents one of the major known causes for the onset of hepatocellular carcinoma, and obese individuals have a 2-fold increased risk of hepatocellular carcinoma–related mortality.[3–5]

On the basis of the degree of disease severity, NAFLD can be subdivided into NAFL and nonalcoholic steatohepatitis (NASH). Usually, the term NAFL refers to the accumulation of lipids within hepatocytes, without evident markers of inflammatory activity or cell damage, whereas NASH is associated with liver steatosis, inflammation, and hepatocyte damage (ie, hepatocyte ballooning), with or without fibrosis.[6]

The diagnosis of NAFLD occurs in the presence of hepatic steatosis and the concomitant absence of other types of liver pathologies.[7] Nowadays, the diagnosis is based mainly on liver biopsies, an invasive procedure with several degrees of potential complications. However, other types of noninvasive biomarkers currently are available or under investigation.[8]

Different theories have been postulated to explain the onset and progression of NAFLD. If previously the two-hits hypothesis was considered the principal model for NAFLD pathogenesis, at present the multiple-hits hypothesis is the one that best recapitulates the process at the basis of the

disease. Briefly, in the two-hits hypothesis, an imbalance between hepatic lipid input and output was observed, which eventually resulted in triglyceride accumulation within the hepatocytes (first hit). This renders the liver more exposed to other forms of injuries, which ultimately lead to the activation of inflammatory processes and hepatic stellate cells (HSCs), with consequent extracellular matrix deposition and onset of NASH and fibrosis (second hit).[9] Although initially considered as the most reliable model to explain NAFLD development, scientific advances made clear that the two-hits hypothesis is too simplistic to explain the vast complexity of this disease. Contrarily, the multiple-hits hypothesis considers NAFLD as the resultant combination of parallel insults acting on genetically predisposed individuals.[10]

Notably, differently from the previous hypothesis, the multiple-hits hypothesis also takes into consideration the contribution of extrahepatic tissue to liver inflammation, including the gut and adipose tissue. Gut-derived molecules such as endotoxin, a key component of many bacteria present in the microbiota, might contribute to the exacerbation of both hepatic lipid accumulation and inflammation.[11] Moreover, adipokines secreted by the adipose tissue may impact NAFLD perpetration by regulating hepatic fat accumulation, insulin resistance, and fibrosis.[12]

Genome-wide association studies have identified several genes involved in NAFLD pathogenesis. Nonsynonymous polymorphisms in patatin-like phospholipase 3 (PNPLA3), a multifunctional enzyme involved mainly in triacylglycerol hydrolysis, have been associated with the severity of NAFLD in both pediatric and adult individuals (rs738409 C/G). In subjects carrying 2 minor G alleles (rs738409 G/G), fatty liver progresses directly to NASH.[13,14] Intriguingly, the absence of PNPLA3 in mouse liver or in cultured hepatocytes determines a decreased accumulation of triglycerides, finally conferring protection against NAFLD.[15,16] Further studies surely are needed to clarify the role of PNPLA3 in liver steatosis and its sequelae. In addition to PNPLA3, other genes have been correlated with NAFLD susceptibility, including the transmembrane 6 superfamily 2 and the glucokinase regulator, whose genetic variants have been associated with histologic hepatic lipid accumulation.[17–19]

The genetic predisposition alone is not sufficient to promote NAFLD development. Environmental insults (ie, dietary habits, obesity, and so forth) are involved in the disease progression as well, driving toward a progressive inflammatory phenotype, particular to NASH.[10]

To fully elucidate NAFLD and NASH peculiarities and the mechanisms involved in their progression toward severe forms of disease, several animal models have been used. Each model showed advantages and disadvantages, but none reliably reflected all features of human disease. Two major groups can be distinguished: mice that acquire the disease after dietary or pharmacologic manipulation and the genetically modified ones. Among dietary models, the methionine- and choline-deficient diet (MCDD), high-fat diet (HFD) of diverse compositions, high-cholesterol diet, and fructose-based diets are the most representative. However, different criticisms have emerged in regard to administration of these diets because they do not recapitulate the principal physiological characteristic of NAFLD. For instance, although mice fed with MCDD utterly reproduce steatohepatitis, they display weight loss and decreased concentration of circulating cholesterol and triglycerides, thus not completely resembling human disease. On the contrary, a HFD can induce insulin resistance and obesity, but fails to induce severe liver injury.[20,21] Furthermore, the administration of carbon tetrachloride (CCl_4), a hepatotoxin, has been widely used to induce oxidative stress to the liver, followed by accumulation of toxic lipid species and tissue necrosis. However, fibrosis phenotype promptly regresses after discontinuing drug administration.[22] Finally, genetic models including *ob/ob* and *db/db* mice (both characterized by disrupted leptin signaling), as well as genetically engineered rodents, have been used to improve the understanding of the molecular processes involved in NAFLD onset and progression. Despite providing the opportunity to control both genetic and environmental factors, most of these models required concomitant dietary or drug administration to fully develop the disease and often recap only one aspect of NAFLD.[23] In any case, the use of mouse models allowed a deep comprehension of the molecular determinants of NAFLD and facilitated the identification of the potential pharmacologic intervention for NAFLD therapy.

Intriguingly, although several drugs have been tested or currently are under evaluation in clinical trials, no effective therapy has been approved for the treatment of NAFLD.[24] Major changes in lifestyle, such as dietary improvements and increased physical exercise, beneficially impact the management of NAFLD and may delay the progression of the disease, however, for many individuals this is not achievable, with consequent frequent relapse.[25]

Here, we report the impact of the principal nuclear receptors (NRs) in the pathogenesis and treatment of NASH. NRs act as ligand-activated transcription factors, and constitute a superfamily of 48 members divided into 7 subfamilies designated as NR0–NR6.[26] They mediate a wide range of physiological processes, including development, metabolism, and reproduction,[27] in particular, the NR1 subfamily is implicated in energy/nutrient control. These NRs form a heterodimer with retinoid X receptor and are activated by binding with ligands. When the ligand is unbound, NRs are inactivated and linked to co-repressors. The binding of NRs with ligand and retinoid X receptor allows the release of co-repressors and the recruitment of co-activators starting the transcription of target genes.[28] Peroxisome proliferator-activated receptor (PPAR)α, β/δ, and γ (NR1C1-3); farnesoid X receptor (FXR, NR1H4); and liver X receptor α and β (LXR, NR1H2-3) belong to this subfamily, which overall exert a key role in the pathogenesis of NAFLD and NASH.

