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REVIEW

The Role of Nuclear Receptors in the Pathophysiology, Natural Course, and Drug Treatment of NAFLD in Humans

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) describes steatosis, nonalcoholic steatohepatitis with or without fibrosis, and hepatocellular carcinoma, namely the entire alcohol-like spectrum of liver disease though observed in the nonalcoholic, dysmetabolic, individual free of competing causes of liver disease. NAFLD, which is a major public health issue, exhibits intrahepatic triglyceride storage giving rise to lipotoxicity. Nuclear receptors (NRs) are transcriptional factors which, activated by ligands, are master regulators of metabolism and also have intricate connections with circadian control accounting for cyclical patterns in the metabolic fate of nutrients. Several transcription factors, such as peroxisome proliferator-activated receptors, liver X receptors, farnesoid X receptors, and their molecular cascades, finely regulate energetic fluxes and metabolic pathways. Dysregulation of such pathways is heavily implicated in those metabolic derangements characterizing insulin resistance and metabolic syndrome and in the histogenesis of progressive NAFLD forms. We review the role of selected NRs in NAFLD pathogenesis. Secondly, we analyze the role of NRs in the natural history of human NAFLD. Next, we discuss the results observed in humans following administration of drug agonists or antagonists of the NRs pathogenically involved in NAFLD. Finally, general principles of treatment and lines of research in human NAFLD are briefly examined.

Keywords: Cirrhosis; Farnesoid X receptor; Fibrosis; Hepatocellular carcinoma; Liver X

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) designates a heterogenous set of diseases histologically mimicking alcoholic liver disease though observed in insulin-resistant nonalcoholic individuals, in the absence of competing etiologies of liver disease [1, 2]. The NAFLD spectrum and its natural history span simple steatosis through nonalcoholic steatohepatitis (NASH), fibrosis-cirrhosis, and, in a subset of cases, hepatocellular carcinoma (HCC), with or without cirrhosis [3, 4]. On the basis of the epidemic prevalence of disease and its inherent hepatic, metabolic, oncologic, and cardiovascular disease burden, NAFLD is a major public health issue posing heavy costs on health systems [1, 5–7].

From a pathophysiological point of view, intrahepatic storage of triglycerides (TGs) is an example of an adaptive process becoming maladaptive, given that ectopic fat gives rise to lipotoxicity [8, 9]. Fatty changes will primarily result from excess de novo intrahepatic lipogenesis associated with the liver being overwhelmed by an excess of steatogenic substrates in the setting of insulin resistance (IR), impaired glucose disposal/type 2 diabetes (T2D), hyperlipidemia, visceral obesity, and other features of the metabolic syndrome (MetS) [10]. Steatosis will also derive from the failure of the liver to oxidize and export excess lipids [3, 11–13]. Therefore, NAFLD can best be conceptualized as an abnormal storage of TGs resulting from an imbalance between intrahepatic synthesis and catabolism/disposal of fatty substrates [14], which is inextricably linked to IR/T2D and atherogenic dyslipidemia [13, 15].

Nuclear receptors (NRs) are transcriptional factors which, activated by ligands, are master regulators of metabolism and also have intricate connections with circadian control accounting for cyclical patterns in the metabolic fate of nutrients [16]. Several transcription factors, such as peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and farnesoid X receptors (FXRs), finely regulate energetic fluxes and metabolic pathways via the molecular cascades they trigger [17]. Dysregulation of such pathways is heavily implicated in those metabolic derangements typically belonging to the domain of IR and MetS and in the histogenesis of progressive NAFLD forms and their clinical complications in both adults and children [3, 16, 18, 19].

On these grounds, dietary and pharmacological manipulation of NRs has become a major aim in the research concerning NAFLD treatment. The present article critically reviews the role of NRs in the pathogenesis of NAFLD and explores how this information may potentially be exploited in the drug treatment of this condition. All of these pieces of information may be put into perspective on the basis of the analysis of the natural history of NAFLD (Fig. 1).

This article is based on previously conducted studies and does not involve any new studies of human or animal subjects performed by any of the authors.

PATHOPHYSIOLOGY OF PPARs

The PPARs are members of the NR superfamily including PPAR-α, PPAR-β/δ, and PPAR-γ, which play a key role in regulating cellular growth and

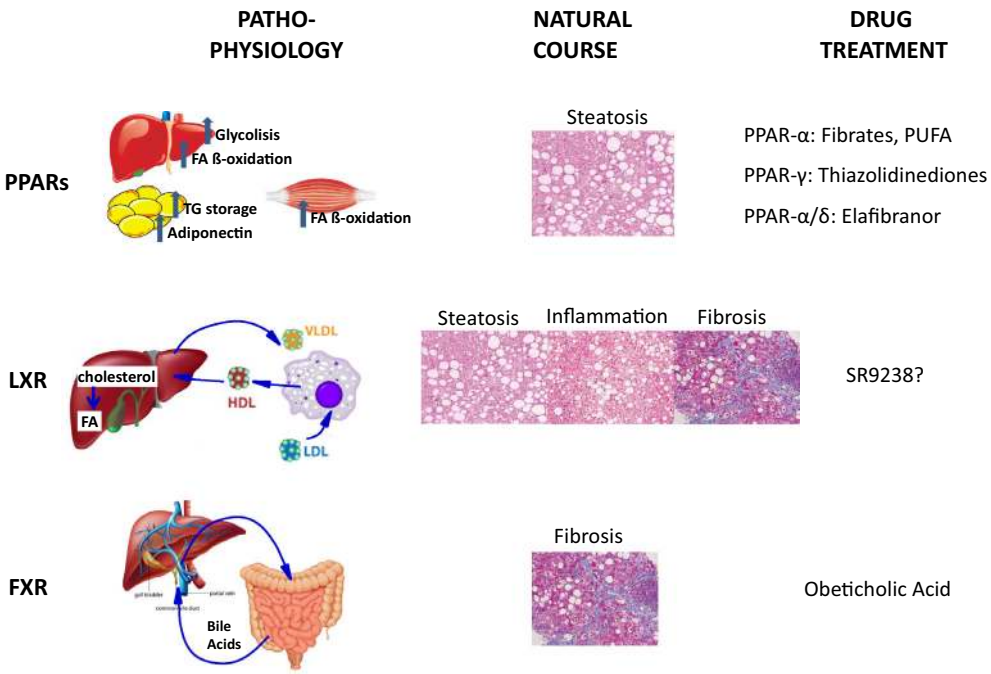

Fig. 1 Overview of the role of nuclear receptors in the pathophysiology, natural course, and treatment of NAFLD based on data discussed in the present review article. This cartoon aims to summarize the specific roles played by PPARs, FXR, and LXR in the development and progression of NAFLD. *Pathophysiology* PPAR-α promotes β-oxidation of FA in the hepatocytes and exerts lipid-lowering activity; PPAR-γ, abundantly expressed in the adipose tissue, promotes adipocyte differentiation and storage of triglycerides and has an insulin-sensitizing activity by protecting non-adipose tissues against excessive fat deposition and by increasing adiponectin secretion; PPAR-β/δ stimulates glycolysis and inhibits gluconeogenesis in the liver, promotes β-oxidation of FA in the muscle, and exerts an anti-inflammatory role; LXR controls cholesterol lipoprotein metabolism and modulates immune, inflammatory, and fibrogenic responses; FXR regulates bile acid homeostasis and also lipoprotein-glucose metabolism. *Natural course* The schematic figure recapitulates the chief role of nuclear receptors in the natural history of NAFLD. *Drug treatment* The potential role of drugs interacting with each of the individual classes of nuclear receptors is also illustrated. *FA* fatty acids, *FXR* farnesoid X receptor, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *LXR* liver X receptor, *NAFLD* nonalcoholic fatty liver disease, *PPAR* peroxisome proliferator-activated receptor, *PUFA* polyunsaturated fatty acids, *TG* triglycerides, *VLDL* very-low-density lipoprotein

differentiation, metabolism, and inflammation [20–22]. PPAR-α (NR1C1) is highly expressed in liver, kidney, and muscle, while PPAR-γ (NR1C3) is mainly present in adipose tissue and PPAR-β/δ appears to be universally expressed. These receptors are classically ligand activated, and the best characterized natural ligands are fatty acids (FA) and their derivatives. Activated PPARs form a heterodimer with retinoid X receptor (RXR) and interact with PPAR response elements in the target genes, regulating their expression. In the liver specifically, PPARs modulate a whole spectrum of physiological processes including cholesterol and bile acid (BA) homeostasis, glucolipidic metabolism, inflammatory response, regenerative mechanisms, and cell differentiation and cycle [20–22].

Role of PPAR-α in Metabolism

In hepatocytes, PPAR-α acts as a nutritional sensor, modulating the rates of FA catabolism, lipogenesis, and ketone body synthesis in response to feeding and starvation [22]. PPAR-α controls the expression of genes regulating peroxisomal/mitochondrial β-oxidation and transport of FA in humans and mouse models. Conversely PPAR-α regulates the glycolysis-gluconeogenesis pathway in mice but not in humans [22]. PPAR-α activation reduces plasma TG-rich lipoprotein by enhanced FA uptake, conversion into acyl-CoA derivatives, and catabolism in the β-oxidation pathways in mouse models [22, 23]. Another mechanism accounting for the PPAR-α lipid-lowering activity in humans and rodents is increased lipolysis via induction of lipoprotein lipase (LPL), which catalyses the hydrolysis of lipoprotein TGs into free FA and monoacylglycerol [22].

Role of PPAR-α in NAFLD

The effect of PPAR-α system activation in improving the NAFLD spectrum has been studied in several animal models, which recapitulate human disease to a partial extent [20, 22].

Two experimental diet-induced NAFLD mouse models have been used with different steatogenic mechanisms. High-fat diet (HFD) induces fatty liver by upregulation of genes involved in de novo lipogenesis [e.g., PPAR-γ and sterol regulatory element-binding protein-1 (SREBP1)] opposed by PPAR-α upregulation which is, however, not sufficient to efficiently catabolize the extra load of FA [24–26]. Methionine and choline deficient (MCD) diet causes fatty liver by downregulation of genes for FA esterification and very-low-density lipoprotein (VLDL) secretion without significantly affecting PPARs expression [26, 27].

With regard to NASH, the HFD model reproduces mild NASH together with typical MetS traits whereas the MCD diet efficiently induces evolution of NASH to fibrosis, histologically similar to human disease, but without the dysmetabolic phenotype [26, 28].

In the MCD diet NASH mouse model, PPAR-α-deficient mice showed histologically more severe NASH [29], and treatment with a potent PPAR-α agonist (Wy-14,643) reversed fibrosis and NASH in wild-type mice [30]. Mice lacking PPAR-α expression (PPAR-α-null) fed an HFD developed increased oxidative stress, histological steatosis, hepatic inflammation, and higher NAFLD Activity Score (NAS) compared to age-matched wild-type mice fed a standard/HFD or PPAR-α-null mice fed a standard diet [31].

A recent study on a PPAR-α mutant mouse model lacking its DNA-binding-dependent activity on FA metabolism showed that PPAR-α agonism (by Wy-14,643) inhibited hepatic inflammatory responses and the transition from steatosis toward NASH and fibrosis via a direct anti-inflammatory effect [32]. Another study, conducted on a NASH model in HFD-fed *foz/foz* obese/diabetic mice, showed that PPAR-α agonist Wy-14,643 improved metabolic indices, steatosis, and ballooning with substantially reduction of NAS and resolution of NASH according to Kleiner's criteria [33], along with suppression of liver nuclear factor (NF)-kB and c-Jun N-terminal kinase activation, reduced tumor necrosis factor (TNF)-α, and monocyte chemoattractant protein (MCP)1 expression and decreased macrophage-neutrophil infiltration in the liver [34]. However, hepatic histological inflammatory score did not

improve and adipose tissue inflammation (increased inflammatory cellular infiltrate and MCP1 expression) and hypoadiponectinemia persisted, although adipose tissue TNF-α expression was reduced. These findings suggest that residual hepatic inflammatory changes result from unsuppressed adipose tissue inflammation via inflammatory chemokines, thus potentially limiting the therapeutic efficacy of PPAR-α agonists in NASH [34].

