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Nonalcoholic Fatty Liver Disease: Pathogenesis and Potential for Nuclear Receptors as Therapeutic Targets

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is a consequence of insulin resistance encompassing a spectrum that extends from simple hepatic steatosis through to nonalcoholic steatohepatitis (NASH), a condition that may progress to cirrhosis with its associated complications. A subset of nuclear receptors act as intracellular sensors for cholesterol metabolites, free fatty acids, and a range of other lipophilic molecules with pivotal roles in energy homeostasis and inflammation. These receptors represent attractive drug targets for the management of NAFLD and NASH as well as related conditions such as type 2 diabetes and the broader metabolic syndrome. To date, human studies have concentrated on peroxisome proliferator-activated receptor (PPAR) agonists, particularly those directed at PPARγ. However, these drugs have significant limitations, so alternate approaches to nuclear receptor targeting are being explored.

Keywords: Nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; liver disease; liver cirrhosis; hepatic fibrosis; nuclear receptors; fibrates; thiazolidinediones; adiponectin; fatty acids; bile acids

NAFLD and NASH

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in affluent societies, representing the hepatic metabolic consequence of relative overnutrition and altered diet composition in the setting of reduced physical activity and sedentary behaviors. While infrequently recognized prior to 1980,[1] it is now not only an important cause of liver damage in its own right but also a component of, and contributor to, virtually all forms of liver disease that occur in individuals with an elevated fat mass. Thus, NAFLD and hepatic steatosis represent important cofactors in accelerating disease progression in disorders as diverse as alcoholic liver disease, chronic hepatitis C, and hemochromatosis.[2–5]

The clinical entity of NAFLD represents a spectrum of liver diseases including nonalcoholic fatty liver (NAFL or simple steatosis) and the sometimes progressive, inflammatory form termed nonalcoholic steatohepatitis (NASH). When progressive, NASH leads to the development of hepatic fibrosis and cirrhosis that may present as subacute liver failure.[6] End-stage NASH cirrhosis may require liver trans-

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plantation and occasionally is an etiologic factor for the development of hepatocellular carcinoma.[7–9] In addition, it is now recognized that the majority of cases of so-called "cryptogenic" cirrhosis likely represent "burnt out" NASH, since this entity shares many of the pathophysiological associations of the classical disease.[10,11]

The distinction between NAFL and NASH can only be made on liver biopsy. Since this procedure is associated with a small but definite risk of morbidity and mortality, biopsy is neither feasible, nor practical, for large natural history cohort studies. Hence, the most reliable estimate of prevalence is for NAFLD, which can be diagnosed by excluding other liver disorders, identifying its key metabolic associations and linking these to an imaging modality such as a hepatic ultrasound or magnetic resonance spectroscopy to demonstrate fatty infiltration. Such studies reveal that the prevalence of NAFLD may be as high as 30%. Estimates for NASH are lower and the figures less reliable, principally due to sample size considerations, but vary between 5.7% and 17%.[13–15] Natural history studies clearly document significant liver-related morbidity and mortality attributable to NASH, almost exclusively in those that have progressed to advanced fibrosis or cirrhosis.[16–20] The mortality rate is probably similar to that of persons with chronic hepatitis C-associated cirrhosis,[20,21] and the majority of patients with NASH-related cirrhosis succumb to liver-related causes.[21] In persons with NAFLD without cirrhosis, morbidity from cardiovascular disease and type 2 diabetes is high due to shared pathogenic mechanisms.

Pathogenesis of NAFLD and NASH

Over the past decade, significant insights have been gained into the etiology and pathogenesis of NAFLD based on both human data and observations in animal models.[22] Unusually, the former has often led the way in directing experimental research, particularly with regard to our understanding of the pathophysiological hallmarks of NAFLD and in the appreciation of the role of adipokines.[23–26] In broad terms, the development of NAFLD can be considered as involving both extra- and intrahepatic events. The former comprises the metabolic milieu consequent to the development of an

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elevated fat mass and reduced physical activity, which sets the stage for the deposition of lipid in the liver. In turn, it should be noted that many of the signals that lead to hepatic fat deposition are also pro-inflammatory and pro-fibrotic in their own right.