PPARs in Hepatic Physiology and NASH

The PPAR subfamily members play a crucial role in the regulation of lipid metabolism in different tissues. Although they usually are considered as master regulators of fatty acids, PPARs now also have been recognized for playing an

important role in lipid and carbohydrate metabolism, as well as in inflammation and cellular proliferation.[29] There are 3 PPAR isotypes, termed PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3), which display a different tissue distribution. PPARα is found mainly in tissue with high fatty acid catabolism, such as the liver. PPARβ/δ is distributed ubiquitously, with a hepatic expression that varies from low to moderate in human beings and rats, and from moderate to high in mice. On the contrary, PPARγ is present at high levels in white adipose tissue. Intriguingly, although PPARγ is expressed weakly in healthy liver, its expression is correlated positively with liver steatosis in obese patients.[30–33]

PPARα

In the liver, PPARα coordinates different pathways involved in fatty acid metabolism as well as inflammation.[31,34,35] Upon binding to fatty acid derivatives formed during lipolysis or to synthetic ligands (ie, fibrates), PPARα mediates the transcriptional regulation of several genes that are widely implicated in the adaptive or protective response exerted by this nuclear receptor in the liver.[36–39] PPARα directly regulates the expression of fatty acid transporters involved in cellular fatty acid uptake, and controls the expression of the principal enzymes of peroxisomal β-oxidation, the catabolic process by which fatty acids are broken down to produce energy.[40–44] Moreover, activated PPARα up-regulates the mitochondrial hydroxymethylglutaryl-Coenzyme A (CoA) synthase, the rate-limiting enzyme of ketogenesis. PPARα fasting knock-out mice show impaired fatty acid β-oxidation, hypoglycemia, and an inability to produce ketone bodies.[45,46] Notably, PPARα also is able to regulate the hepatic lipogenic program. Indeed, in addition to the direct induction of sterol regulatory element-binding protein 1c (SREBP1c), PPARα also indirectly can coordinate SREBP1c expression through cross-regulation with the LXR signaling pathway.[47,48] Although these functions may appear conflicting, it is plausible that in a fed state PPARα controls de novo lipogenesis to provide lipids for storage. On the contrary, during fasting PPARα activity shifts to fatty acid uptake and fatty acid β-oxidation. In this way, PPARα is able to supply energy to peripheral tissues via ketogenesis. Finally, PPARα shows an anti-inflammatory activity in a murine model of systemic inflammation. Indeed, lipopolysaccharide (LPS)-induced acute-phase response is inhibited by fenofibrate treatment in hepatic-specific PPARα mice, but not in PPARα-deficient mice.[49]

Early evidence regarding the hepatoprotective role of PPARα in NAFLD comes from preclinical studies. PPARα-null mice subjected to HFD show massive hepatic lipid accumulation owing to inhibition of fatty acid uptake and β-oxidation.[45] Moreover, both HFD-fed mice and obese Zucker rats treated with selective PPARα agonists show improved insulin sensitivity, suggesting that PPARα is active in the early pathologic stages to guarantee a healthy liver.[50] Interestingly, mice with a hepatocyte-specific deletion of PPARα fed with a standard diet develop steatosis in aging, without becoming overweight, thus indicating that hepatic

PPARα regulates liver as well as whole-body fatty acid homeostasis.[51]

In addition to steatosis, PPARα also can ameliorate NASH pathology. Indeed, in mice, MCDD-induced steatohepatitis and fibrosis can be reversed by treatment with the PPARα agonist Wy-14,643. The activation of PPARα prevents intrahepatic lipid accumulation and inflammation by lowering the number of activated macrophages and HSCs, finally promoting the normalization of the histologic changes typical of NASH.[52,53] Moreover, mice lacking adipose triglyceride lipase, which fail to generate endogenous PPARα agonists, are more prone to develop hepatic inflammation when challenged with LPS and MCDD compared with wild-type mice.[54]

The contribution of PPARα to early stages of NASH have been studied in apolipoprotein-E2 (APO-E2) knock-in mice, which mimic human type III hyperlipoproteinemia.[55] The whole-body deletion of PPARα in APO-E2 knock-in mice fed a Western diet exacerbates hepatic steatosis and inflammation. On the contrary, APO-E2 knock-in mice treated with fibrates show induction of PPARα activity. This results in the down-regulation of proinflammatory genes and in the up-regulation of genes involved in lipid catabolism. Overall, these changes inhibit NASH progression.[56,57]

The hepatoprotective effects of PPARα activity are partially mediated by Vanin 1, a pantetheinase expressed in liver and secreted in serum that regulates tissue adaptation to stress. The concentration of serum Vanin 1 reflects PPARα activation in the liver. Vanin 1 ablation in mice as well as inhibition of Vanin 1 activity in rats results in hepatic steatosis in response to fasting associated with a change in the expression of inflammatory and oxidative genes.[58,59]

Finally, the healthy benefits of PPARα also are attributable to fibroblast growth factor 21 (FGF21), a hepatokine secreted from the liver directly into the bloodstream, which upon binding to a specific receptor complex in target tissues improves systemic insulin sensitivity and lipid turnover. In the liver, activation of PPARα results in a significant increase of both hepatic and serum levels of FGF21, and PPARα *null* mice are FGF21-deficient. Notably, mice lacking both PPARα and FGF21 or FGF21 alone are more prone to develop hepatic steatosis when fed a MCDD.

Intriguingly, in human beings the expression of PPARα negatively correlates with the presence of NASH and the severity of steatosis.[60] The administration of PPARα agonists (discussed in more detail later) in pilot studies and clinical trials has further assessed the clinical relevance of the contribution of PPARα to NAFLD/NASH.

Generally, PPARα controls energy and nutrient homeostasis, both directly, via activation of genes encoding enzymes involved in fatty acid metabolism, and, indirectly, by means of FGF21. This capacity, coupled with its anti-inflammatory actions, results in the overall protection against hepatic fatty acid accumulation and progression toward NASH.

PPARβ/δ

PPARβ/δ is the least studied in this family of nuclear receptor, although its expression is fundamental for embryo

development. Indeed, disruption of the PPARβ/δ gene lead to embryonic death in the first days of development owing to impaired placenta development and giant cell differentiation.[61] Despite its role in embryogenesis, PPARβ/δ plays a role in the regulation of energy metabolism in several organs, including the liver.[62] In the liver, PPARβ/δ is highly expressed in hepatocytes, HSCs, and Kupffer cells, thus indicating a potential role of this nuclear receptor in inflammation and fibrosis.[63] Intriguingly, the hepatic action of PPARβ/δ and PPARα in fatty acid β-oxidation and transportation appears moderately redundant. However, PPARβ/δ fails to compensate for the absence of PPARα in PPARα-null mice fed with a HFD.[45,62,64]

Several monounsaturated fatty acids (MUFAs) can bind to and activate PPARβ/δ, inducing balanced control of both hepatic fatty acids and glucose metabolism.[65] PPARβ/δ mediates the activation of the principal enzyme designated to endogenous MUFAs synthesis, the stearoyl-CoA desaturase 1 (SCD1), finally resulting in a positive loop of regulation that culminates in a liver safeguard.[65,66] Indeed, animals with liver-specific adenovirus-mediated PPARβ/δ activation fed with a HFD show less hepatic damage, despite increased lipid accumulation. This is mostly owing to the induced expression of SCD1, which avoids lipotoxicity by converting saturated fatty acids into MUFA.[65] The saturated fatty acid:MUFA ratio is fundamental to preserve cellular homeostasis; indeed, a shift toward saturated fatty acid has been related to several pathologic conditions.[67] Furthermore, hepatic PPARβ/δ overexpression or activation in db/db mice inhibits the expression of SREBP1c, the master regulator of lipid biogenesis, finally leading to improved hepatic steatosis.[68] Interestingly, the expression of SCD1 can be induced by SREBP1c.[69] Therefore, it is plausible that both PPARβ/δ and SREBP1c contribute to the fine-tuning of this enzyme in the liver to limit the accumulation of toxic lipid species with consequential detrimental effects.