Moreover, a protective effect of PPAR-α expression or activation by fibrates on liver steatosis and inflammation has been shown in the apolipoprotein-E2 knock-in (APO-E2KI) mouse model mimicking human type III hyperlipoproteinemia [35, 36].

In humans, a recent paired liver biopsy study on 85 consecutive patients showed that liver PPAR-α gene expression was negatively correlated with IR, visceral adiposity, severity of steatosis, presence of NASH, ballooning, NAS, and fibrosis, and positively correlated with adiponectin. At 1 year, histological improvement was associated with an increased expression of PPAR-α and its target genes. Liver PPAR-β/δ and PPAR-γ expression did not correlate with any histological feature nor with gluco-lipidic metabolism [37]. However, as further discussed below (see "Fibrates and Polyunstaurated FA" and "Lipid-Lowering Agents"), PPARα agonists such as fibrates are effective in reducing steatosis in rodents [38], but have failed to provide convincing results in humans [16, 39].

Diet affects development and progression of NAFLD via PPARs system. Dietary monounsaturated FA (MUFA), polyunsaturated FA (PUFA), and proteins can activate PPARs which stimulate lipid oxidation and reduce inflammation and IR, leading to improvement of hepatic steatosis [40]. Moreover, these nutrients can inhibit the expression of transcription factor SREBP-1 regulating the expression of genes involved in hepatic de novo lipogenesis, and thereby reduce liver fat [40]. For example, obese individuals are prone to the risk of developing steatosis owing to an increased SREBP-1c/PPAR-α ratio associated with n-3 long chain PUFA depletion and IR, which favors lipogenesis over FA oxidation [41]. Consistently, in mice, the supplementation of n-3 long chain PUFA abolishes HFD-induced enhancement in hepatic SREBP-1c/PPAR-α ratios, promoting increased FA oxidation and steatosis attenuation [42], decreases oxidative stress, IR [43], and has an additive effect, with ursodeoxycholic acid (UDCA), in alleviating histological features, in HFD-induced NASH [44].

Finally, conflicting evidence links genetic variants of PPAR-α with susceptibility to human NAFLD [45, 46]. The results of MUFA and/or PUFA supplementation in NAFLD is discussed below (see "Fibrates and Polyunstaurated FA").

Role of PPAR-γ in Metabolism

PPAR-γ is abundantly expressed in the adipose tissue where it enhances the activation of genes promoting adipocyte differentiation and storage of TG [14, 16, 20, 21]. Moreover, PPAR-γ is a key regulator of glucose homeostasis through its insulin-sensitizing activity by protecting non-adipose tissues against excessive fat deposition and by balancing the secretion of adipocytokines [47].

Role of PPAR-γ in NAFLD

PPAR-γ is generally increased in fatty liver associated with obesity both in mouse models, except for steatosis induced by MCD diet as previously detailed [26, 27], and in humans [20,

21]. In accordance with this statement, studies in mice fed an HFD have shown that hepatocyte-specific PPAR-γ-knockout protected from hepatic steatosis and PPAR-γ-knockdown by RNA interfering-adenoviral vector injection improved fatty liver [48, 49]. Although these studies would suggest a deleterious effect of PPAR-γ on NAFLD, in mouse liver the net effect of thiazolidinedione PPAR-γ agonists results in improved hepatic steatosis and protection from NASH and hepatic fibrosis via increased insulin sensitivity in adipose tissue and skeletal muscle, overcoming the direct steatogenic effects in hepatocytes [21, 50]. PPAR-γ agonism also upregulates the secretion of adiponectin and the expression of its receptors in the liver and adipose tissue, thus improving insulin sensitivity and upregulating hepatic FA oxidation [51]. Interestingly, increased PPAR-γ expression improves fibrosis in vitro and in mice by inhibiting the activation of hepatic stellate cells [52, 53].

In humans, the C161T, Pro12Ala PPAR-γ single nucleotide polymorphism (SNP) has been specifically associated with the development and histological progression of NAFLD, although a recent meta-analysis found this association to be valid for East Asian populations, but not for European populations [54].

Role of PPAR-β/δ in Metabolism

PPAR-β/δ regulate hepatic glucose utilization and lipoprotein metabolism and exert an anti-inflammatory role [55, 56]. Data on PPAR-β/δ physiopathology are mainly derived from experimental studies in animal models.

A study has shown that the activation of hepatic PPAR-β/δ stimulates glucose utilization and inhibits gluconeogenesis in HFD-fed mice [57]. PPAR-β/δ knockout mice show glucose intolerance and hypertriglyceridemia [58, 59]. Conversely, PPAR-β/δ agonists have a plasma TG-lowering effect [60].

Role of PPAR-β/δ in NAFLD

The effect of PPAR-β/δ on liver lipid metabolism is still controversial. Liver PPAR-β/δ upregulation through adenoviral infection has been associated with either improved (in obese *db*/*db* mice) [61] or increased hepatic steatosis (in HFD-fed mice liver) [57]. However, despite increased steatosis, HFD-fed mice showed less liver damage because PPAR-β/δ activation increased the production of protective MUFA and, conversely, reduced serum concentrations of lipotoxic saturated FA [57].

Moreover, one study showed that PPAR-β/δ-deficient mice treated with an hepatotoxic agent developed more liver necrosis, inflammation, and enhanced expression of profibrotic genes compared to control mice, suggesting that PPAR-β/δ protect the liver from inflammation and fibrosis [62].

Several studies conducted in mouse models with specific agonists of PPAR-β/δ (such as GW501516, GW0742, and L-165041) reported a beneficial effect of this NR activation on fatty liver by modulation of FA metabolism (increased β-oxidation and reduced FA synthesis) and reduced IR and inflammatory activity [63–65]. Histological features of NASH also improved following treatment with PPAR-β/δ agonists [66, 67].

Two clinical trials on overweight patients have shown that PPAR-β/δ agonists (GW501516 or MBX-8025) improved metabolic features (IR, plasmatic TGs, nonesterified FA [NEFA], apolipoprotein B-100, LDL-cholesterol), liver enzymes, and liver fat content [68, 69]. However, it should be noted that the one study assessing the effect of PPAR-β/δ agonist

GW501516 on steatosis was a very small pilot study [68].

The recently discovered dual PPAR-α/δ agonist GFT505 has shown protective effects on liver steatosis, inflammation, and fibrosis, mediated by both PPAR-α-dependent and -independent mechanisms, in murine models of NAFLD/NASH and liver fibrosis [70]. GFT505 reduced hepatic and peripheral IR in obese humans [71]. GFT505 will be further discussed in "Dual PPAR-α/δ Agonists" below.

PATHOPHYSIOLOGY OF FXR AND ITS ROLE IN NAFLD

Once deemed to be simple detergents, physicochemically facilitating the digestive processes, BA are instead master regulators of metabolic pathways [72]. FXR, a member of the nuclear hormone receptor superfamily, together with TGR5, a G protein-coupled BA receptor (GPBAR1), acts as a BA sensor regulating their intrahepatocyte levels and mediates the signaling effects exerted by BA on gluco-lipidic metabolism [73, 74]. FXR is mainly expressed in the liver and gut. BA are the natural ligands of FXR, and chenodeoxycholic acid (CDC) shows the highest affinity [74]. Similar to other NRs, activated FXR forms a heterodimer with RXR that binds to the promoter region of the target genes, small heterodimer partner (SHP) and fibroblast growth factor (FGF)-19, increasing their expression and promoting pathways finally leading to reduced expression of genes involved in BA synthesis (mainly *CYP7A1*) [74, 75]. In particular, FGF-19 is secreted from ileum into the portal circulation and acts as an enterohepatic signal to downregulate CYP7A1 via FGF-4 activation [76]. Moreover, FXR critically regulates lipid and glucose metabolism by multiple mechanisms [77]. Interestingly, a study conducted in 2166 German subjects reported that SNPs in the FXR-encoding gene *NR1H4* were strong determinants of fasting glucose and free FA serum levels independent of unhealthy body fat accumulation [78]. In the liver, BA downregulate steatogenesis via the FXR-SHP pathway [79]. A study conducted in 40 biopsy-proven NAFLD cases found that FXR and SHP and BA transporters [sodium-taurocholate cotransporting polypeptide (NTCP) and bile salt export pump (BSEP)] were significantly upregulated in NASH compared to simple steatosis suggesting that FXR may play a major role in NAFLD progression [80].

Normal BA synthesis is essential in maintaining normal liver histology: mice with reduced BA synthesis develop overt steatosis, which is reversed either by BA feeding or administration with obeticholic acid (OCA), an FXR agonist, demonstrating that the hepatoprotection exerted by BA is indeed FXR-dependent [81]. Consistently, the age-dependent decline in FXR activity is a major factor in the development of fatty liver observed in aging mice [82].

Analysis of animal models of alcoholic liver disease has shown that FXR-deficient mice are more exposed to developing steatohepatitis and fibrosis following ethanol feeding [83]. Conversely, the FXR agonist 6ECDCA reverses steatosis and decreases the oxidative stress induced, in rodents, by feeding ethanol with protein-deficient diet [84].

Similarly, once challenged with steatogenic diets, hepatic FXR-deficient mice, or mice in which FXR is acetylated, develop a liver phenotype fully recapitulating human NASH and cholestasis [85–87]. However, treatments capable of disrupting the intestinal FXR/ceramide axis signaling have led to a reduced grade of steatosis [88].

Either direct pharmacological FXR agonism or drug interventions/surgery eventually leading to FXR upregulation have consistently been reported to improve NAFLD histology. For example, deficiency in the electroneutral Na(+)/H(+) exchanger NHE1 (Slc9a1) leads to FXR upregulation, reduced cellular stress, and preserved insulin signaling even upon HFD feeding [89]. In agreement with these observations, treatment with hepatic FXR agonists has resulted in beneficial effects in NAFLD animal models. Administration of the dual FXR/TGR5 agonist (INT-767) for 6 weeks resulted in significantly improved histological features of NASH associated with an increase in the proportion of intrahepatic monocytes with the anti-inflammatory phenotype [73]. Similarly, WAY-362450, a potent synthetic and orally active FXR agonist, attenuated fatty liver by acting through multiple steatogenic mechanisms [90]. Finally, OCA, a semisynthetic BA-selective ligand for FXR, has been shown to improve liver steatosis, inflammation, and fibrosis in preclinical models and in NAFLD patients (see "Drugs Interacting with FXR" below) [91, 92]. As far as surgical experimental models are concerned, vertical sleeve gastrectomy (VSG) produces weight loss independent of SHP status in mice; SHP molecular ablation induces a pro-inflammatory milieu, which is exacerbated after VSG despite weight loss [79].

FXR systemic expression has led to novel therapeutic strategies targeting cholesterol and TG metabolism, fatty liver, and cholestasis [74, 93, 94]. In contrast to such a systemic therapy, however, postprandial BA release will selectively activate intestinal FXR. By mimicking this tissue-selective effect, the gut-restricted FXR agonist fexaramine significantly induces enteric FGF-15. Fexaramine reduces obesity, systemic low-grade inflammation, and hepatic IR resulting from high-calorie diet by enhancing thermogenesis and browning of white adipose tissue, without activating hepatic FXR [95].

PATHOPHYSIOLOGY OF LXR AND NAFLD

Cholesterol and FA play a key role not only in lipid and energy metabolism but are also involved in multiple and complex biological phenomena such as the gut–liver axis [96], the liver–brown adipose tissue interaction [97], homeostasis of cell membranes, endoplasmic reticulum stress, inflammation [98, 99], atherogenesis [99, 100], T2D, obesity [101, 102], and cancer [103].

