Toxicology Research

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Toxicol. Res.*, 2018, **7**, 664

Role of xenobiotics in the induction and progression of fatty liver disease

James E. Klaunig, * Xilin Li and Zemin Wang

Non-alcoholic fatty liver disease is a major cause of chronic liver pathology in humans. Fatty liver disease involves the accumulation of hepatocellular fat in hepatocytes that can progress to hepatitis. Steatohepatitis is categorized into alcoholic (ASH) or non-alcoholic (NASH) steatohepatitis based on the etiology of the insult. Both pathologies involve an initial steatosis followed by a progressive inflammation of the liver and eventual hepatic fibrosis (steatohepatitis) and cirrhosis. The involvement of pharmaceuticals and other chemicals in the initiation and progression of fatty liver disease has received increased study. This review will examine not only how xenobiotics initiate hepatic steatosis and steatohepatitis but also how the presence of fatty liver may modify the metabolism and pathologic effects of xenobiotics. The feeding of a high fat diet results in changes in the expression of nuclear receptors that are involved in adaptive and adverse liver effects following xenobiotic exposure. High fat diets also modulate cellular and molecular pathways involved in inflammation, metabolism, oxidative phosphorylation and cell growth. Understanding the role of hepatic steatosis and steatohepatitis on the sequelae of toxic and pathologic changes seen following xenobiotic exposure has importance in defining proper and meaningful human risk characterization of the drugs and other chemical agents.

Received 8th December 2017,
Accepted 9th May 2018

DOI: 10.1039/c7tx00326a

rsc.li/toxicology-research

Pathogenesis and etiology of non-alcoholic fatty liver disease

Etiology

Fatty liver disease is a major public health burden affecting up to one-third of the general population in Western Countries. Fatty liver disease is the result of accumulation of fat (steatosis) in hepatocytes. The diagnosis of steatosis is made when fat in the liver exceeds 5–10% by weight. Fatty liver disease has been classified into two general categories; alcoholic (ASH) or non-alcoholic (NASH) steatohepatitis.[1] In the case of the former, chronic alcohol consumption results in fatty liver due to production of toxic metabolites of alcohol including aldehydes. In NASH, multiple (non-alcohol) factors including nutritional, drugs and hepatic-toxicants and metabolic disorders have been identified in the initiation and progression of the disease. While different in etiology, the pathologic sequelae from the accumulation of hepatocellular fat in alcoholic or non-alcoholic steatosis are commonly accompanied by a progressive inflammation of the liver and resulting hepatic fibrosis (steatohepatitis). Non-alcoholic fatty liver disease consists of a spectrum of liver damage from simple steatosis (non-alcoholic fatty liver disease), to steatohepatitis (NASH), fibrosis and cirrhosis, and hepatocellular carcinoma (Fig. 1). The early stage of fat accumulation is reversible and is characterized by simple steatosis. Continual fat accumulation results in hepatocyte damage, inflammation and fibrosis producing the nonalcoholic steatohepatitis (NASH) phenotype. Non-alcoholic fatty liver disease progresses to NASH in approximately 10% of patients, and cirrhosis in 2–3% (15–25% of those that have NASH) patients usually over a period of 10–20 years.[2] Some patients with advanced NASH may eventually develop hepatocellular carcinoma (HCC). Multiple factors are involved in the progression from simple steatosis to nonalcoholic fatty liver to NASH to cirrhosis including diet, genetic predisposition and the gut microbiota. These factors form the background by which xenobiotics, including drugs and environmental agents, may influence the progression to steatohepatitis through modulation of multiple pathways including oxidative stress, mitochondrial function, fatty acid biosynthesis and inflammation.

Pathogenesis

The pathogenesis of non-alcoholic fatty liver disease is multifactorial. Genetic background, dietary factors, gut microbiota and other factors act simultaneously in the initiation and progression of non-alcoholic fatty liver disease. In addition, recent studies have suggested a link between xenobiotic exposure

Indiana University, School of Public Health, Bloomington, Indiana, USA.
E-mail: jklauni@indiana.edu

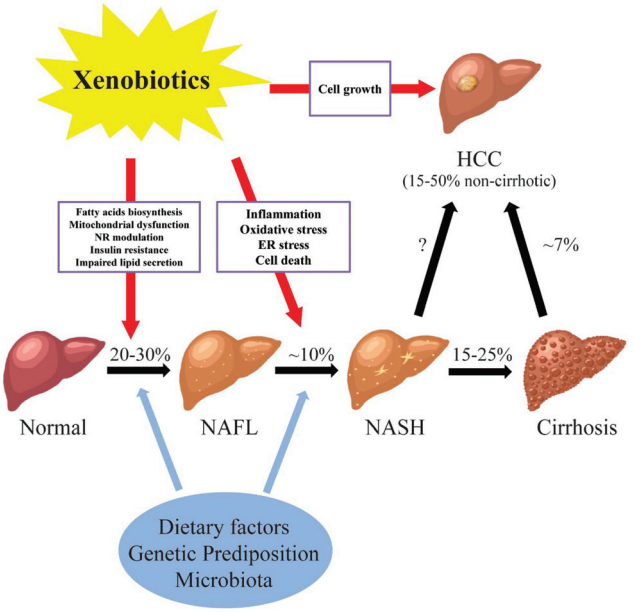

Fig. 1 The potential roles of xenobiotics in non-alcoholic fatty liver diseases. Abbreviations: NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; NR, nuclear receptor; ER, endoplasmic reticulum; % prevalence/incidence; missing value ("?"), the magnitude of non-cirrhotic liver cancer developed from NASH is not well-defined. NAFLD consists of a wide spectrum of liver damages from simple steatosis (NAFL), to steatohepatitis (NASH), fibrosis and cirrhosis, and hepatocellular carcinoma (HCC). The pathogenesis of NAFLD is multi-factorial. Genetic background, dietary factors, gut microbiota and other factors act simultaneously in the initiation and progression of NAFLD. In addition, recently studies have shown the link between xenobiotics and the courses of NAFLD. Liver is the major organ for the metabolism and transportation of drugs and environmental agents. Exposure to these compounds may lead to the lipid accumulation in the liver possibly through increased fatty acid biosynthesis, mitochondrial dysfunction, modulation of nuclear receptors, insulin resistance, and impaired lipid excretion. Moreover, the progression of steatohepatitis from simple steatosis may be induced by xenobiotics *via* oxidative stress, inflammation, ER stress, and cell death. Finally, chemical exposure may stimulate the cell growth in the liver, which further increases the chance of developing HCC in NAFLD.