Mice with hepatocyte PPARβ/δ activation show high circulating levels of phosphatidylcholine (18:0/18:1), which promotes muscle fatty acid uptake and catabolism via PPARα. On the contrary, hepatic PPARβ/δ ablation shows the opposite effect. Notably, administering phosphatidylcholine (18:0/18:1) to db/db mice improves metabolic homeostasis, thus corroborating the protective role for PPARβ/δ in liver steatosis.[70] Another mechanism through which PPARβ/δ elicits amelioration of NAFLD resides in its capacity to regulate hepatic very-low-density lipoprotein receptor (VLDLR). Indeed, the expression of VLDLR correlates negatively with the abundance of PPARβ/δ in steatotic liver biopsy specimens, and the absence of the nuclear receptor in mice and primary cultured hepatocytes resulted in increased VLDLR levels.[71] However, several studies have shown that VLDLR expression is up-regulated by several PPAR agonists, including PPARβ/δ ones.[72–74] The administration of the PPARβ/δ agonist GW501516 increases VLDLR levels and triglycerides accumulation in the liver of wild-type mice, but in PPARβ/δ knockout animals this effect was blunted.[74] In macrophages, VLDL particles bind to PPARβ/δ and lead to the activation of a downstream pathway, eventually inducing triglyceride accumulation.

Notably, in this context the expression of VLDLR also increases when PPARβ/δ expression is null.[75] Overall, this suggests that PPARβ/δ is essential for orchestrating the transcriptional response of VLDL particles and finely modulates the level of VLDLR, probably on the basis of the available ligands. However, it also is possible that VLDLR is required to guarantee the action of exogenous PPARβ/δ ligands.

In addition to its function in hepatic metabolism, PPARβ/δ also has a major impact on inflammation.[29,76,77] However, the exact role of PPARβ/δ activation in liver inflammation is not well established, given the conflicting results obtained until now. On one hand, the activation of PPARβ/δ has been correlated with the induction of anti-inflammatory signals. Indeed, CCl4-treated PPARβ/δ-null mice show higher levels of liver fibrosis than wild-type mice, owing to induced HSC proliferation. Moreover, the administration of GW0742 as well as KD3010, 2 PPARβ/δ agonists, to the wild-type mice resulted in amelioration of a fibrosis condition both in the CCl4-fibrotic model and in the cholestasis-induced fibrosis model.[78,79] On the other hand, activating PPARβ/δ using the synthetic ligand GW501516 or L165041 in CCl4-treated mice enhanced the fibrotic response owing to increased expression of proinflammatory and profibrotic genes, as well as HSC stimulation.[80,81] Further studies are needed to clarify the contribution of PPARβ/δ to inflammation-driven hepatic injuries.

PPARγ

In mammals, PPARγ exists as 2 protein isoforms, both deriving from a single gene, which differ in length and tissue expression.[82,83] Although PPARγ2 (G2 isoform) is expressed mainly in adipose tissue, where it governs lipid storage and adipocytes differentiation, PPARγ1 (G1 isoform) also can be found ubiquitously at low levels in non–white adipose tissue (WAT) such as liver, spleen, and heart.[83,84] Moreover, PPARγ1 is expressed abundantly in macrophages, where it regulates cholesterol homeostasis, macrophage activation, and repression of inflammation.[85–88] Notably, the high abundance of PPARγ messenger RNA in the liver is a manifest feature of the steatotic liver in both human beings and experimental animal models.[33,89,90] Mice treated with HFD show up-regulation of PPARγ with concomitant induction of liver steatosis.[91] Accordingly, hepatocyte PPARγ selective ablation exerts a protective effect against hepatic steatosis in HFD-fed mice as well as ob/ob mice.[92,93] Indeed, evidence in liver-specific PPARγ knockout mice indicates that PPARγ induces hepatic lipid accumulation by promoting the synthesis of new fatty acids together with their increased uptake.[93] Furthermore, the treatment with PPARγ ligand rosiglitazone results in an increased steatogenic effect in the liver of KK-A^y mice, which recapitulates the features of human NAFLD, including altered adipokine expression, obesity, dyslipidemia, and insulin resistance.[94] However, rosiglitazone administration to NASH patients ameliorates insulin sensitivity and histologic markers of steatosis.[95,96] In a murine model of MCDD-induced fibrosis, rosiglitazone treatment prevents NASH development.[97] Moreover,

adenovirus-mediated PPARγ overexpression in mice fed with MCDD for 2 months causes the resolution of liver fibrosis via decreased HSC proliferation and cell-cycle arrest and apoptosis.[98] Indeed, the activated phenotype of HSCs may be reversed to quiescent ones upon PPARγ ligands, thus pointing at PPARγ capacity to modulate proinflammatory and profibrogenic gene expression.[99,100] Although activation of PPARγ elicits a harmful outcome in hepatocytes with the promotion of NAFLD progression, in HSCs its activity exerts beneficial effects that result in the resolution of NASH. Indeed, the disruption of PPARγ expression in macrophages and HSCs aggravates the fibrogenic response to CCl$_4$-induced liver injury.[101] Finally, PPARγ expression in liver macrophages, both Kupffer cells and infiltrating monocytes, is necessary for an alternative macrophage activation (M2) pathway, which is associated with decreased release of inflammatory cytokines and growth factors, therefore resulting in attenuated fibrosis.[102] Indeed, macrophage PPARγ deletion predisposes animals to develop diet-induced obesity and insulin resistance, as well as worsens CCl$_4$-induced liver fibrosis.[101,103] If overall PPARγ activation is driving or diminishing hepatic damage is still not completely clear. Further studies aimed at identifying a proper therapy to selectively balance desirable and detrimental effects is of primary importance.

PPARs and NASH Treatments

The first line in the management of NASH patients is represented by lifestyle modifications, which includes weight loss through a proper dietary regimen and concomitant increased physical exercise.[7,104] Indeed, by losing up to 10% of body weight, NASH patients showed diminished inflammation and regression of fibrosis.[105,106] However, because lifestyle modifications are not easily achieved and kept over time, the treatment of NASH patients also required a combination with pharmacologic intervention.

Since now, several drugs have been tested and most of them have been designed to specifically target NRs in the liver. However, NR-based therapies usually showed poor efficacy in human beings. Today, new combined drugs targeting NRs, including dual-/triple-agonists and NR modulators, are emerging as promising pharmacologic interventions in NASH patients, with minimal negative metabolic effects[107] (Figure 1, Table 1).

Given the hepatoprotective role of PPARα, therapies aimed at restoring its expression or activity are widely considered beneficial for the treatment of NASH patients. Fibrates represent the first class of PPARα ligands able to improve lipid accumulation and inflammation. However, despite their extensive use in the treatment of hypertriglyceridemia, fibrates do not show any benefit in the treatment of NAFLD and its sequelae. Indeed, fibrates are weak PPARα agonists, and their administration has been limited owing to dose-related adverse events.[35,108,109]

Although few data still exist concerning the role of PPARβ/δ in NASH, it is widely recognized that active PPARβ/δ is able to attenuate insulin resistance and the inflammatory process. A PPARβ/δ agonist (GW501516) originally was designed, however, despite the promising results in the initial trial, the drug has been withdrawn because of safety concerns.[110] Seladelpar (also known as MBX-8025) is another selective PPARβ/δ agonist and its administration in preclinical and clinical studies ameliorated the serum lipid profile, lowered liver enzyme levels, and contrasted the accumulation of lipotoxic lipid species, thus indicating beneficial properties for NAFLD. However, it has been suspended from a phase II trial because of unexpected histologic findings.[111–114] In any case, the development of new pharmacologic drugs targeting PPARβ/δ has to take into consideration a preponderant side effect: the activation of this nuclear receptor triggers inflammatory pathways in the epidermis and enhances keratinocyte proliferation, thus inducing psoriasis.[115,116]

A dual-agonist targeting PPARα and PPARβ/δ has been developed, namely Elafibranor (GFT505, Genfit, France). Treatment with this drug improves NASH conditions in human beings, with a regression in fibrosis stage.[117] However, Elafibranor recently failed to pass the phase III clinical trial that investigates the efficacy against NASH and the safety of long-term administration.