LXRs, which comprise LXR-α and LXR-β, belong to the nuclear hormone receptor superfamily of ligand-activated transcription factors which, in the hepatic tissue, serve as lipid sensors and participate in regulating the expression of master genes which modulate the metabolism of cholesterol and FA [104].

Orchestrated collaboration between LXR and SREBP-1 is a main step in the molecular cascade of events characterizing steatogenesis. Steatosis is commonly found either in the setting of NAFLD [105] or associated with hepatitis C virus (HCV) infection [106]. Both these two pathogenically interconnected diseases span steatosis to inflammatory and fibrotic changes in humans [107, 108]. Consistently, inverse agonism of LXR-α and LXR-β obtained through administration of a powerful synthetic compound (SR9238) has been reported to suppress hepatic lipogenesis, inflammation, and steatosis in an experimental NAFLD model in mice [109] and UDCA inhibits LXR-α-mediated hepatic lipogenesis [110].

On these grounds, by investigating the connections of LXR-α with other intrahepatic lipid transporters and histological inflammatory and fibrotic changes in 40 NAFLD patients, a study found a positive correlation with the intrahepatic expression of ABCG5/8, CD36, and SREBP-1c; that the expression of NPC1L1 was negatively correlated with intrahepatic inflammation and LXR-α intensity and, notably, that LXR-α expression was directly correlated with the degree of steatosis, as well as with inflammatory and fibrotic changes [111]. These data identify LXR as a potential target for NAFLD treatment.

NATURAL HISTORY OF NAFLD AT A GLANCE AND ROLE OF NRS

Although the basic pathological steps of NAFLD [112] and the natural history of disease [6] are closely interconnected and largely overlap one another, for the sake of clarity, they can be dissected into four distinct phases: steatosis, NASH, fibrosis-cirrhosis, and HCC.

Steatosis

Fatty changes affecting more than 5% of the hepatocytes, usually associated with minor 'sterile' inflammatory changes [113], make up 70–75% of all NAFLD cases and are a common finding in the general population in most countries worldwide [4]. Risk factors for the development of nonalcoholic steatosis include age, gender, ethnicity, western-type lifestyle habits (sedentary and hypercaloric, high-fat, high simple sugar, low in fiber diet) which, against a background of genetic predisposition, lead to the development of either individual features or the full-blown MetS [3, 5]. Gain or reduction of as little as 2.7 kg affects the risk of developing steatosis or its reversal [114]. A study conducted with proton magnetic resonance spectroscopy in 922 subjects who participated in a population screening for NAFLD suggested that metabolic factors increase the risk of developing steatosis more than genetic factors [115].

Does simple steatosis evolve into NASH? The consistent findings that NASH individuals have an increased mortality compared to those with steatosis had originally led to the notion of two different conditions with a low potential, if any, for steatosis to progress to NASH [11, 116]. Challenging such a view, however, several recent reports have now clearly proven the progression from steatosis to NASH in individual cases [117–119]. A recent report by Singh et al. [120] reconciles these seemingly opposite views. These authors, by conducting a meta-analysis of 11 studies including 150 patients with simple steatosis and 261 with NASH, all of whom had biopsy-proven disease, were able to show that the rate of progression of one stage of fibrosis takes place in over 14.3 years for individuals with simple steatosis [95% confidence interval (CI) 9.1–50.0] versus 7.1 years for those with NASH (95% CI 4.8–14.3) [120]. In other words, it would probably take as long as 57.2 years for steatosis versus 24.4 years for NASH to progress from early, non-fibrosis disease to cirrhosis, which confirms that, although they form a disease spectrum, these two conditions follow a fairly distinct course.

Although, sometimes, simple steatosis is alluded to as 'nonalcoholic fatty liver' it is, nevertheless, a disease which is worth treating not only to untrigger its potential progression to NASH but also to reduce the risk of developing T2D [121]. Further to weight loss [122, 123], various drugs appear able to promote the reversal of steatosis, notably including

statins [124], pentoxifylline, vitamin E, thiazolidinediones, and OCA [125].

Role of PPARs in Hepatic Steatogenesis

PPARs play a major role in the steatogenesis and, probably, the progression of NAFLD. Steatogenic drugs such as amiodarone, valproic acid, and tetracycline affect PPAR signaling in a mouse precision-cut liver slices model [126]. Reduced expression of PPAR-α, a master regulator of FA oxidation, and activation of the Jun amino-terminal kinase (JNK) signaling pathway promote hepatic steatosis and hypertriglyceridemia in mice [127] and steatogenesis in patients with type II citrullinemia [128]. Consistently, PPAR-α activation improves hepatic IR and steatosis in high-fructose-diet-fed mice [129].

PPAR-γ is upregulated in the liver of obese patients [130, 131]. Conversely, dietary short-chain FA supplementation prevented and reversed HFD-induced metabolic abnormalities in mice by decreasing PPAR-γ expression and activity therefore switching metabolic pathways from the synthesis of lipids to their utilization [132]. Interestingly, genetic PPAR-γ variants are associated with the development and histological progression of human NAFLD [133].

Nonalcoholic Steatohepatitis

NASH, histologically defined by concurrent steatosis with inflammatory changes associated with ballooning hepatocyte degeneration, affects approximately 25–30% of those with NAFLD [4].

The ballooned hepatocyte has lost its normal shape as a result of a combination of cytoskeletal injury, storage of oxidized fat microvesicles, and dilated endoplasmic reticulum. Ballooning is probably the best morphological evidence of what has been alluded to as 'multi-organelle failure', which mirrors unbalanced oxidative stress in a lipid-rich milieu and all these events eventually conducing to activation of immunologic pathways [11, 134].

Age, gender, and genetic and metabolic factors are independently associated with NASH [135–141]. At variance with vitamin E and OCA, thiazolidinediones do not reverse hepatocyte balloning suggesting that IR plays a key role in the early phases of disease only [142].

Role of LXR in Hepatic Inflammation

Once activated by elevated intracellular cholesterol levels, LXRs induce the expression of genes controlling the absorption, efflux, transport, and excretion of cholesterol. Moreover, LXRs modulate immune, inflammatory, and fibrogenic responses and are thus identified as integrators of metabolic and inflammatory signaling and an ideal target for treatment strategies [143–146].

Interestingly, LXR expression correlates with the degree of hepatic fat deposition, as well as with hepatic inflammation and fibrosis in human NAFLD [111].

Fibrosis and Cirrhosis

Fibrosis (not NASH) is the strongest predictor of hepatic mortality in NAFLD [147]. However, NASH, together with age, gender, and genetic and endocrine-hormonal variables, is among the independent predictors of fibrosis [148–158].

Cirrhosis (usually associated with steatohepatitis and hence defined as NASH-cirrhosis) features advanced fibrosis

coupled with deeply distorted (nodular) liver histological architecture [159].

Recent studies have found that features of the MetS (body mass index [BMI], T2D, and steatosis) together with features of cardiovascular risk (carotid plaques and intima-media thickness) are the independent correlates of advanced fibrosis/cirrhosis [160–162]. In particular, the Rotterdam study conducted with transient elastography in 3041 participants found that the combined presence of T2D and steatosis was strongly associated with liver stiffness (8.0 kPa), suggestive of clinically relevant fibrosis [161].

Role of FXR in Hepatic Fibrogenesis

CDC is the natural agonist of FXR, the nuclear hormone receptor which regulates gluco-lipidic metabolism, senses BA, and inhibits BA synthesis by inducing SHP gene expression [163]. Moreover, FXR plays a major role in experimental hepatic fibrosis and in fibrosing kidney disorders observed in diabetic humans [164, 165]. Interestingly, the expression of FXR is reduced in human and mice fibrotic livers [166]. Experimental evidence supports that an FXR-SHP regulatory cascade promotes the reversal of hepatic fibrosis, suggesting that FXR ligands might be effective antifibrotic agents [163]. Consistently, a phase 2 trial has shown that the administration of OCA, a semisynthetic derivative of CDC, reduced surrogate markers of liver inflammation and fibrosis in patients with NAFLD and T2D [167]. The role of OCA is further discussed in "Drugs Interacting with FXR" below.

Hepatocellular Carcinoma

HCC is the end stage of NAFLD as well as of other chronic liver disorders due to varying etiology. At variance with other etiologies, however, NAFLD-HCC is associated with a less striking prevalence in the male gender and may occur in the absence of cirrhosis [168–170]. Given that these NAFLD individuals escape surveillance programs, NAFLD-HCC often undergoes a diagnostic delay which curtails the chances for radical treatment and accounts for a worse prognosis compared to other etiologies of disease, such as hepatitis B virus (HBV)-HCC and HCV-HCC [171, 172].

Several risk modifiers, such as genetics (*PNPLA3* polymorphisms), age, features of the MetS (BMI and T2D), dietary habits (coffee, fish and vegetables protecting from, and alcohol predisposing to developing HCC) and drugs (statins and certain oral glucose-lowering agents seem to exert a protective effect) are strongly associated with the development of NAFLD-HCC [155, 173–179]. Moreover, concurrent T2D worsens the prognosis of HCC by reducing both disease-free survival and overall survival [180].

Role of NRs in HCC

There is paucity of data on the role, if any, of NRs, specifically as regards the development of NAFLD-HCC. Nevertheless, data extrapolated from various etiologies support a role for vitamin D receptor (VDR), PPAR-γ, and FXR pathways [181–185].

NAFLD TREATMENT BASED ON NR-TARGETED DRUGS

On the basis of the pivotal role of NRs in hepatic metabolic pathways and on the promising results observed in animal models of NAFLD, drugs which interfere with some of these NRs are among the strongest candidates for treating human NAFLD [14, 16]. Nevertheless, findings

from experimental studies have not been systematically replicated in humans and several clinical trials utilizing pharmacological manipulation of NRs have yielded conflicting results (see Table S1 in the supplementary material).

Fibrates and Polyunstaurated FA

Treatment with drugs interacting with PPAR-α, such as fibrates and PUFA, has failed to improve NAFLD histology in humans. In particular, despite certain favorable metabolic effects, fibrate monotherapy improves liver histology only to a minimal extent [186–190]. Similarly, PUFA supplementation may ameliorate some metabolic parameters and probably reduces liver fat content, but has no effect on liver inflammation and fibrosis [191–196]. Paradoxically, a recent study suggested that PUFA treatment may even worsen IR and liver histology in diabetic patients with NASH [197]. Accordingly, despite improving dyslipidemia [198, 199], this class of drugs has a quite limited scope in treating NAFLD per se [125].

Thiazolidinediones

Although extensively evaluated in human NAFLD, the thiazolidinedione PPAR-γ agonists have failed to deliver fully convincing results so far. Overall, trials on thiazolidinediones showed an improvement in hepatic and systemic IR (despite a significant weight gain), and a reduction of hepatic steatosis and necro-inflammation [200–210]. In a phase II, double-blind, placebo-controlled, 24-month study, pioglitazone significantly improved aminotransferase levels, steatosis, ballooning, and inflammation in NASH patients with impaired glucose tolerance or T2D [202]. The seminal PIVENS study, a large multicenter, 96-week clinical trial, randomized 247 non-diabetic patients with biopsy-proven NASH to pioglitazone (30 mg/day), vitamin E (800 IU/day), or placebo for 24 months [207]. The primary endpoint was an improvement in the composite of NAS ≥2 points with at least a 1-point improvement in hepatocellular ballooning and a 1-point improvement in either the lobular inflammation or steatosis score, and no worsening of fibrosis. This primary endpoint was achieved in 19% of subjects in the placebo group compared to 34% in the pioglitazone group ($P = 0.04$, not significant). Although pioglitazone did not meet the prespecified significance level for the primary outcome (prespecified α error of 0.025), it was associated with significantly higher reductions in the individual histological features of steatosis, inflammation, and hepatocellular ballooning, as well as with the resolution of steatohepatitis in a significant proportion of subjects and with improvements in IR and liver enzymes [207]. A recent network meta-analysis by Singh et al. [125] confirmed that thiazolidinediones may improve steatosis, hepatocellular ballooning, and probably lobular inflammation in NASH patients. Nevertheless, it should be pointed out that in NASH, thiazolidinediones do not reverse or may even worsen mitochondrial abnormalities [142], and that their benefit on liver fibrosis remains unproven [125].