(including pharmaceuticals and non-pharmaceutical chemicals) and the initiation and progression of the clinical course of non-alcoholic fatty liver disease.[3,4] The liver is the major organ for the metabolism of drugs and environmental agents and it has been demonstrated that exposure to some of these agents may lead to the lipid accumulation in hepatocytes through increased fatty acid biosynthesis, mitochondrial dysfunction, modulation of nuclear receptor activation, insulin resistance, and impaired lipid excretion (Fig. 1). Moreover, the progression from simple steatosis to steatohepatitis may be induced by xenobiotics *via* oxidative stress, inflammation, endoplasmic reticulum (ER) stress, and cell death. Finally, exposure to chemicals may stimulate the cell growth in the liver, which further increases the chance of developing HCC in patients with NASH. The initiation and progression of non-alcoholic fatty liver disease to NASH involves multiple pathways. Initially a 2-hit hypothesis for NASH was proposed.[5] The first hit is hepatic steatosis which sensitizes the hepatocytes to further injury (second hits) from inflammatory cytokines, oxidative stress and mitochondrial dysfunction. The second hit eventually leading to steatohepatitis and fibrosis. The two-hit hypothesis has been expanded to a multi-hit model (the modified two hit hypothesis) which takes into account genetic predisposition, diet, and environmental factors functioning together during the progression of non-alcohol fatty liver disease. As with the two hit hypothesis, a number of parallel mechanisms including insulin resistance, lipotoxicity, inflammation, mitochondria malfunction, oxidative stress, and endoplasmic reticulum stress may all be involved at various stages of the disease.[6,7]

Inflammation

The hallmarks of NASH include inflammation, hepatocyte injury, fibrosis, and cell death. Accumulation of fatty acids in hepatocytes can induce hepatotoxicity exclusive of other pathways. Hepatocytes injured by lipid accumulation can recruit innate immune cells involving Toll-like receptors (TLRs), Kupffer cells, dendritic cells, natural killer cells, lymphocytes, and neutrophils, triggering the inflammatory response, which further promotes simple steatosis to NASH.[8] Changes in the gut microbiota from dietary fat may lead to the dysfunction of the tight junction of epithelial cells in the intestine. This increased gut permeability facilitates the delivery of endotoxin, bacteria, and virus to the liver, and induces inflammation.[9,10] Reactive oxygen species (ROS) generation are also involved in the pathogenesis of NASH. One hypothesis put forth is that free oxygen radicals produced by lipid peroxidation may damage the mitochondria DNA and result in the apoptosis and necrosis of hepatocytes.[11] In addition, accumulated fatty acids in hepatocytes may induce lipotoxicity by generating excessive lipid free radicals that induce oxidative stress. Although rare, hepatocellular neoplasms may be developed from advanced NASH or cirrhosis. In general, the sustained cell death and compensatory proliferation in response to stress and injury in NASH appears to make the liver more susceptible to neoplasm development.[12] In non-alcohol fatty liver disease several molecular mechanisms linked to liver tumor promotion have been described. Cytokines such as TNFα and interleukin 6 (IL-6) released from expanded adipose tissues are key activators of pro-oncogenic signaling pathways (NFκB, JNK, *etc.*). These cytokines, induced by high fat diet (HFD) feeding, have been demonstrated to promote liver tumor formation in diethyl nitrosamine initiated mice.[13] Secondly, Kupffer cells are a key regulator in liver injury and hepatocarcinogenesis.[14] Leptin, a hormone elevated in non-alcohol fatty liver disease, activates Kupffer cells resulting in further inflammatory and fibrogenic factors being released.[15,16] While the endotoxin-mediated inflammatory response in Kupffer cells is normally suppressed by adiponectin,[17] reduced adiponectin mRNA and adiponectin protein levels were found in NASH patients that may amplify the ongoing inflammation.[18]

Fibrosis

Hepatic fibrosis involves the extracellular accumulation of collagen in the sinusoidal and extra hepatocyte areas of the liver lobule. Fibrosis occurs in 10–15% of NASH patients and the presence of fibrosis constitutes a poorer prognosis for liver function and increased mortality.[19–21] Multiple causes for the formation of hepatic fibrosis including excessive alcohol consumption, hepatitis viral infection, and cholestasis have been demonstrated. In contrast, studies that have examined the role of xenobiotic exposure in the induction of hepatic fibrosis is limited.[22] Vinyl chloride is an organochloride that is used as an intermediate in chemical processing and has been linked to hepatotoxicity (hepatomegaly, hepatic fibrosis) as well as hepatic hemangiosarcomas in workers.[23] Vinyl chloride has been shown, using ultrasound, to induce both peri-sinusoidal and perivascular fibrosis in humans. Vinyl chloride is metabolized and detoxified in the liver by CYP2E1, aldehyde dehydrogenase 2 (ALDH2) and glutathione S-transferase theta 1 (GSTT1) enzymes. The genetic polymorphism of CYP2E1 might be associated with the observed idiosyncratic response seen in patients exposed to vinyl chloride.[24] In experimental animal models, carbon tetrachloride has been used as a model compound to induce liver damage and produce a similar hepatic fibrosis to that seen in humans. Carbon tetrachloride, like vinyl chloride is also metabolized in the liver by CYP2E1 resulting in the generation of reactive metabolites that induce oxidative damage and lead to hepatocyte cell death.[25] The resulting hepatocyte necrosis leads to the activation of Kupffer cells and the release of cytokines. This promotes the activation of hepatic stellate cells leading to remodeling of extracellular matrix and fibrosis.[26] Thioacetamide has also been widely used as a model chemical to induce fibrosis in rodents. Mice treated by 100 mg kg^{-1} Thioacetamide 3 times per week for 8 weeks developed hepatic fibrosis accompanied with inflammation and centrilobular necrosis.[27] In rats, administration of thioacetamide also produced fibrotic livers and hepatocyte damage.[28] While not completely understood, the thioacetamide induced hepatic fibrosis in rodents has been associated with increased oxidative damage and lipid peroxidation.[22]

Evidence on the induction of hepatic fibrosis by other chemical agents is limited. Nevirapine, an inhibitor of HIV replication, appears to exacerbate the already present fibrosis in patients with hepatitis C.[29] Nevirapine treatment alone did not induce liver toxicity in rats. However, when co-treated with D-galactosamine which produces liver damage similar to viral hepatitis in humans, Nevirapine activated hepatic stellate cells and induced bridging fibrosis in rats after only eight days.[29] While the mechanism of the Nevirapine effects on fibrosis are not fully understood, apoptotic cell death pathways, toll-like receptors, and maturation of dendritic cells have been suggested as target pathways for the effect. The appearance of liver fibrosis is an important determinant in the morbidity and mortality of patients with NASH and ASH mortality.[20,21] While several chemical agents have been identified as either inducing fibrosis or amplifying the already present hepatic fibrosis, this area of research requires further study. In particular, on how the fibrosis induction and modulation occurs with chemical exposure in fatty liver disease.