Perhaps the strongest clinical association of NAFLD is with the rising epidemic of obesity and type 2 diabetes (diabesity). In turn, the pathophysiological correlates appear to be that of insulin resistance, which is almost universal in NAFLD,[23–25] and the metabolic syndrome, a disorder closely related to the presence of visceral obesity. Increased fat mass and more particularly altered body fat topography, manifested by visceral obesity, are present in virtually all persons with NAFLD. Visceral adipose tissue is less susceptible to the antilipolytic effects of insulin and in the presence of insulin resistance leads to increased delivery of free fatty acids *via* the portal circulation to the liver for esterification to triglycerides, contributing to increased hepatic storage of fat. However, only a minority of free fatty acids reaching the liver arises from the visceral/splanchnic compartment, with the majority coming from the peripheral compartment[27,28] as a result of the development of insulin resistance and failure to suppress lipolysis in adipose tissues. It is noteworthy that while insulin resistance is characterized by resistance to the gluco-regulatory actions of insulin, the lipogenic effects of insulin in tissues such as the liver is preserved.[29] Recent careful and detailed metabolic studies suggest that in persons with NAFLD, only one-third of liver fat arises from *de novo* lipogenesis and dietary fatty acids.[30] Triglyceride efflux from the liver in the form of very low-density lipoproteins is also reduced, contributing to development of steatosis in NAFLD.[31]

Expansion of the adipocyte mass and infiltration of the liver with macrophages[32] results in the elaboration of a variety of cytokines and chemokines with effects on energy metabolism, lipid homeostasis, inflammation, and wound healing. Thus, lipid overload modifies the pattern of adipokines/cytokines elaborated by adipose tissue, including increased production of leptin, tumor necrosis factor-α (TNF), angiotensinogen and free fatty acids, whereas adiponectin which is secreted by peripheral, and more particularly, visceral adipose tissue[33] is paradoxically reduced.[34–36] The basis for this seemingly contradictory association is unknown. Of the adipokines, TNF is a well-described pro-inflammatory protein whose expression is increased in the livers of patients with NASH,[37] suggesting local autocrine and paracrine effects on the liver. Serum TNF levels however do not appear to be elevated,[26] indicating that release of TNF by adipose tissue in NAFLD contributes to the peripheral insulin resistance, without systemic effects on the liver. Serum leptin levels are increased in some,[38] but not all reports of patients with NASH.[39] In the liver of patients with NASH, elevated leptin levels may serve an adaptive, anti-steatotic role, protecting the organ from the effects of "lipotoxicity".[40] However, the presence of hepatic steatosis, despite the presence of hyper-leptinemia, suggests the development of leptin resistance, at least at the level of the hepatocyte.[38] In animal models, including a model of steatohepatitis, leptin has been shown to play an important pro-fibrogenic role,[41–43] although correlations between serum

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leptin and hepatic fibrosis in human NASH are lacking.[38,39] The latter may relate to the long natural history of hepatic fibrosis in NASH in which single-point sampling of serum levels is likely to be inadequate.