Major concerns about treatment with thiazolidinediones in NAFLD are long-term durability of their effects and safety. Indeed, on the one hand, treatment discontinuation may lead to subsequent worsening of liver histology with recurrence of steatosis and inflammation [211], and, on the other hand, treatment extension over time has not been associated with additional improvement of NASH histological features [206].

Thiazolidinediones promote pre-adipocyte differentiation into small, insulin-sensitive adipocytes and induce weight gain attributable to an increase in adipose tissue mass through a redistribution of fat from ectopic sites, such as the liver and muscle, to the more physiological reservoir, the peripheral subcutaneous adipose tissue [206, 212]. Recent data suggest that a plateau phenomenon in the improvement in liver steatosis and inflammation probably occurs under PPAR-γ agonists, once the individual subcutaneous adipose tissue expandability reaches its storage capacity [213, 214]. Moreover, thiazolidinediones are associated with long-term safety issues, such as an increased risk of congestive heart failure, bone fractures, and bladder cancer [215, 216]. For these reasons, the risk/benefit ratio of long-term treatment with PPAR-γ agonists should be carefully assessed. Even though current guidelines suggest that pioglitazone can be used to treat biopsy-proven NASH in non-diabetic patients, thiazolidinediones are not specifically licensed for the treatment of NASH implying that off-label administration poses an additional burden of responsibilities on the individual prescribing physician [2, 217].

Dual PPAR-α/δ Agonists

Dual PPAR-α/δ agonists are a novel promising class of drugs for NAFLD treatment. In phase II clinical trials, GFT505 (elafibranor) has been proven able to improve insulin sensitivity, lipid profile, and liver enzymes in patients with MetS or prediabetic abdominal obesity [70, 71]. The GOLDEN trial, a randomized, placebo-controlled, three-arm (placebo, GFT505 80 mg, and GFT505 120 mg) 1-year phase IIb study which evaluated 274 subjects with biopsy-proven NASH and a NAS ≥3, was completed in March 2015 [218]. The primary outcome of disappearance of NASH without worsening of fibrosis was not achieved. Nevertheless, after controlling for baseline severity and center effect, patients in the 120 mg arm had a nearly twofold higher relative risk of achieving the primary endpoint compared to placebo. Of note, in the 120 patients with moderate-to-severe activity (NAS >4) from centers recruiting at least one patient for each arm, the response rate was 29% and 5% in the 120 mg and placebo arms, respectively ($P = 0.01$). GFT505 120 mg significantly improved ballooning, inflammation, and steatosis, compared to placebo. Moreover, resolution of steatohepatitis was associated with a significant regression of fibrosis, and with improvement in markers of hepatocellular necrosis and cardio-metabolic risk. This trial demonstrated a favorable safety profile of GFT505 [219]. The most common adverse events were minor gastrointestinal complaints; moreover, a mild dose-dependent increase in creatinine was noted [91, 218, 219].

Drugs Interacting with FXR

BA specifically targeting FXR may be a promising approach to treat NAFLD. UDCA was the first BA proposed as a potential treatment for NASH. However, most of the clinical trials conducted with this tertiary BA failed to demonstrate any significant benefit on NASH histology [220–223]. The recently discovered FXR-antagonistic properties of UDCA may account for its limited therapeutic efficiency [224].

Conversely, OCA, a CDC derivative, is a potent FXR agonist which improves insulin sensitivity and gluco-lipid metabolism and exerts anti-inflammatory and marked antifibrotic effects in preclinical models [91]. A

proof-of-concept, phase 2, randomized, placebo-controlled, 6-week study in T2D patients with NAFLD showed that OCA significantly increased insulin sensitivity and reduced markers of liver inflammation and fibrosis [167]. The recently published randomized, placebo-controlled, 72-week FLINT trial compared OCA 25 mg with placebo in 282 patients with biopsy-proven NASH and NAS $\geq$4 without cirrhosis [92]. The primary endpoint was a decrease of at least two points in NAS, without worsening of fibrosis. A planned interim efficacy analysis of the histological primary outcome showed a significant superiority of OCA over placebo, which supported the decision not to perform end-of-treatment biopsies in 64 subjects. OCA treatment was significantly associated with the primary outcome and with improvement in steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis, but not with NASH resolution [92]. A recent network meta-analytic review supported direct efficacy of OCA in reversing hepatocyte balloning; moreover, OCA was associated with significant efficacy in fibrosis regression on direct comparison [125]. These positive findings, however, are counterbalanced by pruritus and increased CVR (surge in IR, total and LDL cholesterol, and a decrease in high-density lipoprotein cholesterol) [92].

TREATMENT OF NASH

Overview

Here we provide an overview of how NAFLD treatment can be effected based on currently available non-pharmacological, pharmacological, endoscopic, or surgical intervention. This topic has been extensively examined by our group recently [225, 226].

In principle, NAFLD management needs to cover various aims including amelioration of MetS and its individual features and prevention of extrahepatic NAFLD features such as T2D and atherosclerosis. For example a consistent set of data suggests that NAFLD precedes the development of T2D [3, 227] and, consistently, evidence supports that the reversal of NAFLD protects from developing T2D [121]. Similarly, a recent study suggests that the reduction in the severity of NAFLD is associated with a reduced progression in carotid intima-media thickness [228]. Moreover, effective antifibrotic treatment may potentially halt the progression of NASH to cirrhosis and, further to standard principles of treatment of cirrhosis of any etiology [159], specific aims in NASH cirrhosis may include action in preventing portal vein thrombosis (PVT) and chemoprevention of HCC [229, 230].

The available weaponry spans from simple lifestyle changes to drugs (licensed for conditions other than NAFLD) to endoscopic and surgical procedures (aimed to treat obesity and indirectly improving NAFLD). However no 'ideal', 'one-fits-all' approach is available to target all the aims listed above. Rather, a highly tailored management approach should best be implemented on the basis of age, stage of liver disease, and systemic co-morbidity [231].

Lifestyle Changes

Diet

A 7–10% weight loss is associated with histologically significant rewards such as reduced liver fat content, remission of NASH, and fibrosis reduction [232]. Moreover, quality of life is also improved in these patients as a result of weight loss [233]. Further to total ingested calories, saturated fats, carbohydrates,

and fructose-rich beverages all need to be restricted and, conversely, increases in MUFA, long-chain PUFA, and caffeine should be promoted [234].

Physical Exercise

There is clear meta-analytic evidence that exercising reduces liver fat content irrespective of weight loss [235]. This is of interest given the low cost and widespread availability of this option and that most patients fail to maintain weight loss over time. Aerobic training is superior to progressive resistance training [236]. On the basis of guidelines, cardiorespiratory exercise training should be regularly performed [237].

Drug Treatment

Lipid-Lowering Agents

Cardiovascular disease is the leading cause of mortality in NAFLD patients and guidelines recommend the use of statins in these individuals. However, statins are underutilized in patients with NAFLD owing to implicit concerns of hepatotoxicity [238, 239].

There is no relationship linking reduction in LDL cholesterol values and raised transaminases, and hypertransaminasemia is linked to statin dosage and physicochemical properties [240, 241]. Moreover, statins improve symptom-free survival from cardiovascular disease selectively in those patients in whom coronary artery disease coexists with NAFLD [242]. Recent evidence also suggests that statin use improves liver histology and protects from NASH, in a dose-dependent manner, both in diabetic and non-diabetic individuals [124, 243].

Although fibrates do not improve NAFLD histology, they do offer a safe and effective treatment for dyslipidemic patients with NAFLD [226].

Antidiabetics

NAFLD is exceedingly common among those with T2D [244]; conversely, a subset of NAFLD individuals have T2D at baseline and a large proportion of them will develop T2D over a 5-year follow-up [227, 244, 245]. Principles of treatment of T2D in NAFLD have recently been discussed elsewhere [226]. Probably, the most exciting novel finding in this arena is the possibility to implement a chemopreventive strategy of HCC with antidiabetic medications such as discussed below.

Antihypertensives

In NAFLD patients, arterial hypertension is the least prevalent among the individual features of the MetS [244] and, nevertheless, it is a major risk factor for the progression of hepatic fibrosis in these patients [120], which raises the possibility that, by treating high blood pressure with sartans, hepatic fibrosis progression may be halted. Proof-of-concept experimental studies consistently support this notion [246–253].

Obesity

Endoscopic Procedures

BioEnterics intragastric balloon safely induces a sustained weight loss with diet support or for preparing patients for bariatric surgery. Those individuals attaining a BMI reduction greater than 10% experience improvement in biochemical surrogate indices of NAFLD, IR, and NAS [254, 255].

Surgery

Bariatric surgery in NAFLD improves transaminases, cardiometabolic risk factors (IR, glucose and lipid metabolism, hypertension), and histological endpoints (hepatic steatosis, steatohepatitis, and fibrosis); however, it is not

qualified as a treatment option for NAFLD per se, but only for the accompanying morbid obesity [256]. Roux-en-Y gastric bypass (RYGB) is more effective than other types of surgery in inducing both weight loss and NAFLD improvement. However, additional studies designed to evaluate liver-specific mortality, liver transplantation, or quality of life are eagerly awaited [256].

NASH-Cirrhosis

General principles of management of cirrhosis are fully covered elsewhere [159, 257–259].

Here we discuss some selected topics of specific relevance to NASH-cirrhosis.

Prevention of Cirrhosis via Antifibrotic Agents

This aim remains largely unaddressed in clinical practice. A recent survey reporting that aspirin use seems to exert a protection from fibrosis in the general population paves the way for randomized clinical trials (RCTs) in individuals with NASH [260]. Moderate-quality network meta-analytic evidence supports the superiority of pentoxifylline and OCA over placebo in improving fibrosis [125].

Prevention of Portal Vein Thrombosis

Portal vein thrombosis (PVT) is a major event dictating the natural history of cirrhosis irrespective of etiology and the prevention of PVT with enoxaparin is associated with decreased hepatic decompensation and improved survival [261]. As regards the relationship of the etiology of cirrhosis with the risk of PVT, a nationwide US survey enrolling, overall, 33,368 patients who underwent liver transplantation reported that NASH cirrhosis was the strongest risk factor independently associated with PVT [229]. These data attest that NASH is a prothrombotic state and suggest that PVT prevention may be particularly indicated in this population. RCTs should assess which anticoagulants should be used in preventing PVT effectively and safely in this specific patient population.

Chemoprevention of HCC

The molecular bases of this topic have extensively been examined [176, 230]. In short, on the basis of current evidence metformin [262, 263], statins [264, 265], or their combination [266] may potentially be useful in this setting. However, most data derive from observational studies and RCTs are warranted before chemoprevention of HCC can be licensed for clinical practice.

CONCLUSIONS

Our understanding of the role of NRs in NAFLD pathophysiology, natural history, and treatment is preliminary (Fig. 1) and additional studies are required. PPAR-γ agonists seem to improve steatosis and necro-inflammation in NAFLD, but thiazolidinediones are not approved for the treatment of NASH, data in diabetic patients are scarce, and long-term safety and efficacy in NASH patients have not been established [91]. The dual PPAR-α/δ agonist elafibranor exhibits promising results and favorable safety profile, but its efficacy should be further confirmed. The FXR agonist OCA has the potential to prevent progression to cirrhosis by improving all the histological features of NASH, notably including fibrosis. However, additional research is needed to confirm this promise and address concerns about tolerability and side effects. Finally, drugs interacting with several NRs, either discussed here, such as LXR, or not discussed in the present review, such as pregnane X

receptor (PXR), constitutive androstane receptor (CAR), liver receptor homolog-1 (LRH-1), estrogen receptor beta (ERβ), thyroid hormone receptor beta (TRβ), and VDR, whose importance has been demonstrated in NAFLD animal models, await urgent evaluation in humans [267–269].