Genetic predisposition

Genetic predisposition has also been identified as a potential risk factor that correlates with non-alcohol fatty liver disease susceptibility between individuals. Romeo *et al.* identified a single nucleotide polymorphism in the patatin-like phospholipase domain-containing 3 (PNPLA3) that changes a highly conserved codon 148 from isoleucine to methionine and is significantly correlated with intra-hepatic fat content.[30] Variants in PNPLA3 have been partially explained the susceptibility of non-alcohol fatty liver disease among different ethnic groups, in which Hispanics (G allele frequency: 0.49%) tend to have a higher risk to develop hepatic steatosis whereas African American (G allele frequency: 0.17%) is more protective from non-alcohol fatty liver disease. While the mechanism underlying $PNPLA3^{148M}$ associated hepatic steatosis is not well understood, it appears to modify three pathways: (1) increasing the biosynthesis of triglycerides in the cell due to elevated acetyltransferase activity;[31] (2) reducing the lipolytic ability of the hepatocyte;[32] (3) and depleting of TAG long-chain polyunsaturated fatty acids.[33] In addition, $PNPLA3^{148M}$ appears to modify the profibrogenic capability of hepatic stellate cells (which are involved in the fibrosis seen in NASH).[34] An additional polymorphism, TM6SF2, has also been identified that appears to confer susceptibility to non-alcohol fatty liver disease.[35] TM6SF2 results in an increased hepatic susceptibility to steatosis due to impaired functioning of apolipoprotein B and VLDL secretion.

Diet

The role of diet has been well documented in the initiation and progression of non-alcohol fatty liver disease. Hepatocytes absorb fatty acids proportional to the fatty acid concentrations in the blood. An overabundance of saturated fats, sugars and cholesterol in the diet may lead to the steatosis in the liver. Increased lipid in the hepatocytes has been linked to an increased production of reactive oxygen species, mitochondrial dysfunction, and apoptosis.[36] In addition, excess storage of saturated fat in white adipose tissue can lead to the hypertrophy and hyperplasia of adipocytes, which in turn recruit macrophages and release proinflammatory cytokines into circulation and liver.[37] Besides saturated fats, excess fructose consumption has also been linked to the pathogenesis of non-alcohol fatty liver disease through an increase in lipogenesis and an inhibition of fatty acid β-oxidation. Fructose is converted to fructose-1-phosphate, a precursor for fatty acids synthesis in hepatocytes.[38] Fructose appears to contribute to the changes involved in the progression from simple steatosis to NASH by inducing oxidative stress, causing mitochondrial dysfunction and ER stress.[39] In addition, fructose consumption and fibrosis has also been noted in patients with non-alcohol fatty liver disease.[40] It is important to also note the role of

increase calories in the induction of steatosis. In particular, a hyper caloric diet along with high fat is an important contributor to the NASH in Western Cultures.

Excessive carbohydrates in the diet can activate sterol regulatory element-binding protein-1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP) in the liver, increasing *de novo* fatty acids synthesis.[41] When obesity develops, the overexpression of tumor necrosis factor α (TNFα) in the adipose tissue inhibits phosphorylation of insulin receptor substrates, impairs insulin-mediated suppression of hormone-sensitive lipase, and increases the release of LCFA into circulation.[42] Mitochondrial and peroxisomal β-oxidation is the major pathway of fatty acid catabolism in hepatocytes. Carnitine palmonitoyl transferase 1 (CPT1) and peroxisome proliferator-activated receptor α (PPARα) play important roles in the process. CPT1 catalyzes the transfer of acyl group from cytoplasm into mitochondria, where β-oxidation normally carried out. PPARα regulates the expression of many genes associated with fatty acids transport, oxidation, and synthesis. PPARα null mice develop hepatic steatosis spontaneously and the administration of PPARα activators reverses methionine choline-deficient diet (MCD) induced NASH and fibrosis in mice.[43,44] Lastly, the accumulated fatty acids in the liver are esterified into triglycerides and exported into circulation by VLDL. The biogenesis of VLDL is controlled by mitochondrial triglyceride transfer (MTT). Studies have shown that drugs can modify MTT function that in turn will lead to hepatic steatosis.[45,46]

Rodent models for non-alcohol fatty liver disease

Several rodent models for non-alcohol fatty liver disease have been described that allow for the further investigation of the cellular and molecular changes that occur during the progression from steatosis to cirrhosis. Given the strong association between diet and fatty liver disease in humans, rodent models for the initiation and progression of non-alcohol fatty liver disease have for the most part been diet based. A summary of some of the model diets that have been used for the induction of steatosis and NASH are shown in Table 1. One diet, a methionine choline-deficient diet reproduces the steatosis, inflammation and fibrosis seen in human liver in mice.[47] The lack of methionine and choline in the rodent diet leads to the impaired biosynthesis and secretion of VLDL. This results in the rapid accumulation of lipids in hepatocytes resulting in toxicity, oxidative stress, and inflammation in the liver. However, the methionine choline-deficient diet does not replicate the metabolic profile seen in human non-alcohol fatty liver disease.[48] In patients with non-alcohol fatty liver disease, the metabolic syndrome (obesity and dyslipidemia) is prevalent. However, in the methionine choline-deficient diet model, rodents exhibited a decrease body weight, the absence of insulin resistance, and a decreased triglyceride in the circulation. A modification of the methionine choline-deficient diet using a choline deficient high fat diet (CD-HFD), while not physiological, has been shown to better mimic the liver