PPARγ activators thiazolidinediones have been broadly used in the management of diabetes as insulin sensitizers, and their effectiveness in NASH was shown recently. However, side effects such as weight gain and risk of heart failure associated with thiazolidinediones (ie, pioglitazone) may limit their use.[118–120] A dual PPARα/γ agonist, saroglitazar, has been approved in India for the treatment of diabetic patients. However, this drug decreases biomarkers of hepatic health in both NAFLD and NASH patients.[121]

The protective effects of PPARs against fibrosis and inflammation lead to the generation of PPAR pan agonist, with the belief that, differently from targeting a single isotype, orchestrating all the PPARs together will induce an optimal metabolic response able to contrast and reduce NASH, given the combined effect of single PPAR agonists. Bezafibrate was one of the earlier PPAR pan agonists developed. Its administration in mice fed with MCDD improved hepatic steatosis and inflammation when used in combination with the PPARβ/δ agonist GW501516.[122] Lanifibranor (IVA337), another PPAR pan agonist targeting all 3 isotypes, decreased liver steatosis, hepatocyte ballooning, and fibrosis in different mouse models of NASH.[123,124] It was proved to be effective against skin and lung fibrosis.[125,126] Lanifibranor currently is being tested in a phase 2b clinical trial.

FXR–FGF15/19 in the Gut–Liver Axis

FXR was described in 1995[127] and it is the master regulator of bile acid (BA) homeostasis.[128] It has a specific tissue distribution in the gastrointestinal tract with a peak in the liver and ileum, as well as in the kidney and adrenal glands.[127,129,130] BA homeostasis is the result of the crosstalk between the liver and the intestine orchestrated by tissue-specific FXR activities. This NR reduces BA de novo synthesis in the liver, promotes BA secretion in bile,
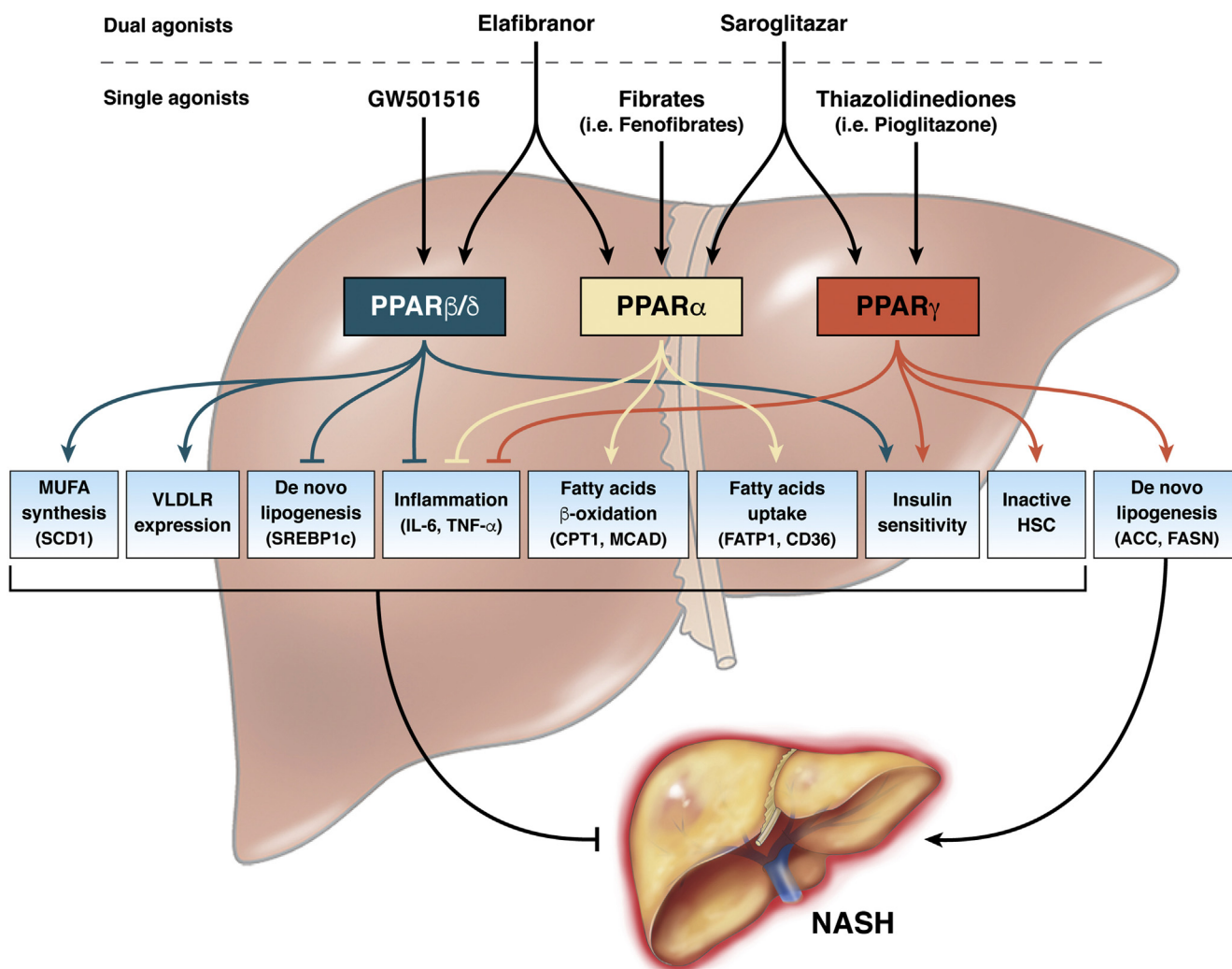
Figure 1. Role of selective agonists of PPARs in NASH. In the liver, the 3 existing isoforms of PPAR can be activated by selective agonists. Although initially developed as a single agonist, able to selectively activate 1 single isoform, recently, dual agonists simultaneously targeting 2 PPAR isoforms represented the better therapeutic strategy to limit the detrimental effects of NASH. The beneficial effect of PPAR activation in the liver is the result of a complex cross-talk between different cellular and molecular pathways, which overall down-regulate lipid accumulation and contrast inflammation, thus contributing to improved liver health. Despite the role of PPARα and PPARβ/δ in NASH pathology being unambiguously clear, some concerns remain for PPARγ. Indeed, when activated in hepatocytes, PPARγ acts to promote fatty acid accumulation, steatosis, and progression toward NASH. On the contrary, the activation of PPARγ in HSCs exerts beneficial effects that result in the resolution of NASH. *Red lines* indicate the pathways down-regulated by PPAR activation, whereas *green lines* specify the pathways induced by PPAR agonism. ACC, acetyl CoA carboxylase; CD36, fatty acid translocase CD36; CPT1, carnitine palmitoyltransferase 1; FASN, fatty acid synthase; FATP1, Fatty acid transport protein 1; IL6, interleukin 6; MCAD, medium-chain acyl-coenzyme A dehydrogenase.

increases BA intestinal re-absorption, and decreases hepatic basolateral BA re-uptake.