Adiponectin possesses potent anti-inflammatory properties by modulating the endothelial cell inflammatory response through inhibition of nuclear factor-κB (NF-κB) activation and blockade of TNF release.[44,45] Adiponectin also suppresses macrophage function and the proliferation and migration of vascular smooth muscle cells[46,47] and induces anti-inflammatory cytokines in leukocytes and modulates lymphopoesis.[48] In addition to these potent anti-inflammatory activities, adiponectin plays an important role in lipid physiology being antilipogenic and protecting nonadipose tissues such as the liver from lipid accumulation.[49] This effect is mediated by a direct effect of the hormone to increase β-oxidation of free fatty acids and decreasing *de novo* free fatty acid production within hepatocytes.[50] Adiponectin plays a more direct role in impairing the development of hepatic fibrosis. Thus, knockout mice develop extensive liver fibrosis after chronic liver injury, and conversely, the administration of adiponectin before and after carbon tetrachloride treatment prevents hepatic fibrosis in wild-type mice.[51] These effects suggest a hepatoprotective role for this adipokine in NAFLD. Adiponectin levels are reduced in persons with visceral obesity and type 2 diabetes.[52,53] In persons with NAFLD, hepatic steatosis and the presence of hepatic inflammation correlates with the presence of hypo-adiponectinemia, though probably for reasons similar to that discussed for leptin, no correlation was observed with the extent of hepatic fibrosis.[26] The roles of other circulating adipokines such as resistin and angiotensinogen released by adipose tissues in contributing to the genesis of NAFL and NASH have not been explored.

The intrahepatic consequence of lipid deposition and more especially the transition from hepatic steatosis to steatohepatitis and liver fibrosis has been most extensively studied in animal models. Among the many pathophysiological processes that have been noted to be operative in these models of steatohepatitis and fibrosis and for which human data are either supportive or for which data has not been gathered include oxidative stress, pro-inflammatory cytokine and chemokine release, hepatocellular apoptosis, bacterial overgrowth, microvascular damage, and microsomal, peroxisomal, and mitochondrial injury.[13,22,54] As previously discussed, while up to a third of the adult population in affluent nations have evidence of NAFL, only a minority progress to have the inflammatory lesion of NASH. Thus, the development of NASH must also reflect host genetics and disease susceptibility, particularly the balance between pro-inflammatory and cytoprotective pathways.

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The best studied model for the transition from hepatic steatosis to steatohepatitis is that consequent to the feeding of rodents a diet deficient in methionine and choline and high in sucrose (40%) and fat (10%) (the MCD diet).[55,56] Rats and mice fed the MCD diet lose body and liver weight, develop severe steatosis, and subsequently develop necro-inflammation, elevations in serum transaminases, and hepatic fibrosis.[55–57] The increased oxidation of intrahepatic lipid by microsomal, peroxisomal, and mitochondrial pathways results in the generation of lipid peroxidation products that are damaging to the cell and are intensely pro-inflammatory. In particular, lipid oxidases such as Cyp2e1 (also increased in human NASH), Cyp4a10, and Cyp4a14 have been shown to generate reactive oxygen species in the MCD model. Lipid mediators also incite apoptosis, a frequent finding in humans with NASH.[58,59] A variety of cytokines, chemokines, and adhesion molecules are up regulated in MCD-fed mice including IL-6, TNF, TGF-β, ICAM1, VCAM1, and MCP1. In humans, TNF expression is increased in the livers of patients with NASH,[37] though circulating levels may not be elevated.[26] This and animal data suggest that TNF may not be an obligate requirement for the development of NASH, with oxidative stress mediating inflammatory recruitment directly through activation of transcription factors such as NFκB.[60] For a detailed review of other animal models of NASH, including genetic models, the reader is referred to other detailed reviews.[61,62]

Nuclear Receptors in the Pathogenesis of NAFLD and NASH

Members of the nuclear receptor superfamily function as intracellular ligand-activated transcription factors and have critical roles in a diverse range of cellular processes.[63] A subset of nuclear receptors that heterodimerize with the retinoid X receptor α (RXRα) are low-affinity receptors for important metabolic intermediates and are key regulators of metabolic and adaptive/defensive processes, particularly in the liver. Ligands for these heterodimers include cholesterol metabolites such as oxysterols and bile acids, free fatty acids, ecosinoids, and a structurally diverse range of xenobiotic compounds. To date, their significance in the development of hepatic steatosis, inflammation, and fibrosis in NAFLD has not been extensively characterized, except for the peroxisome proliferator-activated receptors (PPARs).