Table 1 Dietary models of steatosis and steatohepatitis in rodents

Models	Kcal from fat in diet	Added sugars in water	Treatment duration (week)	Obesity	Insulin resistance	Steatosis	Lobular inflammation	Hepatocytes ballooning	Perisinusoidal fibrosis	HCC	Ref.
MCD	20%	−	Up to 52	−	−	+	+	+	+	−	48
CD-HFD	NP	−	48	+	NP	+	+	+	+	+	49
HFD	60%	−	Up to 50	+	+	+	+	−	+	−	50
HFTFD	58%	+	16	+	+	+	+	+	+	−	58
ALIOS	45%	+	Up to 16	+	+	+	+	+	−	−	54
HFD32	64%	−	60	+	+	+	+	−	+	+	50
AMLN	40%	+	Up to 30	+	+	+	+	+	+	−	56
WD	42%	−	Up to 60	+	+	+	+	−	+	+	52
WD	42%	+	Up to 52	+	+	+	+	+	+	+	58

Abbreviations: HCC, hepatocellular carcinoma; MCD, methionine choline deficient; CD-HFD, choline deficient high fat diet; HFD, high fat diet; HFTFD, high fructose trans-fat diet; ALIOS, American lifestyle induced obesity syndrome; HFD32, high fat diet 32% weight from fat; AMLN, Amylin liver NASH model; WD, western diet; NP, no report.

changes seen in humans with non-alcoholic liver disease in mice.[49]

A second dietary approach in rodent models of nonalcoholic fatty liver disease utilizes a high fat diet. In these models, between 30% to 85% of the calories are derived from fat.[50] The high fat diets produce obesity, insulin resistance, dyslipidemia, and hepatic steatosis. The development of steatohepatitis and fibrosis with these diets depends on the composition of the high fat diet, the test species, and the duration of time on diet. In an effort to replicate the human diet, a high fat diet commonly referred to as the western diet, with 42% of calories from fat, 43% of calories from carbohydrates, and 0.15% cholesterol by weight comparable with that of unhealthy human diet in western countries has been developed. The western diet model has seen increase use in studying NASH.[51–53]

Additional studies have shown that dietary cholesterol and fructose may promote the development of NASH by inducing insulin resistance, oxidative stress, and inflammation.[54] Mice fed with a higher level of cholesterol and fructose in the diet exhibited clinically relevant characteristics of non-alcohol fatty liver disease/NASH seen in humans.[55,56] With the Western diet, simple hepatic steatosis in mice occurs as early as 6 weeks and progresses to NASH and fibrosis in 20–30 weeks.[52,57] With the supplementation of sugar water, Asgharpour *et al.* were able to observe hepatocellular neoplasms in mice fed with western diet at 52 weeks.[58] The metabolic features, histopathology, as well as transcriptomic patterns in the western diet treated mouse liver matches with that seen in human non-alcohol fatty liver disease. Thus, there is strong support that the western diet used in rodent models best approximates the physiopathology and etiology of the spectrum of liver damages induced by non-alcohol fatty liver disease in humans.

Chemical induced non-alcohol fatty liver disease

Drugs

Besides dietary factors, the formation of hepatic steatosis and its progression to NASH has also been linked to the pharmaceuticals and environmental chemicals.[1,4,59,60] Pharmaceuticals drug induced steatosis is one of the more common manifestations of drug induced liver injuries (Table 2). Chronic treatment with amiodarone has produced steatosis in patients through impaired mitochondrial function and inhibition of lipid oxidation.[61] Similarly, long term use of valproic acid, an antiepileptic drug, is associated with fatty liver.[62] Valproic acid induced steatosis appears to correlate with the induction of metabolic disorders leading to fatty acid retention in human liver. Chronic exposure to valproic acid has been shown to increase hepatic steatosis in mice on a high fat diet by impairing mitochondrial β-oxidation.[63] Other drugs such as tamoxifen, tetracycline, methotrexate, and corticosteroids have also been associated with steatosis in the human liver.[4,64–67]

Table 2 Drugs induced steatosis and steatohepatitis

Chemicals	Hepatic effects	Potential mechanism	Ref.
Tamoxifen	Steatosis/ steatohepatitis	Increase fatty acids biosynthesis	65
Amiodarone	Steatosis/ steatohepatitis	Inhibit mitochondrial fatty acid oxidation	55
Valproic acid	Steatosis	Induce metabolic disorders; impair mitochondrial functions	57
Tetracyclines[a]	Steatosis	Upregulate lipogenic genes	61
Methotrexate	Steatosis/ steatohepatitis	Induce oxidative stress	63 and 64
Corticosteroids	Steatosis	Inhibit mitochondrial fatty acid oxidation; increase *de novo* fatty acids synthesis	55

[a] Tetracyclines compounds, including tetracycline, doxycycline and minocycline, may induce acute microvesicular steatosis. Hepatic steatosis and steatohepatitis are important considerations in drug induced liver injury (DILI). Histological features of micro- or macrovesicular steatosis may present after acute or chronic use of these compounds. The potential mechanisms of chemical induced steatosis and steatohepatitis may include the impairment of mitochondrial function, upregulation of *de novo* fatty acids synthesis, induction of lipotoxicity and oxidative stress.

Non-pharmaceutical chemicals

Given the confirmed role for the induction of steatosis and non-alcohol fatty liver disease by selective pharmaceuticals, recent efforts have also focused on the possible linkage environmental chemical exposure and fatty liver changes. Despite this, few studies have been done to show a causal effect of chemicals on NASH. Recently, Cave and colleagues have proposed novel nomenclature to describe the spectrum of toxicant-associated fatty liver diseases (TAFLD) including steatosis, steatohepatitis (TASH).[3] The use of the terms TAFLD and TASH were proposed to highlight the unique differences between the mechanisms underlying the drug-induced liver injury and hepatotoxicity induced by industrial toxicants compared with dietary induced NASH. Many classes of industrial chemicals, including halogenated hydrocarbons, volatile organic mixtures, POPs, pesticides, and some nitro-organic compounds, have been associated with TAFLD.[3] The specific mechanisms of TAFLD/TASH appear in many cases to be chemical-specific. While insulin resistance, proinflammatory cytokines, and apoptotic cell death have been reported in TAFLD/TASH,[68] modulation of nuclear receptors by these environmental and industrial chemicals may also be critical in the onset of steatosis, the progression to steatohepatitis, and the development of fibrosis and hepatocellular carcinoma. The term TASH was first used to describe vinyl chloride induce liver disease in workers. Studies from the Cave group showed that vinyl chloride exposure resulted in impairment of mitochondrial beta oxidation leading to an increased sensitivity of the liver to steatohepatitis from high fat diet.[68] They have proposed that the mitochondrial dysfunction seen in the initial hit with vinyl chloride may be due to the formation of protein

adducts from vinyl chloride metabolites. Metabolomics data from vinyl chloride workers showed increased serum lipid peroxides and altered acyl carnitines consistent with incomplete hepatic lipid peroxidation was also apparent.[69] In addition, a potential role for PPAR receptors was suggested.