BAs are amphipathic detergents synthesized in the liver via a multistep reaction that converts cholesterol into BAs.[131] In the classic pathway, cholesterol is oxidized by the rate-limiting enzyme cytochrome P450 7A1 (CYP7A1) to produce cholic acid (CA). Alternative or acidic pathways lead to the formation of chenodeoxycholic acid (CDCA) through 27-hydroxylase. CA and CDCA are conjugated with taurine or glycine to form less-toxic, more hydrophilic bile salts. BAs are stored in the gallbladder and then secreted into the small intestine, where they contribute to the digestion of lipids, cholesterol, and fat-soluble vitamins.[132] In the intestine, gut microbiota via bile salt hydrolase mediates the deconjugation of CA and CDCA to secondary BAs: deoxycholic acid and lithocholic acid or ursodeoxycholic acid, respectively.[133] Finally, BAs are reabsorbed back through the portal circulation into the liver,[132] recycling 95% of BAs and reducing their de novo synthesis.

At the distal ileum, BAs are reabsorbed by the apical sodium-dependent bile acid transporter.[134] In enterocytes, BAs are able to activate FXR, inducing the fibroblast growth

Table 1. PPARs, FXR–FGF19, and NAFLD/NASH Clinical Trials

Trial identifier	Trial phase (status)	Disease	Intervention
PPAR			
NCT03008070	Active, not recruiting	NASH	Drug: lanifibranor
			Drug: placebo
NCT02285205	Completed	Type 2 diabetes	Drug: oral administration of lobeglitazone
		NAFLD	
NCT00252499	Terminated, has results	Fatty liver insulin resistance	Drug: rosiglitazone
			Drug: fenofibrate
			Drug: placebo for rosiglitazone
			Drug: placebo for fenofibrate
NCT00633282	Completed	NAFLD	Behavioral: lifestyle intervention
			Drug: pioglitazone
			Drug: berberine
NCT01694849	Completed	NASH	Drug: elafibranor 80 mg
			Drug: elafibranor 120 mg
			Drug: placebo
NCT03639623	Recruiting	Liver transplant complications	Drug: saroglitazar
		NAFLD	
NCT00062764	Completed, has results	NASH	Drug: Actos (pioglitazone)
NCT00013598	Completed	Fatty liver	Drug: pioglitazone
		NASH	
NCT03883607	Recruiting	NASH	Drug: elafibranor 80 mg
			Drug: elafibranor 120 mg
NCT03953456	Recruiting	NAFLD	Drug: elafibranor 120 mg
			Drug: placebo
NCT02704403	Recruiting	NASH with fibrosis	Drug: elafibranor
			Drug: placebo
NCT03617263	Recruiting	NAFLD in women with PCOS	Drug: saroglitazar magnesium 4-mg tablet
			Drug: placebo
NCT03061721	Active, not recruiting	NASH	Drug: saroglitazar magnesium 1 mg
		NAFLD	Drug: saroglitazar magnesium 2 mg
			Drug: saroglitazar magnesium 4 mg
			Drug: placebo
NCT03863574	Recruiting	NASH	Drug: saroglitazar magnesium 2 mg
			Drug: saroglitazar magnesium 4 mg
			Drug: placebo
NCT02265276	Unknown	Fatty liver	Drug: saroglitazar
			Drug: pioglitazone
NCT04193982	Not yet recruiting	NAFLD	Drug: saroglitazar
			Drug: vitamin E
			Drug: combination drug
			Behavioral: lifestyle changes
NCT02891408	Completed	NASH	Drug: firsocostat
			Drug: fenofibrate
NCT01289639	Terminated, has results	Fatty liver	Drug: fenofibrate
			Drug: pioglitazone
			Drug: placebo
NCT00262964	Completed, has results	NAFLD	Drug: niacin
			Drug: fenofibrate
			Drug: placebo
NCT02781584	Recruiting	NASH	Drug: SEL
		NAFLD	Drug: firsocostat
			Drug: cilofexor
			Drug: fenofibrate
			Drug: vascepa
NCT03646292	Not yet recruiting	NAFLD	Drug: pioglitazone
		Type 2 diabetes	Drug: empagliflozin
			Drug: combination of pioglitazone and empagliflozin
NCT00994682	Completed, has results	Type 2 diabetes mellitus	Drug: pioglitazone study drug
		NASH	Drug: placebo
		NAFLD	Drug: pioglitazone open label
NCT02365233	Terminated, has results	Type 2 diabetes mellitus	Drug: DPP4 inhibitor
		NAFLD	Drug: pioglitazone
			Drug: Lantus insulin
FXR–FGF19			
NCT01265498	Completed, has results	NAFLD	Drug: obeticholic acid
		NASH	Drug: placebo

Table 1. Continued

Trial identifier	Trial phase (status)	Disease	Intervention
NCT02855164	Active, not recruiting	NASH	Drug: tropifexor (LJN452)
			Drug: placebo
NCT03976687	Recruiting	NASH	Drug: EYP001a
		Healthy	
NCT01999101	Completed	NAFLD	Drug: Px-104
NCT04065841	Recruiting	NASH	Drug: tropifexor
			Drug: licogliflozin
NCT03836937	Recruiting	NAFLD	Drug: obeticholic acid
NCT00501592	Completed, has results	Diabetes mellitus, type II	Drug: INT-747
		Fatty liver	Drug: placebo
NCT04328077	Not yet recruiting	NASH	Drug: TERN-101
			Other: placebo
NCT02808312	Completed	NASH	Drug: GS-9674 (30 mg)
			Drug: GS-9674 (10 mg)
NCT02654002	Completed	NASH	Drug: GS-9674
			Drug: placebo
NCT02854605	Completed	NASH	Drug: GS-9674
			Drug: placebo to match GS-9674
NCT02918929	Completed	Presumptive NAFLD	Drug: EDP 305
			Drug: placebo
NCT02633956	Completed, has results	NASH	Drug: obeticholic acid
			Drug: atorvastatin
			Drug: placebo
NCT02548351	Active, not recruiting	NASH	Drug: obeticholic acid
			Drug: placebo
NCT03439254	Active, not recruiting	Compensated cirrhosis	Drug: obeticholic acid (10 mg)
		NASH	Drug: obeticholic acid (10–25 mg)
			Drug: placebo
NCT03912532	Recruiting	NASH	Biological: NGM282
			Other: placebo
NCT02443116	Active, not recruiting	NASH	Biological: NGM282
			Other: placebo
NCT04210245	Recruiting	Compensated cirrhosis	Biological: aldafermin
		NASH	Other: placebo

Actos (pioglitazone) (Takeda Global R&D Centre Ltd, Tokyo, Japan); DPP4, Dipeptidyl peptidase-4; PCOS polycystic ovarian syndrome; SEL, Selonsertib.

factor FGF15/19 (mouse and human, respectively) expression. FGF19 is an enterokine that travels through the portal circulation, reaches the liver, and binds to the FGF receptor $4/\beta$-Klotho complex. The binding leads to the activation of the c-jun N-terminal kinase-dependent pathway, which ultimately down-regulates CYP7A1 expression and reduces BA synthesis.[135] In enterocytes, FXR increases BA intestinal re-absorption and secretion in the portal vein, up-regulating the intestinal BA binding protein (which shuttles BAs from the apical to the basolateral membrane),[136,137] and the heterodimeric organic solute transporter α/β.[138] Furthermore, FXR reduces hepatic basolateral BA re-uptake, negatively regulating the sodium–taurocholate cotransporter protein and organic anion transporting polypeptide expression.