PPARs. The PPAR family encompasses PPARα, PPARγ, and PPARδ. PPARα is most prominently expressed in liver, kidney, heart, and skeletal muscle[64] and can be activated by eicosanoids, free fatty acids, and drugs of the fibrate class.[65] Activation results in increased uptake and oxidation of free fatty acids, increased triglyceride hydrolysis and up-regulation of ApoA-I and II. The net effect is increased fatty acid oxidation, decreased serum triglycerides, a rise in high-density lipoprotein, and an increase in cholesterol efflux.[66] PPARα activation also has anti-inflammatory effects via inhibition of cyclooxygenase 2, IL-6 and C-reactive protein,[67] effects that are at least in part mediated by reduced expression of p50-NF-κB as well as an increase in IκB-α leading to prevention of p50-NF-κB nuclear translocation.[68]

Acyl CoA oxidase (AOX) is the rate-limiting enzyme in peroxisomal fatty acid β-oxidation for the preferential metabolism of very long chain fatty acids. Consistent with these activities, *Aox*-null mice have defective peroxisomal β-oxidation and exhibit steatohepatitis, increased PPARα, Cyp4a10, and Cyp4a14 expression (both PPARα-regulated genes) and raised H_2O_2 levels by 4–5 months of age.[69] As expected, when *Aox*-null mice are interbred with *Ppar*α-null

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mice, the double knockouts exhibit less steatosis with reduced inflammation, cellular injury, and adenoma development. This effect is possibly a consequence of the inhibition of PPARα-induced CYP4A activity, thereby decreasing the production of toxic dicarboxylic acids.[70] *Ppar*α-null mice fed the MCD diet display greater levels of steatohepatitis compared to wild-type mice on the same diet.[71,72] An intervention study with the PPARα agonist Wy-14643 supports this premise by reducing or reversing MCD diet-induced steatohepatitis and fibrosis in these mice.[73] Most importantly, the data highlight the critical role of fatty acid disposal pathways in the pathogenesis of hepatic steatosis and steatohepatitis.

PPARγ is most highly expressed in adipose tissue but is also found in vascular endothelium, pancreatic β cells, hepatocytes, hepatic stellate cells, and macrophages. PPARγ activation by fatty acids, prostaglandins, or drugs of the thiazolidinedione class results in terminal differentiation and proliferation of subcutaneous adipocytes. This results in increased fatty acid uptake by adipocytes, thereby preventing lipotoxicity in nonadipose tissues such as the liver and pancreatic beta cells.[74,75] Other beneficial effects include increases in plasma adiponectin levels[76,77] and adiponectin receptor expression in the liver.[78] The overall effect of PPARγ activation is thus an increase in insulin sensitivity and glycemic control, coupled with a reduction in circulating free fatty acids. PPARγ activation has also been shown to preserve hepatic stellate cells in a quiescent phenotype and thus prevent or reverse hepatic fibrosis.[79] This is not entirely surprising as the quiescent stellate cell has a lipid-rich cytoplasm and thus shares some phenotypic features of adipocytes.[80] For these reasons, PPARγ agonists are an attractive therapeutic option as they reverse many of the pathophysiological abnormalities present in NASH as well as have direct antifibrotic effects on the liver.

PPARδ is a powerful metabolic regulator with actions on fat, skeletal muscle, liver, and heart. Its activation by fatty acids enhances fatty acid transport and oxidation, improves glucose homeostasis via inhibition of hepatic glucose output, reduces macrophage inflammatory responses, and dramatically increases circulating high density lipoprotein levels.[81] Thus, selective PPARδ agonists have the potential to target multiple components of the metabolic syndrome including obesity, dyslipidemia, hyperglycemia, insulin resistance, and possibly NASH.[82]