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REFERENCES

1. Loria P, Adinolfi LE, Bellentani S, et al. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. Dig Liver Dis. 2010;42:272–82.

2. Nascimbeni F, Pais R, Bellentani S, et al. From NAFLD in clinical practice to answers from guidelines. J Hepatol. 2013;59:859–71.

3. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. Dig Liver Dis. 2015;47:181–90.

4. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA. 2015;313:2263–73.

5. Non-alcoholic fatty liver disease study group, Lonardo A, Bellentani S, et al. Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. Dig Liver Dis. 2015.

6. Lonardo A, Sookoian S, Chonchol M, Loria P, Targher G. Cardiovascular and systemic risk in nonalcoholic fatty liver disease—atherosclerosis as a major player in the natural course of NAFLD. Curr Pharm Des. 2013;19:5177–92.

7. Wong RJ, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the US. Hepatology. 2014;59:2188–95.

8. Yamaguchi K, Yang L, McCall S, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology. 2007;45:1366–74.

9. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology. 2012;142(711–725):e716.

10. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343–51.

11. Lonardo A, Loria P, Argo C, Caldwell S. Perspectives on cellular dysfunction in nonalcoholic steatohepatitis: a case of 'multiorganelle failure'? Proceedings of a virtual workshop on nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol. 2011;5:135–9.

12. Lonardo A, Lombardini S, Ricchi M, Scaglioni F, Loria P. Review article: hepatic steatosis and insulin resistance. Aliment Pharmacol Ther. 2005;22(Suppl 2):64–70.

13. Loria P, Marchesini G, Nascimbeni F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. Atherosclerosis. 2014;232:99–109.

14. Mazzoccoli G, Vinciguerra M, Oben J, Tarquini R, De Cosmo S. Non-alcoholic fatty liver disease: the

role of nuclear receptors and circadian rhythmicity. Liver Int. 2014;34:1133–52.

15. Loria P, Lonardo A, Anania F. Liver and diabetes. A vicious circle. Hepatol Res. 2013;43:51–64.

16. Machado MV, Cortez-Pinto H. Nuclear receptors: how do they position in non-alcoholic fatty liver disease treatment? Liver Int. 2014;34:1291–4.

17. Wang Y, Viscarra J, Kim SJ, Sul HS. Transcriptional regulation of hepatic lipogenesis. Nat Rev Mol Cell Biol. 2015;16:678–89.

18. Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Jarvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis. 2010;42:320–30.

19. Nobili V, Svegliati-Baroni G, Alisi A, Miele L, Valenti L, Vajro P. A 360-degree overview of paediatric NAFLD: recent insights. J Hepatol. 2013;58:1218–29.

20. Tailleux A, Wouters K, Staels B. Roles of PPARs in NAFLD: potential therapeutic targets. Biochim Biophys Acta. 2012;1821:809–18.

21. Peyrou M, Ramadori P, Bourgoin L, Foti M. PPARs in liver diseases and cancer: epigenetic regulation by microRNAs. PPAR Res. 2012;2012:757803.

22. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARalpha action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. J Hepatol. 2015;62:720–33.

23. Qiu L, Lin J, Xu F, et al. Inhibition of aldose reductase activates hepatic peroxisome proliferator-activated receptor-alpha and ameliorates hepatosteatosis in diabetic db/db mice. Exp Diabetes Res. 2012;2012:789730.

24. Inoue M, Ohtake T, Motomura W, et al. Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. Biochem Biophys Res Commun. 2005;336:215–22.

25. Patsouris D, Reddy JK, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha mediates the effects of high-fat diet on hepatic gene expression. Endocrinology. 2006;147:1508–16.

26. Machado MV, Michelotti GA, Xie G, et al. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. PLoS One. 2015;10:e0127991.

27. Rinella ME, Elias MS, Smolak RR, Fu T, Borensztajn J, Green RM. Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. J Lipid Res. 2008;49:1068–76.

28. Kucera O, Cervinkova Z. Experimental models of non-alcoholic fatty liver disease in rats. World J Gastroenterol. 2014;20:8364–76.

29. Ip E, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. Hepatology. 2003;38:123–32.

30. Ip E, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. Hepatology. 2004;39:1286–96.

31. Abdelmegeed MA, Yoo SH, Henderson LE, Gonzalez FJ, Woodcroft KJ, Song BJ. PPARalpha expression protects male mice from high fat-induced nonalcoholic fatty liver. J Nutr. 2011;141:603–10.

32. Pawlak M, Bauge E, Bourguet W, et al. The transrepressive activity of peroxisome proliferator-activated receptor alpha is necessary and sufficient to prevent liver fibrosis in mice. Hepatology. 2014;60:1593–606.

33. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313–21.

34. Larter CZ, Yeh MM, Van Rooyen DM, Brooling J, Ghatora K, Farrell GC. Peroxisome proliferator-activated receptor-alpha agonist, Wy 14,643, improves metabolic indices, steatosis and ballooning in diabetic mice with non-alcoholic steatohepatitis. J Gastroenterol Hepatol. 2012;27:341–50.

35. Shiri-Sverdlov R, Wouters K, van Gorp PJ, et al. Early diet-induced non-alcoholic steatohepatitis in APOE2 knock-in mice and its prevention by fibrates. J Hepatol. 2006;44:732–41.

36. Lalloyer F, Wouters K, Baron M, et al. Peroxisome proliferator-activated receptor-alpha gene level differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice. Arterioscler Thromb Vasc Biol. 2011;31:1573–9.

37. Francque S, Verrijken A, Caron S, et al. PPARalpha gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. J Hepatol. 2015;63:164–73.

38. Harano Y, Yasui K, Toyama T, et al. Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, reduces hepatic steatosis and lipid

peroxidation in fatty liver Shionogi mice with hereditary fatty liver. Liver Int. 2006;26:613–20.

39. Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia. 2012;55:885–904.

40. de Wit NJ, Afman LA, Mensink M, Muller M. Phenotyping the effect of diet on non-alcoholic fatty liver disease. J Hepatol. 2012;57:1370–3.

41. Pettinelli P, Del Pozo T, Araya J, et al. Enhancement in liver SREBP-1c/PPAR-alpha ratio and steatosis in obese patients: correlations with insulin resistance and n-3 long-chain polyunsaturated fatty acid depletion. Biochim Biophys Acta. 2009;1792:1080–6.

42. Dossi CG, Tapia GS, Espinosa A, Videla LA, D'Espessailles A. Reversal of high-fat diet-induced hepatic steatosis by n-3 LCPUFA: role of PPAR-alpha and SREBP-1c. J Nutr Biochem. 2014;25:977–84.

43. Valenzuela R, Espinosa A, Gonzalez-Manan D, et al. N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. PLoS One. 2012;7:e46400.

44. Kim JK, Lee KS, Lee DK, et al. Omega-3 polyunsaturated fatty acid and ursodeoxycholic acid have an additive effect in attenuating diet-induced nonalcoholic steatohepatitis in mice. Exp Mol Med. 2014;46:e127.

45. Chen S, Li Y, Li S, Yu C. A Val227Ala substitution in the peroxisome proliferator activated receptor alpha (PPAR alpha) gene associated with non-alcoholic fatty liver disease and decreased waist circumference and waist-to-hip ratio. J Gastroenterol Hepatol. 2008;23:1415–8.

46. Dongiovanni P, Rametta R, Fracanzani AL, et al. Lack of association between peroxisome proliferator-activated receptors alpha and gamma2 polymorphisms and progressive liver damage in patients with non-alcoholic fatty liver disease: a case control study. BMC Gastroenterol. 2010;10:102.

47. Kintscher U, Law RE. PPARgamma-mediated insulin sensitization: the importance of fat versus muscle. Am J Physiol Endocrinol Metab. 2005;288:E287–91.

48. Moran-Salvador E, Lopez-Parra M, Garcia-Alonso V, et al. Role for PPARgamma in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. FASEB J. 2011;25:2538–50.

49. Yamazaki T, Shiraishi S, Kishimoto K, Miura S, Ezaki O. An increase in liver PPARgamma2 is an initial event to induce fatty liver in response to a diet high in butter: PPARgamma2 knockdown improves fatty liver induced by high-saturated fat. J Nutr Biochem. 2011;22:543–53.

50. Gupte AA, Liu JZ, Ren Y, et al. Rosiglitazone attenuates age- and diet-associated nonalcoholic steatohepatitis in male low-density lipoprotein receptor knockout mice. Hepatology. 2010;52:2001–11.

51. Liu S, Wu HJ, Zhang ZQ, et al. The ameliorating effect of rosiglitazone on experimental nonalcoholic steatohepatitis is associated with regulating adiponectin receptor expression in rats. Eur J Pharmacol. 2011;650:384–9.

52. Zhang F, Lu Y, Zheng S. Peroxisome proliferator-activated receptor-gamma cross-regulation of signaling events implicated in liver fibrogenesis. Cell Signal. 2012;24:596–605.

53. Yu J, Zhang S, Chu ES, et al. Peroxisome proliferator-activated receptors gamma reverses hepatic nutritional fibrosis in mice and suppresses activation of hepatic stellate cells in vitro. Int J Biochem Cell Biol. 2010;42:948–57.

54. Lee YH, Bae SC, Song GG. Meta-analysis of associations between the peroxisome proliferator-activated receptor-gamma Pro12Ala polymorphism and susceptibility to nonalcoholic fatty liver disease, rheumatoid arthritis, and psoriatic arthritis. Genet Test Mol Biomarkers. 2014;18:341–8.

55. Bojic LA, Huff MW. Peroxisome proliferator-activated receptor delta: a multifaceted metabolic player. Curr Opin Lipidol. 2013;24:171–7.

56. Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, et al. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab. 2008;7:496–507.

57. Liu S, Hatano B, Zhao M, et al. Role of peroxisome proliferator-activated receptor delta}/{beta in hepatic metabolic regulation. J Biol Chem. 2011;286:1237–47.

58. Lee CH, Olson P, Hevener A, et al. PPARdelta regulates glucose metabolism and insulin sensitivity. Proc Natl Acad Sci USA. 2006;103:3444–9.

59. Sanderson LM, Boekschoten MV, Desvergne B, Muller M, Kersten S. Transcriptional profiling reveals divergent roles of PPARalpha and PPARbeta/delta in regulation of gene expression in mouse liver. Physiol Genom. 2010;41:42–52.

60. Akiyama TE, Lambert G, Nicol CJ, et al. Peroxisome proliferator-activated receptor beta/delta regulates very low density lipoprotein production and catabolism in mice on a Western diet. J Biol Chem. 2004;279:20874–81.

61. Qin X, Xie X, Fan Y, et al. Peroxisome proliferator-activated receptor-delta induces insulin-induced gene-1 and suppresses hepatic lipogenesis in obese diabetic mice. Hepatology. 2008;48:432–41.

62. Shan W, Nicol CJ, Ito S, et al. Peroxisome proliferator-activated receptor-beta/delta protects against chemically induced liver toxicity in mice. Hepatology. 2008;47:225–35.

63. Wu HT, Chen CT, Cheng KC, Li YX, Yeh CH, Cheng JT. Pharmacological activation of peroxisome proliferator-activated receptor delta improves insulin resistance and hepatic steatosis in high fat diet-induced diabetic mice. Horm Metab Res. 2011;43:631–5.

64. Lee MY, Choi R, Kim HM, et al. Peroxisome proliferator-activated receptor delta agonist attenuates hepatic steatosis by anti-inflammatory mechanism. Exp Mol Med. 2012;44:578–85.

65. Serrano-Marco L, Barroso E, El Kochairi I, et al. The peroxisome proliferator-activated receptor (PPAR) beta/delta agonist GW501516 inhibits IL-6-induced signal transducer and activator of transcription 3 (STAT3) activation and insulin resistance in human liver cells. Diabetologia. 2012;55:743–51.