In hepatocytes, FXR activation induces the small heterodimer partner (SHP), reducing CYP7A1 expression and BA synthesis.[129] Notably, in the liver, FXR activation promotes BA excretion but does not reduce BA synthesis with the same intensity of the FGF15/19 pathway.[139]

FXR and NASH: Preclinical Studies

The role of FXR activation on the development and protection against NASH has been evaluated in several studies using different mouse models and FXR agonists. Systemic activation of FXR prevents hepatic steatosis, inflammation, and fibrosis. In mice fed a HFD, the administration of FXR agonists (GW4064 and obeticholic acid [OCA]) improved glucose tolerance, and reduced body weight, fat mass, and hepatic triglyceride accumulation, ameliorating steatosis severity.[140,141] In mice fed a HFD and in LDLR$^{-/-}$ mice, a model of Western diet, the administration of GW4064 and WAY-362450, 2 synthetic FXR agonists, reduced triglyceride and cholesterol levels, and WAY-362450 treatment also decreased high-density lipoprotein (HDL) levels.[141,142] C57BL/6 mice fed a MCDD, a well-established NASH model, and treated with WAY-362450 for 4 weeks, showed a reduction of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, inflammatory cell infiltration, and hepatic steatosis.[143] These positive effects of WAY-362450 were abolished in

FXR$^{-/-}$ mice fed a MCDD. FXR$^{-/-}$ mice showed worse liver damage than wild-type mice.[144] Interestingly, FXR$^{-/-}$ mice fed a MCDD developed hepatic cholestasis owing to the high concentration of BAs in the liver and the inhibition of genes involved in fatty acid uptake and triglyceride accumulation.[144]

It has been shown that hepatic FXR activation via SHP induction decreases lipogenesis and increases fatty acid oxidation through PPARα, leading to protection against NASH development.[145,146] Interestingly, FXR induces PPARα expression and fatty acid oxidation only in human beings because the murine PPARα promoter does not present a functional FXR responsive element. In addition, FXR regulates hepatic glucose metabolism and immune response. In db/db mice the administration of GW4064 for 5 days activated glycogenesis and ameliorated insulin sensitivity.[147] Animal model studies showed that FXR activation is able to induce the expression of genes involved in the acute-phase response and the activation of Natural Killer T (NKT) cells.[148,149] In mice fed a MCDD and treated with WAY-362450, the activation of FXR reduced inflammatory infiltrates and Monocyte Chemoattractant Protein-1 (MCP1) levels.[143] In line with this, monocytes isolated from FXR$^{-/-}$ mice showed high levels of interleukin 1β, tumor necrosis factor α (TNFα), and interferon γ, and were less responsive to anti-inflammatory drugs.[150] Activation of FXR in HSCs induced SHP expression, reducing hepatic fibrosis.[151,152] Furthermore, in rat HSCs, OCA administration induced PPARγ expression, reducing collagen gene induction.[153]

Mice fed a HFD and treated with fexaramine, an intestinal-specific FXR agonist, showed a reduction of body weight and lipogenesis expression genes and an induction of browning adipose tissue and energy expenditure ameliorating triglyceride levels and steatosis.[154,155] In addition, fexaramine treatment improved insulin sensitivity, reducing fasting serum insulin and increasing serum glucagon-like peptide 1 levels.[155] This FXR agonist is able to change BA composition, up-regulating taurolithocholic acid (TLCA) and lithocholic acid levels, strong agonists of FXR-Takeda G-protein receptor 5, which up-regulates serum glucagon-like peptide 1 levels.[156,157] Furthermore, intestinal FXR activation leads to FGF15/19 induction, which prevents steatosis, inflammation, fibrosis, and the metabolic syndrome, major causes of NASH. Fgf15$^{-/-}$ mice fed a HFD showed severe steatosis.[158,159] FGF15/19 administration down-regulates the expression of genes involved in lipid synthesis such as fatty acid synthase, Scd1, and diacylglycerol O-acyl-transferase 2, and modifies BA composition, increasing tauro-β-muricholic acid (TβMCA) levels.[159] In mice fed a high-fat, high-fructose, and high-cholesterol diet, treatment with FGF19 analog (M70) reduced hepatic inflammation and fibrosis.[159] Furthermore, FGF19 acts on the metabolic syndrome, decreasing total weight and body fat mass, dyslipidemia, and ameliorating glucose homeostasis.[159]

Very recently, it has been shown that high-fructose and high-fat–fed pigs developed NASH, cholestasis, and impaired FXR–FGF19 signaling in the gut–liver axis. In this animal model, the severity of NASH was correlated with the reduction of FGF19 levels that lead to gut dysbiosis and increased colonic levels of choline metabolites and secondary BAs (FXR, NASH, and microbiota).

Several preclinical studies focused on the role of OCA, a selective FXR agonist with 100-fold activity higher than CDCA,[160] in the prevention of NASH development, highlighting the ability of OCA to modulate glucose and lipid homeostasis and to promote hepatic anti-inflammatory and antifibrotic effects.[161] In Zucker fa/fa obese rats, the administration of OCA (10 mg/kg) reduced insulin resistance and hepatic steatosis as well as body weight gain and liver fat deposition.[162] Similar effects were observed in APO-E2$^{-/-}$ mice treated with OCA for 12 weeks.[163] Furthermore, in these mice, drug administration prevented aortic plaque formation, reducing hepatic triglycerides and cholesterol content, although the development of atherosclerosis was not inhibited.[164] OCA administration in a rabbit model of metabolic syndrome reduced visceral fat and improved glucose tolerance.[165] OCA also shows immunomodulatory and anti-inflammatory effects. In vascular smooth muscle cells OCA down-regulated nuclear factor-κB–dependent expression of inducible nitric oxide synthetase (NOS) and cyclo-oxygenase-2.[166] In a mouse model of hepatitis, OCA treatment reduced serum AST, interferon γ, and TNF-α levels.[148] Furthermore, OCA prevents hepatic fibrosis from acting on HSC activation.[152] In a thioacetamide rat model of liver fibrosis, OCA treatment decreased fibrosis and cirrhosis, reducing portal hypertension.[167] Taken together, these data indicate that OCA ameliorates glucose levels and insulin sensitivity, and reduces hepatic lipid synthesis and inflammation, preventing liver damage.