In a recent study, bezafibrate a pan-PPAR agonist was evaluated in the MCD model of steatohepatitis.[83] Administration of the compound to mice for 5 weeks resulted in reductions in ALT, hepatic steatosis, products of lipid peroxidation, necro-inflammation, and the number of activated stellate cells. At the molecular level, bezafibrate increased the expression of enzymes involved in fatty acid β-oxidation and reduced the production of pro-inflammatory

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and pro-fibrotic cytokines and chemokines including TGFβ_1, IL-6, IL1β, MCP1, and TNF and reduced NF-κB activation. Finally, bezafibrate increased the levels of the anti-inflammatory and hepatoprotective protein adiponectin. In another report, administration of the PPARα agonist fenofibrate or the PPARγ agonist rosiglitazone in the Otsuka−Long−Evans−Tokushima fat rat for 28 weeks resulted in improvements in steatosis, serum insulin, TNF levels, and increased expression of select genes involved in fatty acid β-oxidation.[84] Similar protective effects were demonstrated for the PPARδ agonist GW501516 in the MCD model of steatohepatitis.[83]

PXR and CAR. Other nuclear receptors have only recently begun to be examined for their role in the pathogenesis or treatment of NASH. Studies in mice and cell-based systems show that the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) regulate an overlapping set of genes that encode proteins that are involved in oxidative metabolism, conjugation, and the transport of small hydrophobic substrates.[85] These genes include those that encode several cytochrome P450s, glutathione S-transferases, UDP-glucuronosyltransferases, sulfotransferases, multidrug resistance proteins, multidrug resistance-associated proteins, and organic anion-transporting polypeptides. A similar cohort of genes has been identified in the human context using primary cultures of human hepatocytes.[86] PXR and CAR appear to be important receptors in engaging defensive gene programs within the liver, in particular, adaptation to oxidative stress and in the metabolism and transport of a range of toxic molecules, especially bile acids.[87,88] PXR preferentially directs hydroxylation of bile acids,[89] while CAR induces sulfation.[90] It seems likely that bile acids are the major endogenous ligands for PXR,[89] while the endogenous ligand for CAR has not yet been identified, though it has been suggested that both bilirubin and bile acids may indirectly activate CAR and promote their own metabolism and/or elimination.[90,91]

Recent studies reveal that both PXR and CAR also influence lipid metabolism. Treatment of fasted mice with the potent selective PXR agonist pregnenolone 16α-carbonitrile reduced hepatic fatty acid β-oxidation and ketogenesis by inhibiting FoxA2 regulation of carnitine palmitoyltransferase 1A and 3-hydroxy-3-methylglutarate-CoA synthase 2 while promoting triglyceride synthesis through up-regulation of stearoyl-CoA desaturase. The net effect was increased hepatic steatosis.[92] Similarly, CAR activation by phenobarbital treatment decreased expression of genes involved fatty acid β-oxidation and gluconeogenesis in wild type but not *Car*-null mice,[93] an effect that is in part mediated by CAR-mediated interference with the actions of hepatocyte nuclear factor-4.[94] CAR activation by 1,4-bis-(2-(3, 5-dichloropyridyloxy)) benzene in mice receiving the MCD diet for 8 weeks worsened steatosis, liver inflammation, and fibrosis, consistent with suppression of fatty acid disposal through β-oxidation. Importantly, *Car*-null mice were partially protected from the hepatic effects of the MCD diet.[95] The effects of CAR inverse-agonists, such as androstanol and androstenol,[96] in animal models of NAFLD and NASH have

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not yet been explored but could yield useful information regarding the utility of CAR as a drug target for these conditions.