66. Nagasawa T, Inada Y, Nakano S, et al. Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. Eur J Pharmacol. 2006;536:182–91.

67. Li X, Li J, Lu X, et al. Treatment with PPARdelta agonist alleviates non-alcoholic fatty liver disease by modulating glucose and fatty acid metabolic enzymes in a rat model. Int J Mol Med. 2015;36:767–75.

68. Riserus U, Sprecher D, Johnson T, et al. Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. Diabetes. 2008;57:332–9.

69. Bays HE, Schwartz S, Littlejohn T 3rd, et al. MBX-8025, a novel peroxisome proliferator receptor-delta agonist: lipid and other metabolic effects in dyslipidemic overweight patients treated with and without atorvastatin. J Clin Endocrinol Metab. 2011;96:2889–97.

70. Staels B, Rubenstrunk A, Noel B, et al. Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Hepatology. 2013;58:1941–52.

71. Cariou B, Hanf R, Lambert-Porcheron S, et al. Dual peroxisome proliferator-activated receptor alpha/delta agonist GFT505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects. Diabetes Care. 2013;36:2923–30.

72. Ma H, Patti ME. Bile acids, obesity, and the metabolic syndrome. Best Pract Res Clin Gastroenterol. 2014;28:573–83.

73. McMahan RH, Wang XX, Cheng LL, et al. Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. J Biol Chem. 2013;288:11761–70.

74. Neuschwander-Tetri BA. Targeting the FXR nuclear receptor to treat liver disease. Gastroenterology. 2015;148:704–6.

75. Monte MJ, Marin JJ, Antelo A, Vazquez-Tato J. Bile acids: chemistry, physiology, and pathophysiology. World J Gastroenterol. 2009;15:804–16.

76. Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab. 2005;2:217–25.

77. Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. 2009;89:147–91.

78. Heni M, Wagner R, Ketterer C, et al. Genetic variation in NR1H4 encoding the bile acid receptor FXR determines fasting glucose and free fatty acid levels in humans. J Clin Endocrinol Metab. 2013;98:E1224–9.

79. Myronovych A, Salazar-Gonzalez RM, Ryan KK, et al. The role of small heterodimer partner in nonalcoholic fatty liver disease improvement after sleeve gastrectomy in mice. Obesity (Silver Spring). 2014;22:2301–11.

80. Aguilar-Olivos NE, Carrillo-Cordova D, Oria-Hernandez J, et al. The nuclear receptor FXR, but not LXR, up-regulates bile acid transporter expression in non-alcoholic fatty liver disease. Ann Hepatol. 2015;14:487–93.

81. Kunne C, Acco A, Duijst S, et al. FXR-dependent reduction of hepatic steatosis in a bile salt deficient mouse model. Biochim Biophys Acta. 2014;1842:739–46.

82. Xiong X, Wang X, Lu Y, et al. Hepatic steatosis exacerbated by endoplasmic reticulum stress-mediated downregulation of FXR in aging mice. J Hepatol. 2014;60:847–54.

83. Wu WB, Chen YY, Zhu B, Peng XM, Zhang SW, Zhou ML. Excessive bile acid activated NF-kappa B and promoted the development of alcoholic steatohepatitis in farnesoid X receptor deficient mice. Biochimie. 2015;115:86–92.

84. Livero FA, Stolf AM, Dreifuss AA, et al. The FXR agonist 6ECDCA reduces hepatic steatosis and oxidative stress induced by ethanol and low-protein diet in mice. Chem Biol Interact. 2014;217:19–27.

85. Bjursell M, Wedin M, Admyre T, et al. Ageing Fxr deficient mice develop increased energy expenditure, improved glucose control and liver damage resembling NASH. PLoS One. 2013;8:e64721.

86. Wu W, Liu X, Peng X, et al. Bile acids override steatosis in farnesoid X receptor deficient mice in a model of non-alcoholic steatohepatitis. Biochem Biophys Res Commun. 2014;448:50–5.

87. Kim DH, Xiao Z, Kwon S, et al. A dysregulated acetyl/SUMO switch of FXR promotes hepatic inflammation in obesity. EMBO J. 2015;34:184–99.

88. Jiang C, Xie C, Li F, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest. 2015;125:386–402.

89. Prasad V, Chirra S, Kohli R, Shull GE. NHE1 deficiency in liver: implications for non-alcoholic fatty liver disease. Biochem Biophys Res Commun. 2014;450:1027–31.

90. Liu X, Xue R, Ji L, et al. Activation of farnesoid X receptor (FXR) protects against fructose-induced liver steatosis via inflammatory inhibition and ADRP reduction. Biochem Biophys Res Commun. 2014;450:117–23.

91. Filozof C, Goldstein BJ, Williams RN, Sanyal A. Non-alcoholic steatohepatitis: limited available treatment options but promising drugs in development and recent progress towards a regulatory approval pathway. Drugs. 2015;75:1373–92.

92. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2015;385:956–65.

93. Maneschi E, Vignozzi L, Morelli A, et al. FXR activation normalizes insulin sensitivity in visceral preadipocytes of a rabbit model of MetS. J Endocrinol. 2013;218:215–31.

94. Ma Y, Huang Y, Yan L, Gao M, Liu D. Synthetic FXR agonist GW4064 prevents diet-induced hepatic steatosis and insulin resistance. Pharm Res. 2013;30:1447–57.

95. Fang S, Suh JM, Reilly SM, et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat Med. 2015;21:159–65.

96. Ducheix S, Montagner A, Theodorou V, Ferrier L, Guillou H. The liver X receptor: a master regulator of the gut-liver axis and a target for non alcoholic fatty liver disease. Biochem Pharmacol. 2013;86:96–105.

97. Wang GX, Zhao XY, Meng ZX, et al. The brown fat-enriched secreted factor Nrg4 preserves metabolic homeostasis through attenuation of hepatic lipogenesis. Nat Med. 2014;20:1436–43.

98. Rong X, Albert CJ, Hong C, et al. LXRs regulate ER stress and inflammation through dynamic modulation of membrane phospholipid composition. Cell Metab. 2013;18:685–97.

99. Varin A, Thomas C, Ishibashi M, et al. Liver X receptor activation promotes polyunsaturated fatty acid synthesis in macrophages: relevance in the context of atherosclerosis. Arterioscler Thromb Vasc Biol. 2015;35:1357–65.

100. Shchelkunova TA, Morozov IA, Rubtsov PM, et al. Lipid regulators during atherogenesis: expression of LXR, PPAR, and SREBP mRNA in the human aorta. PLoS One. 2013;8:e63374.

101. Miao Y, Wu W, Dai Y, et al. Liver X receptor beta controls thyroid hormone feedback in the brain and regulates browning of subcutaneous white adipose tissue. Proc Natl Acad Sci USA. 2015;112:14006–11.

102. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. Nat Rev Mol Cell Biol. 2012;13:213–24.

103. Gabitova L, Gorin A, Astsaturov I. Molecular pathways: sterols and receptor signaling in cancer. Clin Cancer Res. 2014;20:28–34.

104. Zhang X, Liu J, Su W, et al. Liver X receptor activation increases hepatic fatty acid desaturation

by the induction of SCD1 expression through an LXRalpha-SREBP1c-dependent mechanism. J Diabetes. 2014;6:212–20.

105. Sim WC, Park S, Lee KY, et al. LXR-alpha antagonist meso-dihydroguaiaretic acid attenuates high-fat diet-induced nonalcoholic fatty liver. Biochem Pharmacol. 2014;90:414–24.

106. Garcia-Mediavilla MV, Pisonero-Vaquero S, Lima-Cabello E, et al. Liver X receptor alpha-mediated regulation of lipogenesis by core and NS5A proteins contributes to HCV-induced liver steatosis and HCV replication. Lab Invest. 2012;92:1191–202.

107. Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. Gastroenterology. 2004;126:586–97.

108. Lonardo A, Adinolfi LE, Restivo L, et al. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. World J Gastroenterol. 2014;20:7089–103.

109. Griffett K, Solt LA, El-Gendy Bel D, Kamenecka TM, Burris TP. A liver-selective LXR inverse agonist that suppresses hepatic steatosis. ACS Chem Biol. 2013;8:559–67.

110. Lee JM, Gang GT, Kim DK, et al. Ursodeoxycholic acid inhibits liver X receptor alpha-mediated hepatic lipogenesis via induction of the nuclear corepressor SMILE. J Biol Chem. 2014;289:1079–91.

111. Ahn SB, Jang K, Jun DW, Lee BH, Shin KJ. Expression of liver X receptor correlates with intrahepatic inflammation and fibrosis in patients with nonalcoholic fatty liver disease. Dig Dis Sci. 2014;59:2975–82.

112. Brunt EM. Nonalcoholic fatty liver disease: pros and cons of histologic systems of evaluation. Int J Mol Sci. 2016;17(1):E97. doi:10.3390/ijms17010097.

113. Kubes P, Mehal WZ. Sterile inflammation in the liver. Gastroenterology. 2012;143:1158–72.

114. Zelber-Sagi S, Lotan R, Shlomai A, et al. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. J Hepatol. 2012;56:1145–51.

115. Shen J, Wong GL, Chan HL, et al. PNPLA3 gene polymorphism accounts for fatty liver in community subjects without metabolic syndrome. Aliment Pharmacol Ther. 2014;39:532–9.

116. Yilmaz Y. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? Aliment Pharmacol Ther. 2012;36:815–23.

117. Wong VW, Wong GL, Choi PC, et al. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. Gut. 2010;59:969–74.

118. Pais R, Charlotte F, Fedchuk L, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol. 2013;59:550–6.

119. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015;62:1148–55.

120. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol. 2015;13:643–54 (e641–649; quiz e639–640).

121. Yamazaki H, Tsuboya T, Tsuji K, Dohke M, Maguchi H. Independent association between improvement of nonalcoholic fatty liver disease and reduced incidence of type 2 diabetes. Diabetes Care. 2015;38:1673–9.

122. Lassailly G, Caiazzo R, Buob D, et al. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. Gastroenterology. 2015;149:379–88 (quiz e315–376).

123. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology. 2015;149(367–378):e365 (quiz e314–365).

124. Dongiovanni P, Petta S, Mannisto V, et al. Statin use and non-alcoholic steatohepatitis in at risk individuals. J Hepatol. 2015;63:705–12.

125. Singh S, Khera R, Allen AM, Murad MH, Loomba R. Comparative effectiveness of pharmacological interventions for nonalcoholic steatohepatitis: a systematic review and network meta-analysis. Hepatology. 2015;62:1417–32.

126. Szalowska E, van der Burg B, Man HY, Hendriksen PJ, Peijnenburg AA. Model steatogenic compounds (amiodarone, valproic acid, and tetracycline) alter lipid metabolism by different mechanisms in mouse liver slices. PLoS One. 2014;9:e86795.

127. Lu Y, Liu X, Jiao Y, et al. Periostin promotes liver steatosis and hypertriglyceridemia through downregulation of PPARalpha. J Clin Invest. 2014;124:3501–13.

128. Komatsu M, Kimura T, Yazaki M, et al. Steatogenesis in adult-onset type II citrullinemia is associated with down-regulation of PPARalpha. Biochim Biophys Acta. 2015;1852:473–81.

129. Chan SM, Sun RQ, Zeng XY, et al. Activation of PPARalpha ameliorates hepatic insulin resistance and steatosis in high fructose-fed mice despite increased endoplasmic reticulum stress. Diabetes. 2013;62:2095–105.

130. Westerbacka J, Kolak M, Kiviluoto T, et al. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. Diabetes. 2007;56:2759–65.

131. Pettinelli P, Videla LA. Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. J Clin Endocrinol Metab. 2011;96:1424–30.

132. den Besten G, Bleeker A, Gerding A, et al. Short-chain fatty acids protect against high-fat diet-induced obesity via a PPARgamma-dependent switch from lipogenesis to fat oxidation. Diabetes. 2015;64:2398–408.