FXR and NASH: Clinical Studies

FXR agonists represent an attractive class of drugs for patients with chronic liver disease. Currently, several human clinical trials are testing the safety and effects of these compounds (Table 1). In particular, OCA, a 6-ethyl-CDCA, has been approved for the treatment of primary biliary cholangitis. Clinical trials tested OCA in patients with NAFLD with type II diabetes and NASH.[168,169] In a phase II clinical trial, 64 patients with NAFLD and type II diabetes were randomized to placebo, 25 mg OCA, and 50 mg OCA. The drug improved insulin sensitivity, body weight, serum levels of ALT, serum levels of γ-glutamyltransferase, serum levels of triglycerides, and fibrosis markers. OCA increased serum levels of alkaline phosphatase and LDL, and reduced HDL concentration. As expected, the drug increased FGF19 levels and reduced BA concentration, confirming FXR activation.[168]

In the second trial, a multicenter, randomized, phase III study, the FXR ligand obeticholic acid for noncirrhotic, nonalcoholic steatohepatitis trial (FLINT), 283 patients were treated for 72 weeks and randomized to placebo or 25 mg OCA. FLINT showed that OCA administration improved liver histology (measured as NAFLD Activity Score (NAS) score), steatosis, inflammation, and fibrosis. OCA also reduced body weight and serum ALT and γ-glutamyltransferase levels. In line with previous studies, the drug increased alkaline

phosphatase and LDL levels and reduced HDL concentration. On the contrary, the FXR agonist increased fasting insulin and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and 23% of patients had intense/severe pruritus. A phase II randomized trial in Japan (FLINT-J) showed that high OCA doses (40 mg/d) significantly resolved NASH in patients with mild fibrosis.[169] Trials suggested that high doses of OCA increased the frequency and severity of pruritus. Furthermore, in 2017, the use of OCA (5 mg/d, quantity was lower compared with the dose tested in the FLINT study) was associated with major side effects including liver transplantation and deaths in cirrhotic patients with advanced liver disease (F4 fibrosis), causing a warning by the Food and Drug Administration and European Medicines Agency (EMA) (FDA adds Boxes Warning to highlight correct dosing of Ocaliva February 1, 2018; https://www.fda.gov/Drugs/Drugsafety/ucm594941.htm). To evaluate the side effects and safety of OCA clinical trials are ongoing. In a phase II, double-blind, randomized study, OCA and statin therapy were administered to NASH patients with fibrosis stages 1–4 (clinical trial: NCT02633956).

A phase III, randomized, double-blind, placebo-controlled trial (Randomized Global Phase 3 Study to Evaluate the Impact on NASH With Fibrosis of Obeticholic Acid Treatment [REGENERATE] study; clinical trial: NCT02548351) evaluated OCA safety and efficacy in 2400 patients with NASH with liver fibrosis at stages 2 or 3. Participants received placebo or OCA 10 mg/d or 25 mg/d for 18 months. The REGENERATE trial analyzed the improvement of liver fibrosis and the resolution of NASH.

A phase III trial (Randomized Phase 3 Study Evaluating the Efficacy and Safety of Obeticholic Acid (OCA) in Subjects with Compensated Cirrhosis due to NASH (REVERSE) study; clinical trial: NCT03439254) investigated the OCA effects in 540 compensated cirrhotic NASH patients, evaluating fibrosis improvement using the NASH Clinical Research Network scoring system. Conclusive data from the REVERSE and REGENERATE studies are expected in 2020 and 2022, respectively.

Several nonsteroidal FXR agonists (tropifexor, nidufexor, and turofexorate) have been tested in phase I trials and currently are in phase II. Very recently, a phase II trial on GS-9674 (cilofexor), an FXR agonist close to GW4064, showed that the administration of cilofexor for 24 weeks was well tolerated and ameliorated hepatic steatosis, liver biochemistry, and serum bile acids in patients with NASH (clinical trial: NCT02854605). Severe pruritus was the common side effect, especially in patients receiving high doses of the drug (100 mg).[170] Unlike OCA administration, no changes in lipid profile were observed after cilofexor treatment, highlighting the differences in the molecular structure and properties of these compounds.

Given the role of BA homeostasis in NASH development, clinical trials also have been conducted on the FGF19 analogue.[171] In particular, NGM282 is currently in clinical trials to evaluate the safety, tolerability, and efficacy in NASH patients. NGM282 reduced body weight, body mass index, insulin levels, and HOMA-IR, as well as lipid content
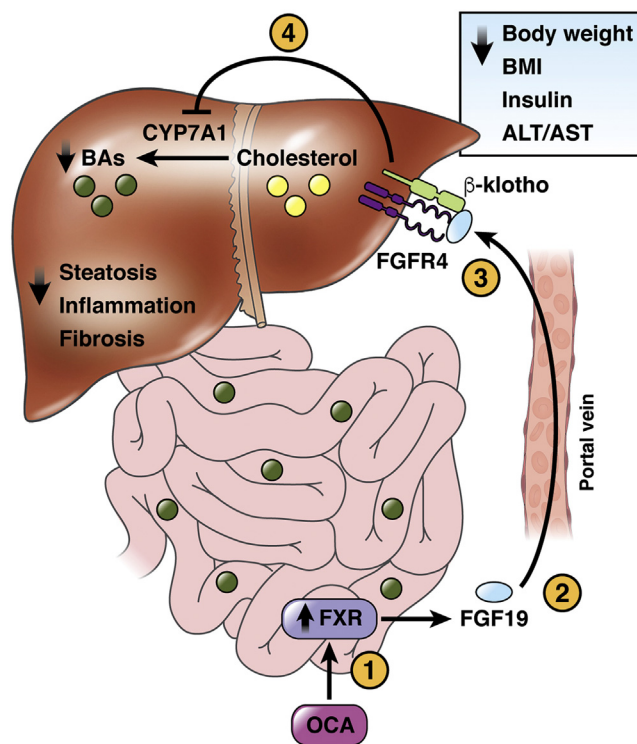
Figure 2. Role of FXR and FGF19 in NASH. OCA-dependent FXR activation induces secretion in the portal circulation of FGF19, which reaches the liver through the portal circulation and binds the receptor FGFR4 with the co-receptor β klotho, repressing CYP7A1 expression and thus reducing BA synthesis. The effects of FXR activation improve liver steatosis, inflammation, and fibrosis. In addition, FGF19 is able to repress CYP7A1 expression, ameliorating body weight, BMI, insulin concentration, and serum ALT/AST levels. BMI, body mass index; FGFR4, fibroblast growth factor receptor 4.

in the liver, serum ALT and AST levels, and fibrosis biomarkers. A total of 84% and 42% of patients showed an improvement in their NAS score and fibrosis stage, respectively. Common side effects were diarrhea, abdominal pain, and nausea.[172] Taken together, these data highlight the importance of the FXR pathway as a promising target for NASH therapy (Figure 2).

LXRs in Hepatic Metabolism

LXRs are the cholesterol sensors and play a central role in the regulation of fatty acids, cholesterol, and glucose metabolism, as well as in the control of inflammation.[173] LXRs exist as 2 isotypes: *LXRα* is expressed mainly in the liver, adipose tissue, kidney, and macrophages, whereas *LXRβ* is expressed ubiquitously.[174–176] LXRs are activated by oxysterols (cholesterol derivatives) such as 24(S),25-epoxycholesterol, 25-hydroxycholesterol, and 22(R)-hydroxycholesterol,[174–176] leading to the excretion of cholesterol as bile acids. At the same time, LXR activation reduces cholesterol synthesis and its uptake, improving cholesterol removal from the body and lipoprotein profile.[174] LXRs regulate reverse cholesterol transport, in which the excess cholesterol reaches the liver and it is eliminated

via feces. In rodents, LXRs induce *CYP7A1* gene expression, the rate-limiting enzyme that converts cholesterol into bile acids. LXR activation also induces gene expression of the adenosine triphosphate (ATP)-binding cassette transporters *ABCA1* and *ABCG1*, which promote cholesterol efflux from macrophages to HDL and apolipoproteins, leading to cholesterol transport to the liver.[177,178]

LXRs play a central role in fatty acid liver metabolism, inducing the expression of SREBP1c, a master regulator of triglycerides and fatty acid synthesis.