Efforts have been made to investigate the mechanisms of environmental chemical induced steatosis and non-alcohol fatty liver disease using rodent models. The Japanese Toxicogenonics Project (TG-GATEs) has included steatosis as an endpoint. In the evaluation of 131 chemicals Using TG-GATEs at multiple doses and time points, 17 chemicals in this database caused steatosis formation in the rat liver.[70] While the underlying mechanism of steatosis induced by each compound may be different, transcriptomic analysis showed genes involved in glucose metabolism, lipid biosynthesis and transportation are modified by the majority of these chemicals.[71] Kaiser *et al.*[72] identified 16 environmental chemicals that have mechanistic support to induce fatty liver in rodents. Mitochondrial malfunction, impaired fatty acid exportation, increased cytokine production, and insulin resistance were suggested as possible mechanisms for the development of steatosis by these chemicals. Interestingly, most of these chemicals share a similar molecular structure with a high degree of chlorination. More recently using two large databases; the Toxicological Reference Database (ToxRefDB) and the Chemical Effects in Biological Systems (CEBS) with the key words fatty change, fatty necrosis, Oil red O-positive staining, steatosis, and lipid deposition, Al-Eryani *et al.* identified 123 chemicals associated with liver steatosis.[73]

Questions remain concerning the potential causal relationship between chemical exposure and the development of non-alcohol fatty liver disease. Treviño and Katz[74] in a recent review suggested a possible linkage between endocrine disruptors and fatty liver diseases in human *via* modulation of xenobiotic nuclear receptors. In addition, the role of these nuclear receptors has been suggested in the pathogenesis of non-alcohol fatty liver disease.[75] Activation of nuclear receptors following xenobiotic exposure results in the induction of phase I and phase II drug-metabolism enzymes.[76,77] Specifically, the peroxisome proliferator activated receptors (PPARs), the pregnane X receptors (PXR), the constitutive androstane receptor (CAR), the liver X receptor (LXR), and the farnesoid X receptor (FXR), and acyl hydrocarbon receptor (AHR) have been shown to participate in non-alcohol fatty liver disease induction.[75,78] These same receptors are activated by xenobiotics in the formation of liver tumors in rodents.[79] The following section reviewed selective nuclear receptor mediated chemicals that have been associated with steatosis and steatohepatitis (Table 3).

Table 3 Selective nuclear receptor mediated chemicals and their effect on NAFLD

Chemicals	Nuclear receptor	Effects on NAFLD	Ref.
Perfluoroalkyl acids (PFOA, PFOS, PFNA, ***etc***.)	PPARα, CAR, PXR	Steatosis, inflammation, cell proliferation	84–87
Phalates(DEHP)	PPARα, PPARγ	Steatosis, inflammation, oxidative stress, cell proliferation	94–97
Dioxins(TCDD)	AhR	Steatosis, inflammation, cell proliferation	98–102
Polychlorinated biphenyls	CAR, PXR, AhR	Steatosis, inflammation	105–108
Cyclodienes	CAR, PXR	Steatosis	109–111
Trichloroethylene and perchloroethylene	PPARα, PPARγ	Steatosis, inflammation, cell cycle	113, 117 and 118
Vinyl chloride	PPARα	Steatosis, inflammation, cell death	69

Abbreviations: PPAR: peroxisome proliferator activated receptors; PXR: the pregnane X receptors, CAR: the constitutive androstane receptor; AhR: aryl hydrocarbon receptor. Modulation of nuclear receptors has been suggested as a potential mechanism underlying the linkage between chemical exposure and NAFLD. The above table summarized some selective nuclear receptor mediated compounds and their effects on NAFLD in rodent studies. PFAAs and DEHP cause extensive micro- and macro-vesicular steatosis, inflammation, oxidative stress and cell proliferation in the liver mainly through the activation of PPARα. TCDD and PCBs may induce steatosis and steatohepatitis in the liver *via* the activation of AhR, CAR, and PXR. TCE and PCE induce steatosis, inflammatory response, and promote cell cycle in the liver. PPARα and PPARγ may play important roles in the pathogenesis. Organochlorine insecticides such as cyclodienes induce fatty changes in the liver after chronic exposure. Activation of CAR/PXR may account for the imbalance of energy homeostasis and lipid accumulation.

Selected chemicals

Perfluoralkyl acids

Perfluoralkyl acids (PFAAs) such as perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorononanoic acid (PFNA) are persistent and widely distributed environmental contaminants.[80] Exposure to PFAAs has been attributed to a wide range of adverse effects in human and animal studies.[80,81] Mechanistically, PFAAs have been shown to activate PPARα which appears to be linked to many of the adverse effects in the rodent liver with exposure.[82,83] The activation of PPARα has been shown to upregulate are critical in hepatic lipid oxidation genes such as CYP4A, acylCoA oxidase 1 (ACOX1), and CPT-1A. The activation of PPARα also enhances the clearance of lipids in the rodent liver.

PFAAs have been shown to cause micro- and macro-vesicular steatosis in the hepatocytes.[84–87] PFOS was found to induce fatty liver in mice in a dose and time dependent manner *via* the upregulation of fatty acid translocase and inhibition of mitochondrial β-oxidation.[88] PFOA also exaggerated high fat diet induced hepatotoxicity as well as lipid accumulation in the liver.[89] In a recent study, short term administration of PFAAs (PFOA, PFNA, and PFHxS) was found to induce hepatic steatosis in mice in a PPARα dependent way.[87] The molecular mechanism underlying the correlation between PFAAs and lipid accumulation is still unresolved. The activation of PPARα leads to an increase of fatty acid oxidation genes in the lipogenic pathway such as CD36, fatty acids synthase, and SREBP-1. An imbalance of this pathway may lead to either an

accumulation of fat or oxidation of fatty acids in hepatocytes.[87] In contrast to the PFAAs, other PPARα activators such as Wy-14643, do not induce hepatic steatosis. However, the PFAAs also activate other xenobiotic nuclear receptors besides PPARα such as CAR and PXR.[90,91] Therefore, the lipogenic effects in the liver following PFAA exposure may possibly be linked to the cross-talk between PPARα and CAR/PXR. Administration of Wy-14643, a potent PPARα agonist, prevented the hepatic steatosis and liver injury in the mouse from dietary administration of a high fat diet.[92] Similarly treatment with the hypolipidemic drug, clofibrate (a PPARα agonist) also decreased high fat diet induced hepatic steatosis and liver inflammation in mice.[93]