In our laboratory, studies in mice fed the MCD diet reveal an increase in serum levels of primary, secondary, and tertiary bile acids as early as 3 days, suggesting that these cholesterol metabolites may influence the early phase of liver injury and contribute to the pathogenesis of NASH. The pattern of hepatic gene expression in these mice suggests predominant activation of PXR and CAR rather than the farnesoid X receptor (FXR, see below) with the net effect being reduced hepatic bile acid uptake and increased bile acid biotransformation to less toxic metabolites and possible PXR- and CAR-mediated adverse effects on lipid homeostasis (London, R.; George, J.; Liddle, C. Unpublished data). However, a human study of bile acid-directed therapy for NASH using ursodeoxycholic acid failed to find any evidence of histological improvement of liver disease after 2 years,[97] though it is unclear what impact the change in bile acid pool composition induced by ursodeoxycholic acid would have on nuclear receptor signaling.

FXR. FXR is a receptor for bile acids, particularly chenodeoxycholic acid.[98] Bile acid activated FXR has several gene targets relevant to bile acid homeostasis. It negatively regulates bile acid uptake into hepatocytes by reducing Na^+-taurocholate cotransporting polypeptide (Ntcp) expression and is a negative regulator of both CYP7A1, an important rate-limiting step in bile acid synthesis and CYP8B1, which determines the ratio of chenodeoxycholic acid to cholic acid synthesized by the liver.[99] FXR has also been recognized as an important regulator of lipid metabolism[100] and, therefore, may be relevant to liver diseases that are characterized by accumulation of fat within hepatocytes, such as NASH. Through an interaction with peroxisome proliferative activated receptor, γ, coactivator 1α (PGC-1α) FXR induces expression of genes that increase gluconeogenesis and promote triglyceride clearance and fatty acid β-oxidation concomitantly with a reduction of lipogenic gene transcription.[101] Moreover, small heterodimer partner (SHP), a nuclear receptor with corepressor activity that is strongly induced by FXR, interferes with sterol regulatory element binding protein-1c (SREBP-1c) expression by inhibiting the activity of the liver X receptor, further reducing hepatic lipogenesis.[102] Not surprisingly, therefore, *Fxr*-null mice exhibit massive hepatic steatosis when challenged with a high cholesterol/high fat diet.[103] Somewhat paradoxically, SHP-deletion in leptin deficient ob/ob mice improves insulin sensitivity and reduces hepatic steatosis through increased production of very low-density lipoprotein and therefore export of triglyceride.[104] Thus, the effects of FXR on NAFLD are complex and to date the role of FXR as a therapeutic target for NASH has not been explored. However, this receptor is pharmacologically tractable with non-bile acid selective agonists such as GW4064[105] and fexaramine[106] having been developed.

In addition to impacting bile acid and lipid homeostasis, FXR appears to have significant actions on the pathological process of hepatic fibrogenesis. Addition of the semisynthetic bile acid FXR agonist 6-ethyl chenodeoxycholic acid (6-ECDCA) to hepatic stellate cells in culture reduces the expression of the pro-fibrotic genes collagen α(1)I, α-smooth muscle actin, tissue inhibitors of metalloproteinase 1 and 2 and TGF-β_1, by a SHP-dependent mechanism. Moreover, 6-EDCA treatment reduces hepatic fibrosis in several rat models of liver injury.[107,108] The impact of FXR agonists on NASH-associated hepatic fibrosis remains to be explored.

LXRs. The LXRs (LXRα and LXRβ) are receptors for oxysterols, particularly 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol, and 24(*S*),25-epoxycholesterol.[109,110]

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LXRα is expressed predominantly in the liver, kidney, gastrointestinal tract, and adipose tissue, while LXRβ exhibits a more homogeneous distribution being found in most tissues.[111] LXRs generally exert actions opposite to that of FXR, promoting cholesterol catabolism by conversion to bile acids, enhancing reverse cholesterol transport, and decreasing sterol absorption from the intestine. Acting through SREBP-1c, LXRs promote fatty acid synthesis and increase circulating triglycerides.[100] In contrast to actions on fat metabolism, activation of LXRs in diabetic Zucker rats lowers blood glucose through decreased gluconeogenesis through repression of phosphoenolpyruvate carboxykinase.[112] Treatment of cultured human primary hepatocytes with the LXR agonist GW3965 results in decreased production very low-density lipoproteins, increased storage of lipid, and reduced insulin sensitivity.[113] Thus, LXR activation causes changes in insulin sensitivity and lipid metabolism that are likely detrimental in NAFLD and NASH. However, LXR antagonism, a property of some oxysterols,[114,115] provides a therapeutic option worthy of further investigation if suitable small molecule LXR antagonists can be successfully developed.