SREBP1c induces several enzymes used in fatty acid biosynthesis such as ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthase, SCD1, and glycerol-3-phosphate acyltransferase, a key enzyme in triglyceride and phospholipid synthesis.[179] Overall, LXR-mediated induction of SREBP1c promotes lipid synthesis and regulates fatty acids and sterol homeostasis.

Furthermore, LXRs are involved in carbohydrate regulatory element-binding protein activation, a transcription factor implicated in the glycolysis and de novo lipogenesis in the liver.[180–182] Carbohydrate regulatory element-binding protein up-regulates liver-pyruvate kinase gene expression, the rate-limiting enzyme of glycolysis. This transcription factor also is involved in the conversion of excess dietary carbohydrates into triglycerides. It works in synergy with LXR and SREBP1c, inducing the expression of genes such as *fatty acid synthase* (*FAS*), *acetyl-CoA carboxylase*, and *SCD1*.

LXRs exert anti-inflammatory functions via direct and indirect mechanisms as shown by the suppression of proinflammatory genes such as *cyclooxygenase-2* and inducible *NOS* after LXR agonist treatment.[183–185] LXR activation also inhibits Toll-like receptor (TLR) ligand-dependent inflammatory pathway through ABCA1 induction.[186,187] In macrophages of atherosclerotic mice, cholesterol induces desmosterol production, an LXR ligand, modulating inflammation and lipid metabolism in a LXR-dependent fashion.[188] Furthermore, in dendritic cells, LXR modulates cell migration via CCL19 and CCL21, which induce the expression of CD38, involved in leukocyte trafficking.[189] Treatment with LXR agonists induces regulatory T cells (Treg) differentiation and inhibits T-helper (Th)1 and Th17 polarization.[190]

LXR and NASH

The role of LXR in the prevention and development of NASH is debated. NASH is characterized by hepatic inflammation resulting from adipose tissue and intestine dysfunction. As described previously, LXR activation is able to modulate inflammation.[191] It has been shown that Small Ubiquitin-like MOdifier (SUMO)ylated forms of LXR down-regulate gene expression of inflammatory genes such as *interleukin 1β* and *NOS*, and inhibits nuclear factor-κB activity.[183,185] In a NAFLD mouse model, LXR activation inhibited the phosphoinositide-3-kinase cascade, reducing TNF-α gene expression and liver injury.[192] These data also were confirmed in a rat model of LPS-induced liver damage, in which treatment with the LXR agonist GW3965 reduced

TNF-α and prostraglandin E2 gene expression.[193] In APO-E2 knock-in mice, treatment with LXR agonist reduced cholesterol levels and inflammation but increased liver triglyceride levels.[194] In bone marrow–derived macrophages, LXR activation inhibited TLR2, TLR4, and TLR9, as well as mitogen-activated protein kinase signaling,[186] reducing the recruitment of these cells. In LXRα/β−/− mice, LPS administration induced proinflammatory cytokine expression and bone marrow–derived macrophage recruitment,[195] showing the role of LXR in the regulation of the inflammatory response in acute liver injury.

On the other hand, hepatic LXR expression is correlated with the severity of NAFLD.[196–198] In patients with NAFLD, LXR expression is up-regulated in liver and monocytes, whereas it is down-regulated in the ileum.[198] In a mouse model of NASH, high levels of 24(S)-hydroxycholesterol and 7β- hydroxycholesterol have been observed.[199] LXR agonists activate hepatic de novo lipogenesis and promote steatosis, inducing the expression of SREBP1c, FAS, and SCD1.[181,200] Notably, in Kupffer cells without SREBP1c expression, 27-hydroxycholesterol reduces HFD-induced steatosis, inhibiting leukocyte recruitment and proinflammatory gene expression.[201] In high-cholesterol diet–fed mice, LXRα deletion promoted cholesterol accumulation and increased serum ALT and AST levels as well as macrophage recruitment and Kupffer cell activation supporting inflammation,[202,203] highlighting the protective role of LXRα in NASH. Furthermore, LXRα/β-/- mice show hepatic fibrosis, as shown by hepatic lipid droplet accumulation and by the induction of profibrotic genes such as *Acta2* and *Col1a1*.[204]

LXRs play a key role in the maintenance of cholesterol homeostasis and they represent a promising therapeutic target in the management of atherosclerosis and cholesterol-related disorders. Several selective LXR agonists such as desmosterol, GW6340, and the LXRβ agonist LXR-623 are well tolerated but less used in the treatment of NAFLD.[205–207] Further studies are needed to evaluate the safety and efficacy of LXR agonists in NASH treatment.

Conclusions

The physiological role of NRs in the gut–liver–adipose axis was explained by Evans and Mangelsdorf[26] in the energy vector of nutrient homeostasis concept. In the fed state, PPARs, FXR, and LXR are involved in nutrient absorption from the gut and distribution from the gut/liver to peripheral tissues (white adipose tissue and muscles). BAs activate intestinal FXR, abetting nutrient acquisition and gut microbiota homeostasis. Absorbed dietary lipids are exported from the liver to peripheral tissue and excess cholesterol is removed from the body via reverse cholesterol transport controlled by the enterokine FGF19/15 (FXR target gene) and/or the activation of LXR by oxysterols. FGF19 stimulates transintestinal cholesterol excretion[208] and reduces postprandial hyperglycemia, promoting hepatic glycogenesis. In the periphery, nutrients are consumed by muscle or stored in WAT thanks to PPARβ/δ and γ. In the fasting state, the retained energy in adipose tissue is metabolized. Through lipolysis, triacylglycerols stored in WAT are

converted into fatty acids and released in the circulation to be used as an energy source by the organs. In the liver, fatty acids activate PPARα, promoting fatty acid catabolism and the production of ATP, ketone bodies, and FGF21. Ketone bodies are used as an energy source in the brain and FGF21 represents a stress signal to prepare other organs for energy deprivation.

Considering that the gut–liver–adipose axis dysfunction and abnormal energy homeostasis are the principal causes of NAFLD/NASH, the dysfunction of energy vectors could be considered as a mechanism by which NRs contributes to NAFLD/NASH development.

Several drugs that act on key pathogenic mechanisms are under development for the treatment of NASH. Agonists of PPARs and FXR have been studied extensively in mouse models, and phase II and III clinical trials currently are ongoing to test the safety and efficacy of these NR-based drugs for treating NASH.

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Correspondence
Address correspondence to: Antonio Moschetta, MD, PhD, Clinica Medica "Cesare Frugoni," Department of Interdisciplinary Medicine, University of Bari "Aldo Moro," Piazza Giulio Cesare 11, 70124 Bari, Italy. e-mail: antonio.moschetta@uniba.it; fax: (39) 0805555388.