Di (2-ethylhexyl) phthalate (DEHP)

Another PPARα activator, di(2-ethylhexyl) phthalate (DEHP), is a common phthalate ester that is widely used as a plasticizer.[94] DEHP induces liver tumors in rodents through the activation of PPARα.[95] Meanwhile, DEHP and its metabolites may also interact PPARγ and in turn affect the regulation of energy metabolism in the liver.[96] DEHP feeding to rats exacerbated the liver effects of concomitantly administered high fat diet to induce non-alcohol fatty liver disease.[97] Similar to the PFAA compounds, DEHP administration induced a dose-dependent modification of gene expression and resulting proteins in both the lipogenesis and lipolysis pathways. Thus, the activation of liver PPARα activation by environmental chemicals remains unclear if this is a beneficial or detrimental effect on the induction and progression of non-alcohol fatty liver disease.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

Exposure to dioxins, specifically, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is associated with abnormal lipid metabolism and diabetes in humans.[98] TCDD is metabolized through acyl hydrocarbon receptor (AhR) mediate enzymes. The disruption of the normal function of AhR may lead to the hepatic steatosis and inflammation.[99] In transgenic mice with constitutively activated AhR, hepatic steatosis developed spontaneously. This was considered to be related to the upregulation of CD36.[100] Short tern administration of TCDD to wild type female mice also produced an increased hepatic steatosis and lipid composition.[101] AhR activation has been shown to also suppress the normal functions of PPARα dependent pathways, resulting in increased insulin resistance and lipid accumulation.[98,102]

Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are polyhalogenated aromatic hydrocarbons that persistent in environment.[103] Epidemiological studies have associated PCBs exposure with obesity, insulin resistance and non-alcohol fatty liver disease.[104,105] PCB126 which possesses dioxin-like AhR activation capability induced fatty change in the liver in female Sprague-Dawley rats. Similarly, PCB126 induced an accumulation of triglycerides in primary cultured hepatocytes through the upregulation of SREBP-1c and microsomal triglyceride transfer protein.[106] PCB126 (similar to TCDD) inhibited PPARα activation and expression of the PPARα downstream genes Acox1 and hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) in rat liver resulting in lipid accumulation in the hepatocytes.[107]

Non TCDD- like PCBs that have limited AhR activity (*e.g.* PCB153, Aroclor 1260) activate the CAR/PXR pathway and inhibit the progression of non-alcohol fatty liver disease. PCB153 treatment alone produced minimal effects on rodent hepatic steatosis but a combination of PCB153 and co administered high fat diet significantly impaired fatty acid β-oxidation and upregulated lipogenic genes.[108] These observations were attributed to the modulation and activation of the nuclear receptors AhR, CAR/PXR by the high fat diet which may make the liver more susceptible to chemically induced toxicities. In contrast to PCB153, Aroclor 1260 increased hepatic inflammation in western diet induced hepatic steatosis in mice.[53] In this study, hepatic *Cyp2b10* and *Cyp3a11* were increased as were an elevation of proinflammatory cytokines release.

Cyclodienes

The cyclodienes are organochlorine insecticides that were widely used but are now banned by EPA due to the adverse effects on human and environmental health. Chronic feeding of cyclodienes to rodents resulted in hepatic steatosis. For example, liver fatty changes were seen in female rats fed the cyclodiene, Chlordecone, for 2 years.[109] Chlordane induced hepatic steatosis in mice after prolonged dietary treatment.[110] Both Chlordane and Chlordecone were found to be activators of CAR/PXR.[111,112] Although not fully understood, the modulation of PXR induced by Chlordecone may partially contribution to the observed fatty changes in the liver through the alterations of cholesterol homeostasis and lipoprotein metabolism.[112]

Tri and tetrachloroethylene

Trichloroethylene (TCE) and Tetrachloroethylene (PERC) are widely used organic solvents and have been reported to induce hepatotoxicity including liver tumors in mice following oral or dermal exposure.[113] TCE activation of PPARα has been suggested to be responsible for the formation of liver tumors in mice.[114] The association between TCE and fatty changes in the liver has been reported.[3] Interestingly, Ramdhan *et al.* showed that TCE increased liver triglyceride levels and steatosis in *Pparα-null* and humanized PPARα mice, but not in m*PPARα* mice.[115] TCE treatment also significantly increased the expression of PPARγ in *Pparα-null* and hPPARα mice and but not in m*PPARα* mice. PPARγ regulates genes in the lipogenesis pathway in the liver, which may account for the observed increase in triglyceride accumulation with TCE treatment.[115] PERC has also been shown to be a PPARα activator[116] and has been associated with altered hepatic lipid metabolism. Increased hepatic triglycerides along with a decrease in serum triglycerides and an induction of *Cyp4a10* and *Acox1* were seen in mice treated with PERC for 24 hours.[117] More recently, non-alcohol fatty liver disease was found to be a susceptibility factor of PERC induced liver effects.[118]

Xenobiotic nuclear receptors

The alteration of drug metabolism enzymes in non-alcohol fatty liver disease is an emerging topic. Studies have shown that both phase I and phase II metabolic enzymes are modulated in models of non-alcohol fatty liver disease.[119] While the mechanism underlying these changes has not been well understood, activation or downregulation of the xenobiotic nuclear receptors has been suggested to be important in regulating the expression of drug metabolism and transport enzymes. Work in our lab showed that nuclear receptors were modulated in the mouse model of the high fat diet induced hepatic steatosis (Table 4). The nuclear receptors modified by the high fat diet are the same receptors involved in drug and other xenobiotic metabolism and activation.[120]

PPARs

The PPAR family of nuclear receptors consists of three members; PPARα, PPARβ/δ, and PPARγ, with PPARα the predominant subtype in hepatocytes.[121] PPARα regulates numerous signaling pathways involving in lipid metabolism in hepatocytes. For example, activation of PPARα increases carnitine palmitoyltransferase 1A (CPT-1A), the rate limiting enzyme in the mitochondrial β-oxidation.[122] The uptake of free fatty acids from circulation to hepatocytes is tightly mediated by CD36, which is also upregulated by PPARα. Other lipid metabolism pathways mediated by PPARα includes: peroxisomal β-oxidation, acyl-CoA formation and hydrolysis, fatty acid biosynthesis, lipoprotein transportation and metabolism, cholesterol transportation, and ketogenesis.[123]