RXRs. The three mammalian RXRs (RXRα, RXRβ, and RXRγ) act as heterodimerization partners for a several nuclear receptors including PPARs, PXR, CAR, FXR, and LXRs and have central roles in a diverse range of cellular processes extending from fetal development and cell survival to homeostasis. RXR heterodimers are classified as either permissive (e.g., PPARs, LXR, and FXR) or nonpermissive (e.g., PXR and CAR) depending on whether the heterodimer can be activated by RXR ligands or not.[116] RXRα is expressed in the liver, kidney, intestine, and adipose tissue and is the predominant partner for nuclear receptors involved in lipid and bile acid metabolism. Collectively, RXR-selective ligands are referred to as rexinoids and include the putative endogenous ligand 9-*cis* retinoic acid. Administration of the rexinoids LGD1069 to ob/ob mice or LG268 to db/db mice results in reduced blood glucose and increased insulin sensitivity, this pattern being consistent with predominant activation of PPARγ/RXRα.[117,118] However, rexinoids also cause elevated serum triglycerides, likely reflecting actions on other RXR-heterodimeric partners, including LXR/RXR. Because of their pleomorphic actions there has been a focus on the development of rexinoids that have relative heterodimeric selectivity and display a favorable pharmacological profile for the treatment of type 2 diabetes and associated conditions (reviewed in ref 119). While potentially useful, the utility of this approach remains to be determined.

ERRα. Estrogen-related receptor α (ERRα) is an orphan nuclear receptor that is expressed predominantly is tissues that preferentially metabolize fatty acids. Unlike the nuclear receptors covered earlier in this review, ERRα binds to DNA as a homodimer or as a heterodimer with other ERRs.[120] It can act as a conduit for PGC-1α target gene activation and is transcriptionally activated by its own expression in a positive feedback loop. In this setting, ERRα appears to be a significant regulator of mitochondrial biogenesis and aids recruitment of PGC-1α at target genes.[121] PGC-1α is a major mediator of adaptive mitochondrial oxidation, is relatively deficient in type 2 diabetes, and has a role in protection from reactive oxygen species. Several nuclear receptors are target

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genes for PGC-1α including PPARγ.[122] *ERR*α-null mice exhibit reduced body weight due to reduced peripheral fat deposits and are highly resistant to obesity induced by a high fat diet.[123] The role of ERRα in animal models of NAFLD and NASH has not been investigated. While there is no known endogenous ligand for this receptor and the ligand-binding pocket is normally obstructed by sidechains, a recent study suggests that pharmacological manipulation may be possible.[124]

Human Studies of Nuclear Receptor Ligands in NASH

The thiazolidinediones (troglitazone, rosiglitazone, and pioglitazone) are highly effective insulin-sensitizing agents;[125,126] however, their utility in NAFLD is limited clinically by weight gain, a direct consequence of the increase in adipose tissue mass caused by these agents.[127,128] In an open label trial of 30 patients with NASH, rosiglitazone 4 mg twice daily for 48 weeks resulted in significant improvements in liver biochemistry and indices of insulin resistance.[129] In almost half of the patients where a repeat biopsy was available there were such marked reductions in steatosis and necro-inflammatory scores that they no longer met histological criteria for a diagnosis of NASH. Improvements in pericellular fibrosis but not in fibrosis stage were also noted. In another controlled trial, pioglitazone and vitamin E were compared to vitamin E alone in 20 patients with NASH.[130] After 6 months follow-up, all patients had significant improvements in liver biochemistry, but posttreatment histology in the vitamin E group only demonstrated a minor reduction in steatosis. The pioglitazone group had reduced inflammation, pericellular fibrosis, and a significant further reduction in steatosis. No weight gain was seen in either group.