133. Gawrieh S, Marion MC, Komorowski R, et al. Genetic variation in the peroxisome proliferator activated receptor-gamma gene is associated with histologically advanced NAFLD. Dig Dis Sci. 2012;57:952–7.

134. Caldwell S. NASH (nonalcoholic steatohepatitis): a case of multiorganelle failure. Free Radic Biol Med. 2014;75(Suppl 1):S6.

135. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121:91–100.

136. Carulli L, Canedi I, Rondinella S, et al. Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. Dig Liver Dis. 2009;41:823–8.

137. Petta S, Camma C, Cabibi D, Di Marco V, Craxi A. Hyperuricemia is associated with histological liver damage in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2011;34:757–66.

138. Younossi ZM, Stepanova M, Negro F, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. Medicine (Baltimore). 2012;91:319–27.

139. Carulli L, Ballestri S, Lonardo A, et al. Is nonalcoholic steatohepatitis associated with a high-though-normal thyroid stimulating hormone level and lower cholesterol levels? Intern Emerg Med. 2013;8:297–305.

140. Reha JL, Lee S, Hofmann LJ. Prevalence and predictors of nonalcoholic steatohepatitis in obese patients undergoing bariatric surgery: a Department of Defense experience. Am Surg. 2014;80:595–9.

141. Dasarathy J, Periyalwar P, Allampati S, et al. Hypovitaminosis D is associated with increased whole body fat mass and greater severity of non-alcoholic fatty liver disease. Liver Int. 2014;34:e118–27.

142. Lonardo A, Bellentani S, Ratziu V, Loria P. Insulin resistance in nonalcoholic steatohepatitis: necessary but not sufficient—death of a dogma from analysis of therapeutic studies? Expert Rev Gastroenterol Hepatol. 2011;5:279–89.

143. Zelcer N, Tontonoz P. Liver X receptors as integrators of metabolic and inflammatory signaling. J Clin Invest. 2006;116:607–14.

144. Torocsik D, Barath M, Benko S, et al. Activation of liver X receptor sensitizes human dendritic cells to inflammatory stimuli. J Immunol. 2010;184:5456–65.

145. Beaven SW, Wroblewski K, Wang J, et al. Liver X receptor signaling is a determinant of stellate cell activation and susceptibility to fibrotic liver disease. Gastroenterology. 2011;140:1052–62.

146. Hong C, Kidani Y, A-Gonzalez N, et al. Coordinate regulation of neutrophil homeostasis by liver X receptors in mice. J Clin Invest. 2012;122:337–47.

147. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2015;149(389–397):e310.

148. Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology. 2004;39:179–87.

149. Fracanzani AL, Valenti L, Bugianesi E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role

for insulin resistance and diabetes. Hepatology. 2008;48:792–8.

150. Singh DK, Sakhuja P, Malhotra V, Gondal R, Sarin SK. Independent predictors of steatohepatitis and fibrosis in Asian Indian patients with non-alcoholic steatohepatitis. Dig Dis Sci. 2008;53:1967–76.

151. Miele L, Beale G, Patman G, et al. The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. Gastroenterology. 2008;135(282–291):e281.

152. Al-Serri A, Anstee QM, Valenti L, et al. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case–control and intra-familial allele association studies. J Hepatol. 2012;56:448–54.

153. McPherson S, Henderson E, Burt AD, Day CP, Anstee QM. Serum immunoglobulin levels predict fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol. 2014;60:1055–62.

154. Yang JD, Abdelmalek MF, Pang H, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology. 2014;59:1406–14.

155. Singal AG, Manjunath H, Yopp AC, et al. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. Am J Gastroenterol. 2014;109:325–34.

156. Petta S, Miele L, Bugianesi E, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. PLoS One. 2014;9:e87523.

157. Loomba R, Schork N, Chen CH, et al. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. Gastroenterology. 2015;149:1784–93.

158. Turola E, Petta S, Vanni E, et al. Ovarian senescence increases liver fibrosis in humans and zebrafish with steatosis. Dis Model Mech. 2015;8:1037–46.

159. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet. 2014;383:1749–61.

160. Roulot D, Czernichow S, Le Clesiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. J Hepatol. 2008;48:606–13.

161. Koehler EM, Plompen EP, Schouten JN, et al. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. Hepatology. 2016;63:138–47.

162. You SC, Kim KJ, Kim SU, et al. Factors associated with significant liver fibrosis assessed using transient elastography in general population. World J Gastroenterol. 2015;21:1158–66.

163. Fiorucci S, Antonelli E, Rizzo G, et al. The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. Gastroenterology. 2004;127:1497–512.

164. Fickert P, Fuchsbichler A, Moustafa T, et al. Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts. Am J Pathol. 2009;175:2392–405.

165. Jiang T, Wang XX, Scherzer P, et al. Farnesoid X receptor modulates renal lipid metabolism, fibrosis, and diabetic nephropathy. Diabetes. 2007;56:2485–93.

166. Lee CG, Kim YW, Kim EH, et al. Farnesoid X receptor protects hepatocytes from injury by repressing miR-199a-3p, which increases levels of LKB1. Gastroenterology. 2012;142(1206–1217):e1207.

167. Mudaliar S, Henry RR, Sanyal AJ, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. Gastroenterology. 2013;145(574–582):e571.

168. Paradis V, Zalinski S, Chelbi E, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology. 2009;49:851–9.

169. Pocha C, Kolly P, Dufour JF. Nonalcoholic fatty liver disease-related hepatocellular carcinoma: a problem of growing magnitude. Semin Liver Dis. 2015;35:304–17.

170. Piscaglia F, Svegliati-Baroni G, Barchetti A, et al. Clinical patterns of hepatocellular carcinoma (HCC) in non alcoholic fatty liver disease (NAFLD): a multicenter prospective study. Hepatology. 2015. doi:10.1002/hep.28368.

171. Giannini EG, Marabotto E, Savarino V, et al. Hepatocellular carcinoma in patients with cryptogenic cirrhosis. Clin Gastroenterol Hepatol. 2009;7:580–5.

172. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in

the United States from 2004 to 2009. Hepatology. 2015;62:1723–30.

173. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology. 2002;123:134–40.

174. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology. 2010;51:1972–8.

175. Sawada N, Inoue M, Iwasaki M, et al. Consumption of n-3 fatty acids and fish reduces risk of hepatocellular carcinoma. Gastroenterology. 2012;142:1468–75.

176. Lonardo A, Loria P. Potential for statins in the chemoprevention and management of hepatocellular carcinoma. J Gastroenterol Hepatol. 2012;27:1654–64.

177. Welzel TM, Graubard BI, Quraishi S, et al. Population-attributable fractions of risk factors for hepatocellular carcinoma in the United States. Am J Gastroenterol. 2013;108:1314–21.

178. Bravi F, Bosetti C, Tavani A, Gallus S, La Vecchia C. Coffee reduces risk for hepatocellular carcinoma: an updated meta-analysis. Clin Gastroenterol Hepatol. 2013;11(1413–1421):e1411.

179. Yang Y, Zhang D, Feng N, et al. Increased intake of vegetables, but not fruit, reduces risk for hepatocellular carcinoma: a meta-analysis. Gastroenterology. 2014;147:1031–42.

180. Wang YG, Wang P, Wang B, Fu ZJ, Zhao WJ, Yan SL. Diabetes mellitus and poorer prognosis in hepatocellular carcinoma: a systematic review and meta-analysis. PLoS One. 2014;9:e95485.

181. Cheung KF, Zhao J, Hao Y, et al. CITED2 is a novel direct effector of peroxisome proliferator-activated receptor gamma in suppressing hepatocellular carcinoma cell growth. Cancer. 2013;119:1217–26.

182. Li Q, Gao Y, Jia Z, et al. Dysregulated Kruppel-like factor 4 and vitamin D receptor signaling contribute to progression of hepatocellular carcinoma. Gastroenterology. 2012;143(799–810):e791–2.

183. Guo F, Xu Z, Zhang Y, et al. FXR induces SOCS3 and suppresses hepatocellular carcinoma. Oncotarget. 2015;6:34606–16.

184. Liu X, Zhang X, Ji L, Gu J, Zhou M, Chen S. Farnesoid X receptor associates with beta-catenin and inhibits its activity in hepatocellular carcinoma. Oncotarget. 2015;6:4226–38.

185. He J, Zhao K, Zheng L, et al. Upregulation of microRNA-122 by farnesoid X receptor suppresses the growth of hepatocellular carcinoma cells. Mol Cancer. 2015;14:163.

186. Nakamuta M, Morizono S, Soejima Y, et al. Short-term intensive treatment for donors with hepatic steatosis in living-donor liver transplantation. Transplantation. 2005;80:608–12.

187. Athyros VG, Mikhailidis DP, Didangelos TP, et al. Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study. Curr Med Res Opin. 2006;22:873–83.

188. Bajaj M, Suraamornkul S, Hardies LJ, Glass L, Musi N, DeFronzo RA. Effects of peroxisome proliferator-activated receptor (PPAR)-alpha and PPAR-gamma agonists on glucose and lipid metabolism in patients with type 2 diabetes mellitus. Diabetologia. 2007;50:1723–31.

189. Fernandez-Miranda C, Perez-Carreras M, Colina F, Lopez-Alonso G, Vargas C, Solis-Herruzo JA. A pilot trial of fenofibrate for the treatment of non-alcoholic fatty liver disease. Dig Liver Dis. 2008;40:200–5.

190. El-Haggar SM, Mostafa TM. Comparative clinical study between the effect of fenofibrate alone and its combination with pentoxifylline on biochemical parameters and liver stiffness in patients with non-alcoholic fatty liver disease. Hepatol Int. 2015;9:471–9.

191. Capanni M, Calella F, Biagini MR, et al. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. Aliment Pharmacol Ther. 2006;23:1143–51.

192. Zhu FS, Liu S, Chen XM, Huang ZG, Zhang DW. Effects of n-3 polyunsaturated fatty acids from seal oils on nonalcoholic fatty liver disease associated with hyperlipidemia. World J Gastroenterol. 2008;14:6395–400.

193. Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol. 2012;56:944–51.

194. Sanyal AJ, Abdelmalek MF, Suzuki A, Cummings OW, Chojkier M, EPE-A Study Group. No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic

steatohepatitis in a phase 2 trial. Gastroenterology. 2014;147(377–384):e371.

195. Scorletti E, Bhatia L, McCormick KG, et al. Effects of purified eicosapentaenoic and docosahexaenoic acids in nonalcoholic fatty liver disease: results from the Welcome study. Hepatology. 2014;60:1211–21.

196. Janczyk W, Lebensztejn D, Wierzbicka-Rucinska A, et al. Omega-3 fatty acids therapy in children with nonalcoholic Fatty liver disease: a randomized controlled trial. J Pediatr. 2015;166(1358–1363):e1351–3.

197. Dasarathy S, Dasarathy J, Khiyami A, et al. Double-blind randomized placebo-controlled clinical trial of omega 3 fatty acids for the treatment of diabetic patients with nonalcoholic steatohepatitis. J Clin Gastroenterol. 2015;49:137–44.

198. Bragt MC, Mensink RP. Comparison of the effects of n-3 long chain polyunsaturated fatty acids and fenofibrate on markers of inflammation and vascular function, and on the serum lipoprotein profile in overweight and obese subjects. Nutr Metab Cardiovasc Dis. 2012;22:966–73.

199. Watts GF, Karpe F. Triglycerides and atherogenic dyslipidaemia: extending treatment beyond statins in the high-risk cardiovascular patient. Heart. 2011;97:350–6.

200. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology. 2003;38:1008–17.

201. Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. Hepatology. 2004;39:188–96.

202. Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355:2297–307.