In patients with NASH, the mRNA level of PPARα is negatively correlated with the severity of steatosis and insulin resistance.[124] A decreased in PPARα may result in an impaired function of mitochondrial and peroxisomal β-oxidation, which further leads to the accumulation of fatty acids in the hepatocytes. Elevation of PPARα gene expression correlated with improved liver histopathology of these patients. Thus, the activation of PPARα has been proposed to be a therapeutic target in non-alcohol fatty liver disease. In animal models, PPARα is activated by chronic high fat diet feeding.[125–127] PPARα is considered a nutritional sensor that allows the adaption based on the rate of lipid catabolism or biosynthesis.[128] Thus, the increased long chain fatty acids influx to hepatocytes may lead to the adaptive activation of PPARα.[129] C57BL/6N mice fed by western diet for 12 weeks had a significant higher level of hepatic phosphatidylcholine, which has been demonstrated as the natural ligand of PPARα.[130,131]

The other two members PPARγ and PPARβ/δ are also present in the liver. PPARγ responses to fatty acids, eicosanoids, and various anti-diabetics drugs and regulate lipid homeostasis mainly in the muscles and adipose tissues.[132] In a hepatic steatosis mouse model, PPARγ mRNA showed a significant increase in the liver.[133,134] This increase may contribute to the abnormal accumulation of triglycerides in the liver, while protecting other tissues from insulin resistance. Mice with liver specific *Pparγ-null* showed a reduced level of hepatic steatosis but hyperlipidemia and muscle insulin resistance.[132] PPARβ/δ is a critical regulator of glucose and lipoprotein metabolism in the liver.[135] Information on the role of hepatic PPARβ/δ rodent models of non-alcohol fatty liver disease is limited. However, some studies have shown that activating PPARβ/δ reduces steatosis, inflammation, and liver fibrosis in the mouse liver,[136–138] suggesting the expression and activity of PPARβ/δ may be impaired in non-alcohol fatty liver disease. In contrast to the rodent models of steatosis, Francque and colleagues reported no changes in gene expression of PPARγ and PPARβ/δ in patients with steatosis and steatohepatitis.[124]

CAR/PXR

A role for CAR/PXR in energy metabolism has been demonstrated.[139] PXR can regulate lipid metabolism in the liver through activation of lipogenic genes (such as CD36 and Stearoyl-CoA desaturase-1 (SCD1)) and induce triglyceride accumulation. PXR can also suppress PPARα function, which further impairs β-oxidation in mitochondria. Reports on the role of CAR in mediating lipid homeostasis are less clear. CAR activation has been associated with the induction of Insig-1, which inhibits the translocation of SREBP-1, an important protein in fatty acids biosynthesis.[140] In contrast, CAR may compete with PPARα for the binding of 3-hydroxyacyl-CoA dehydrogenase and thus inhibit β-oxidation.[139] Moreover, the crosstalk between CAR and PXR makes it more difficult to investigate the functions of CAR alone in the lipid metabolism.

Table 4 Gene expression analysis of xenobiotic nuclear receptors and their target genes in a mouse model of NAFLD[a]

Genes	Fold change over control by qPCR	Fold change over control by RNA-seq[b]
Pparα	**2.27**	1.35
Pparγ	**2.24**	**1.72**
Pparδ/β	**−3.85**	**−2.02**
Cyp4a10	**2.58**	1.25
Car (Nr1i3)	1.48	**1.72**
Pxr (Nr1i2)	**1.56**	1.23
Cyp2b10	21.92	**3.27**
Cyp3a11	**4.20**	**1.83**
Ahr	**2.30**	1.42
Cyp1a1	1.76	1.36
Cyp1a2	**−1.69**	**−1.86**
Lxrα	−1.02	−1.11
Abcg5	**4.08**	**2.42**
Abcg8	**3.58**	**2.06**
Fxr	1.14	1.12
Abcb4	**2.12**	**1.89**
Abcb11	1.11	−1.36

[a] Fatty and normal liver samples were generated from C57BL/6 mice fed with western diet or low-fat control diet for 16 weeks, respectively. [b] Differential expression analysis was done using DESeq-2 package in R. Bold: Statistically different from controls (for qPCR analysis: N = 4–6; student two-tailed t-test, $p < 0.05$; for RNA-seq: $N = 4$; fold change >1.5; adjusted $p < 0.05$). **Study design**: male C57BL/6 (5–6 weeks old) mice were randomly assigned to two groups fed with either control (13.5% calories from fat; TD.120336 Harlan Teklad) or HFD (42% calories from fat; TD.09682 Harlan Teklad). Animals were allowed *ad libitum* to food and water. Liver tissues were sampled at 16 weeks of treatment.

CAR/PXR regulates the expression of CYP3A, which has been reported to be altered in both human and rodent non-alcohol fatty liver disease. A pilot study conducted by Kolwankar *et al.* suggested that hepatic CYP3A4 activity was inversely associated with the severity of steatosis in patients.[141] Subsequently, Fisher *et al.* found no change in the CYP3A4 mRNA level in human fatty liver samples, although a marginal decrease of protein level of CYP3A4 was seen.[142] More recently, biopsies of patients compared to healthy controls.[143] CYP3A mRNA and protein level was decreased in high-fat diet fed rats.[144,145] In the mouse, high fat diet induced changes to mRNA and enzyme activity of *Cyp3a11* showed inconsistent results. Ning & Jeong using a western diet (~42% fat) reported on a significant increase in *Cyp3a11* mRNA and activity level in mice.[146] Kirpich *et al.* using a "pure" high fat diet (~60% fat) reported on the suppression of *Cyp3a11* gene expression.[147] Besides high fat content, the western diet model also contains high levels cholesterol. Previous study showed that high cholesterol diet significantly increased hepatic *Cyp3a11* level in wild-type but not *Pxr-null* mice, suggesting that dietary cholesterol is a ligand of PXR.[148] In a study from our laboratory using the western diet (Table 4), *Pxr* and *Cyp3a11* mRNA level in the liver of mice were significantly higher than that in controls. Therefore, the dietary content of cholesterol may influence the activation of PXR and more work is needed to clarify the modulation of PXR and its downstream *Cyp3a11* induction in high fat diet models for fatty liver disease.