In a more recent controlled trial, 55 patients with NASH were randomized to a hypocaloric diet plus pioglitazone 45 mg per day or diet plus placebo for 6 months.[131] When compared to placebo, the pioglitazone group showed improvements in insulin resistance, HbA1c levels, and liver enzymes. Significant decreases in circulating TNF and elevations in adiponectin levels were also noted. On liver histology, reductions in steatosis and necro-inflammatory scores were evident in the group receiving the PPARγ agonist. However, there was no significant change in fibrosis stage with pioglitazone, possibly due to the short duration of follow-up. Weight gain was the principle adverse effect with a mean increase of over 2 kg. Larger trials of longer duration are currently underway to assess the long-term benefits and safety of these agents.

Trials of PPARα agonists such as the fibrates in human NASH have been disappointing, despite their theoretical attractiveness and their effectiveness in animal models. PPARα expression in human liver is relatively low compared with rodents. Moreover, PPARα almost exclusively controls AOX expression in rodents, which is not the case in humans; therefore, PPARα activation in humans has little impact on fatty acid β-oxidation.[132] In a pilot study, 12 months treatment with clofibrate in 16 patients with NASH and elevated triglycerides had no impact on liver enzyme elevation or triglyceride levels. Likewise, histological improvement was not observed.[133] A 4-week study of gemfibrozil showed improvement in transaminases irrespective of

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initial triglyceride level.[134] Larger trials of PPARα agonists have not eventuated, as other drug targets have appeared more promising.

Dual PPARα/γ agonists are attractive as therapy for NAFLD, NASH, and the metabolic syndrome as they have the potential to improve insulin resistance, reduce circulating free fatty acids and avoid the weight gain associated with pure PPARγ agonists. A number of such dual PPARα/γ agonists have been developed including muraglitazar, tesaglitazar, naveglitazar, and netoglitazone.[135] As expected, in early trials, these agents have reduced circulating triglycerides, increased high-density lipoprotein levels, and improved insulin sensitivity.[136–138] Amelioration in the PPARγ-mediated weight gain via a PPARα-associated decrease in food intake and lipid oxidation has been demonstrated in animals.[136,139] These results are yet to be replicated in humans. Safety concerns have led to the recent withdrawal of muraglitazar and tesaglitazar from phase III trails due to an increased incidence of heart failure and elevations in serum creatinine, respectively.[140]

Summary

Given their pivotal roles in lipid metabolism, energy homeostasis, and inflammation, nuclear receptors are attractive therapeutic targets for the management of NASH and the insulin resistance that precipitates NAFLD. While PPARα and PPARγ agonists have translated through to small human studies, to date most nuclear receptors targets have only been investigated in animal- or cell-based models. Of most interest is compounds that act as agonists for two or all three of the PPARs, though FXR is deserving of more intensive investigation. The challenge is to define both the optimal target(s) and the correct balance in receptor selectivity and binding affinity to ensure efficacy while minimizing untoward side effects.

Abbreviations Used

6-ECDCA, 6-ethyl chenodeoxycholic acid; AOX, acyl CoA oxidase; CAR, constitutive androstane receptor; FXR, farnesoid X receptor; ERRα, estrogen-related receptor α; IL-6, interleukin-6; LXR, liver X receptor; MCD, methionine and choline deficient; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor-κB; PGC-1α, peroxisome proliferative activated receptor, gamma, coactivator 1α; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RXR, retinoid X receptor; SHP, small heterodimer partner; SREBP-1c, sterol regulatory element binding protein-1c; TNF, tumor necrosis factor-α.

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