203. Aithal GP, Thomas JA, Kaye PV, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology. 2008;135:1176–84.

204. Ratziu V, Giral P, Jacqueminet S, et al. Rosiglitazone for nonalcoholic steatohepatitis: 1-year results of the randomized placebo-controlled fatty liver improvement with rosiglitazone therapy (FLIRT) trial. Gastroenterology. 2008;135:100–10.

205. Gastaldelli A, Harrison SA, Belfort-Aguilar R, et al. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. Hepatology. 2009;50:1087–93.

206. Ratziu V, Charlotte F, Bernhardt C, Get al. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. Hepatology. 2010;51:445–53.

207. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362:1675–85.

208. Torres DM, Jones FJ, Shaw JC, Williams CD, Ward JA, Harrison SA. Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open-label trial. Hepatology. 2011;54:1631–9.

209. Mahady SE, Webster AC, Walker S, Sanyal A, George J. The role of thiazolidinediones in non-alcoholic steatohepatitis—a systematic review and meta analysis. J Hepatol. 2011;55:1383–90.

210. Boettcher E, Csako G, Pucino F, Wesley R, Loomba R. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2012;35:66–75.

211. Lutchman G, Modi A, Kleiner DE, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. Hepatology. 2007;46:424–9.

212. Balas B, Belfort R, Harrison SA, et al. Pioglitazone treatment increases whole body fat but not total body water in patients with non-alcoholic steatohepatitis. J Hepatol. 2007;47:565–70.

213. Kim JY, van de Wall E, Laplante M, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest. 2007;117:2621–37.

214. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the metabolic syndrome—an allostatic perspective. Biochim Biophys Acta. 2010;1801:338–49.

215. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In

macroVascular Events): a randomised controlled trial. Lancet. 2005;366:1279–89.

216. Azoulay L, Yin H, Filion KB, et al. The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. BMJ. 2012;344:e3645.

217. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology. 2012;142:1592–609.

218. Genfit (2015) Topline phase IIb results: conference call and webcast transcript. http://www.genfit.com/wp-content/uploads/2015/03/Topline-Phase-2b-results-TC-Transcript.pdf. Accessed 22 Feb 2016.

219. Ratziu V, Harrison S, Francque S, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor-α and -δ, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology. 2016;S0016-5085(16):140–2. doi:10.1053/j.gastro.2016.01.038.

220. Lindor KD, Kowdley KV, Heathcote EJ, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. Hepatology. 2004;39:770–8.

221. Dufour JF, Oneta CM, Gonvers JJ, et al. Randomized placebo-controlled trial of ursodeoxycholic acid with vitamin E in nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol. 2006;4:1537–43.

222. Leuschner UF, Lindenthal B, Herrmann G, et al. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial. Hepatology. 2010;52:472–9.

223. Ratziu V, de Ledinghen V, Oberti F, et al. A randomized controlled trial of high-dose ursodesoxycholic acid for nonalcoholic steatohepatitis. J Hepatol. 2011;54:1011–9.

224. Mueller M, Thorell A, Claudel T, et al. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. J Hepatol. 2015;62:1398–404.

225. Lonardo A, Sookoian S, Pirola CJ, Targher G. Non-alcoholic fatty liver disease and risk of cardiovascular disease. Metabolism. 2015;S0026-0495(15):271–1. doi:10.1016/j.metabol.2015.09.017.

226. Lonardo A, Ballestri S, Targher G, Loria P. Diagnosis and management of cardiovascular risk in nonalcoholic fatty liver disease. Expert Rev Gastroenterol Hepatol. 2015;9:629–50.

227. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost two-fold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2015. doi:10.1111/jgh.13264.

228. Bhatia L, Scorletti E, Curzen N, Clough GF, Calder PC, Byrne CD. Improvement in non-alcoholic fatty liver disease severity is associated with a reduction in carotid intima-media thickness progression. Atherosclerosis. 2015;246:13–20.

229. Stine JG, Shah NL, Argo CK, Pelletier SJ, Caldwell SH, Northup PG. Increased risk of portal vein thrombosis in patients with cirrhosis due to nonalcoholic steatohepatitis. Liver Transpl. 2015;21:1016–21.

230. Singh S, Singh PP, Roberts LR, Sanchez W. Chemopreventive strategies in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol. 2014;11:45–54.

231. Petta S, Valenti L, Bugianesi E, et al. A "systems medicine" approach to the study of non-alcoholic fatty liver disease. Dig Liver Dis. 2016;48:333–42.

232. Marchesini G, Petta S, Dale Grave R. Diet, weight loss, and liver health in NAFLD: pathophysiology, evidence and practice. Hepatology. 2015. doi:10.1002/hep.28392.

233. Tapper EB, Lai M. Weight loss results in significant improvements in quality of life for patients with nonalcoholic fatty liver disease: a prospective cohort study. Hepatology. 2015. doi:10.1002/hep.28416.

234. Ordonez R, Carbajo-Pescador S, Mauriz JL, Gonzalez-Gallego J. Understanding nutritional interventions and physical exercise in non-alcoholic fatty liver disease. Curr Mol Med. 2015;15:3–26.

235. Keating SE, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol. 2012;57:157–66.

236. Slentz CA, Bateman LA, Willis LH, et al. Effects of aerobic vs. resistance training on visceral and liver fat stores, liver enzymes, and insulin resistance by

HOMA in overweight adults from STRRIDE AT/RT. Am J Physiol Endocrinol Metab. 2011;301:E1033–9.

237. WHO (2010) Global recommendations on physical activity for health. WHO, Geneva

238. Rzouq FS, Volk ML, Hatoum HH, Talluri SK, Mummadi RR, Sood GK. Hepatotoxicity fears contribute to underutilization of statin medications by primary care physicians. Am J Med Sci. 2010;340:89–93.

239. Blais P, Lin M, Kramer JR, El-Serag HB, Kanwal F. Statins are underutilized in patients with nonalcoholic fatty liver disease and dyslipidemia. Dig Dis Sci. 2015. **[Epub ahead of print]**

240. Alsheikh-Ali AA, Maddukuri PV, Han H, Karas RH. Effect of the magnitude of lipid lowering on risk of elevated liver enzymes, rhabdomyolysis, and cancer: insights from large randomized statin trials. J Am Coll Cardiol. 2007;50:409–18.

241. Dale KM, White CM, Henyan NN, Kluger J, Coleman CI. Impact of statin dosing intensity on transaminase and creatine kinase. Am J Med. 2007;120:706–12.

242. Athyros VG, Tziomalos K, Daskalopoulos GN, Karagiannis A, Mikhailidis DP. Statin-based treatment for cardiovascular risk and non-alcoholic fatty liver disease. Killing two birds with one stone? Ann Med. 2011;43:167–71.

243. Nascimbeni F, Aron-Wisniewsky J, Pais R, et al. Statins, antidiabetic medications and liver histology in diabetic patients with non-alcoholic fatty liver disease. BMJ Open Gastroenterol. 2016. (In press).

244. Non-alcoholic fatty liver disease study group, Lonardo A, Bellentani S, et al. Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. Dig Liver Dis 2015;47:997–1006.

245. Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44:865–73.

246. Yoshiji H, Noguchi R, Ikenaka Y, et al. Losartan, an angiotensin-II type 1 receptor blocker, attenuates the liver fibrosis development of non-alcoholic steatohepatitis in the rat. BMC Res Notes. 2009;2:70.

247. Sturzeneker MC, Ioshii SO, Villela Baroncini LA, Precoma DB. Olmesartan severely weakened the development of NASH in an animal model of hypercholesterolemia. Atherosclerosis. 2011;216: 97–102.

248. Nakagami H, Kiomy Osako M, Nakagami F, et al. Prevention and regression of non-alcoholic steatohepatitis (NASH) in a rat model by metabosartan, telmisartan. Int J Mol Med. 2010;26:477–81.

249. Toblli JE, Munoz MC, Cao G, Mella J, Pereyra L, Mastai R. ACE inhibition and AT1 receptor blockade prevent fatty liver and fibrosis in obese Zucker rats. Obesity (Silver Spring). 2008;16:770–6.

250. Jin H, Yamamoto N, Uchida K, Terai S, Sakaida I. Telmisartan prevents hepatic fibrosis and enzyme-altered lesions in liver cirrhosis rat induced by a choline-deficient L-amino acid-defined diet. Biochem Biophys Res Commun. 2007;364:801–7.

251. Ibanez P, Solis N, Pizarro M, et al. Effect of losartan on early liver fibrosis development in a rat model of nonalcoholic steatohepatitis. J Gastroenterol Hepatol. 2007;22:846–51.

252. Hirose A, Ono M, Saibara T, et al. Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. Hepatology. 2007;45:1375–81.

253. Fujita K, Yoneda M, Wada K, et al. Telmisartan, an angiotensin II type 1 receptor blocker, controls progress of nonalcoholic steatohepatitis in rats. Dig Dis Sci. 2007;52:3455–64.

254. Ricci G, Bersani G, Rossi A, Pigo F, De Fabritiis G, Alvisi V. Bariatric therapy with intragastric balloon improves liver dysfunction and insulin resistance in obese patients. Obes Surg. 2008;18:1438–42.

255. Lee YM, Low HC, Lim LG, et al. Intragastric balloon significantly improves nonalcoholic fatty liver disease activity score in obese patients with nonalcoholic steatohepatitis: a pilot study. Gastrointest Endosc. 2012;76:756–60.

256. Aguilar-Olivos NE, Almeda-Valdes P, Aguilar-Salinas CA, Uribe M, Mendez-Sanchez N. The role of bariatric surgery in the management of nonalcoholic fatty liver disease and metabolic syndrome. Metabolism. 2015;S0026-0495(15): 258–9. doi:10.1016/j.metabol.2015.09.004.

257. Tripathi D, Stanley AJ, Hayes PC, et al. UK guidelines on the management of variceal haemorrhage in cirrhotic patients. Gut. 2015;64:1680–704.

258. Moore KP, Aithal GP. Guidelines on the management of ascites in cirrhosis. Gut. 2006;55(Suppl 6):vi1–12.

259. Pericleous M, Sarnowski A, Moore A, Fijten R, Zaman M. The clinical management of abdominal

ascites, spontaneous bacterial peritonitis and hepatorenal syndrome: a review of current guidelines and recommendations. Eur J Gastroenterol Hepatol. 2016;28:e10–8.

260. Jiang ZG, Feldbrugge L, Tapper EB, et al. Aspirin use is associated with lower indices of liver fibrosis among adults in the United States. Aliment Pharmacol Ther. 2016;43:734–43.

261. Villa E, Camma C, Marietta M, et al. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. Gastroenterology. 2012;143(1253–1260): e1251–4.

262. Chen HP, Shieh JJ, Chang CC, et al. Metformin decreases hepatocellular carcinoma risk in a dose-dependent manner: population-based and in vitro studies. Gut. 2013;62:606–15.

263. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Anti-diabetic medications and the risk of hepatocellular cancer: a systematic review and meta-analysis. Am J Gastroenterol. 2013;108:881–91 (quiz 892).

264. Singh S, Singh PP, Singh AG, Murad MH, Sanchez W. Statins are associated with a reduced risk of hepatocellular cancer: a systematic review and meta-analysis. Gastroenterology. 2013;144:323–32.

265. Pradelli D, Soranna D, Scotti L, et al. Statins and primary liver cancer: a meta-analysis of observational studies. Eur J Cancer Prev. 2013;22:229–34.

266. Chen CI, Kuan CF, Fang YA, et al. Cancer risk in HBV patients with statin and metformin use: a population-based cohort study. Medicine (Baltimore). 2015;94:e462.

267. George J, Liddle C. Nonalcoholic fatty liver disease: pathogenesis and potential for nuclear receptors as therapeutic targets. Mol Pharm. 2008;5:49–59.

268. Moore DD. Nuclear receptors reverse McGarry's vicious cycle to insulin resistance. Cell Metab. 2012;15:615–22.

269. Ratziu V, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. J Hepatol. 2015;62:S65–75.