CYP2B is the prototypical marker of CAR/PXR activation in the liver. Limited studies have reported the linkage between non-alcohol fatty liver disease and CYP2B6 expression in humans.[119] Concordantly, there is also no robust evidence on the modification of Cyp2b activity in rat models of non-alcohol fatty liver disease.[149,150] In mice, the induction of *Cyp2b10* gene expression and enzymatic activity is inconsistent among studies. Similar with previous findings, our data suggested *Cyp2b10* was marginally induced by western diet feeding (Table 5). Interestingly, the trend of *Cyp2b10* and *Cyp3a11* modulation tends to be comparable within a single study,[147,151] indicating the cross-talk between CAR and PXR upon the effects of dietary components.[152]

AHR

As a major functional member in CYP1A subfamily, CYP1A2 constitutes about 15% of total hepatic CYP enzymes.[153] It is responsible for the metabolism of a variety of drugs including antipsychotics, antihistamines, β-blockers, cyclooxygenase-2 inhibitors.[154] CYP1A2 also is important in the biotransformation of a number of environmental toxicants including aflatoxin B1 and aromatic/heterocyclic amines.[155] The inhibition of CYP1A2 in fatty liver has been consistently shown in human studies. Fisher and colleagues reported a significant decrease of CYP1A2 protein levels as well as microsomal activity with non-alcohol fatty liver disease progression.[142] In cirrhotic livers, the CYP1A2 protein levels decreased to 50% of that of non-cirrhotic liver.[156] In rodents, high fat diets also consistently decrease *Cyp1a2* mRNA and protein level.[146,147] However, the alteration of hepatic *Cyp1a2* in mice on the MCD diet or with transgenic mouse models of non-alcohol fatty liver disease has been inconsistent.[119] AHR is a major upstream inducer of CYP1A2. Activation of AhR by TCDD caused hepatic steatosis in rodents, likely due to the upregulation of CD36.[100] The mRNA level of *Cyp1a2* was also significantly increased with the activation of AhR. Recently, treatment with α-naphthoflavone (an antagonist of AhR) prevented high fat western diet induced obesity and fatty liver in male and female C57BL/6 mice.[157] While the mRNA or protein level of *Cyp1a2* was not reported in this study, the authors suggested that *Cyp1b1* (also mediated by AhR) may be critical in modulation the lipid metabolism in non-alcohol fatty liver disease. These studies suggest that AhR activation (and the resulting increased CYP1A2 expression) is linked to the modulation of hepatic steatosis however more work is needed to understand the observed paradox.

Table 5 Ingenuity pathway analysis predicts hepatic xenobiotic nuclear receptor activation in a mouse model of NAFLD[a]

Upstream nuclear receptor	Predicted activity	Activation *z*-score	*p*-Value
PPARα	+++	4.159	1.08×10^{-50}
PPARγ	+	1.342	5.73×10^{-15}
PPARδ	+++	2.815	9.53×10^{-15}
CAR	+	0.972	1.71×10^{-13}
PXR	+++	3.153	1.13×10^{-19}
AHR	-	-1.740	3.67×10^{-16}
LXR	+	0.886	8.89×10^{-9}
FXR	+	0.943	1.41×10^{-10}

[a] Steatoic and normal liver samples were generated from C57BL/6 mice fed with western diet for 16 weeks. RNA-seq (4 samples per group) was done to profile the whole transcriptome of these samples. Differential expression analysis was done using DESeq-2 package in R. Ingenuity pathway analysis was done to predict the activation of xenobiotic nuclear receptors based on differential expression analysis results. Activation *z*-score and *p*-value were calculated by the build-in algorithms in IPA. +++: strong activation; +: weak activation; –: weak inhibition.

LXR/FXR

LXR has two isoforms LXRα and LXRβ, with LXRα predominantly expressed in the liver.[158] LXR is associated with non-alcohol fatty liver disease because (1) it controls the expression of lipogenic genes such as SREBP-1 and SCD-1, and thus promotes fatty acids biosynthesis; (2) LXR is a regulates cholesterol excretion of the liver *via Abcg5*/*8*; (3) LXR upregulates CYP7A1 and facilitate cholesterol degradation in bile acids.[159] In humans, LXR expression has been correlated with the degree of hepatic fat deposition, as well as with hepatic inflammation and fibrosis.[160] In rat fed with HFD, the elevated levels of *Lxrα* and *Lxrβ* as well as their target genes *Abca1* and *Abcg5* were seen at the stage of hepatic steatosis.[161] Concordantly, increased *Lxrα* mRNA level has been observed in mice fed with high fat high sucrose diet as early as 2 weeks.[162] In high fat high fructose induced non-alcohol fatty liver disease in mice, *Abca1* was also increased after prolonged treatment.[163] Taken

together, evidence strongly support that LXR is activated during the progression of non-alcohol fatty liver disease.

FXR plays critical roles in the bile acids and cholesterol metabolism. Both conjugated and unconjugated forms of bile acids are the endogenous ligands of FXR.[164] It should be noted that FXR is expressed in stellate cells in human and rodent livers. The activation of FXR not only suppresses obesity and hepatic steatosis but also inhibits the formation of extracellular matrix in rodent models.[165,166] Currently, limited study reported FXR and its downstream ATP-binding cassette transporters in dietary models of NAFLD in rodents. In rat, high fat diet seemed to significantly increase the expression of *Fxr* as well as *Abcb11* and *Abcb4*.[161]

Gene expression analysis of hepatic nuclear receptors

Recent studies in our laboratory have examined the changes in nuclear receptor in a mouse model with high fat diet treatment (Table 4).[120] In this study male C57BL/6 were placed randomly into two groups consisting of a control diet (13.5% calories from fat) or a high fat diet (42% calories from fat). Animals were allowed food and water *ad libitum* and mice from each group were sampled after 16 weeks on dietary treatment. Histopathology and receptor activation analysis was performed as well as measurements for steatosis. In this model, sequential hepatic steatosis (8 weeks onwards) was seen along with increased hepatocytes DNA synthesis, lobular inflammation (16–52 weeks), and fibrosis (24–52 weeks).[120] The expression of PPARα, CAR/PXR, AHR, LXR, FXR and their target genes were measured in the livers of mice fed with control and high fat diet for 16 weeks (Table 4). We observed the activation of PPARα, CAR/PXR, and LXR in mice with hepatic steatosis. Consistent with previous results, AHR mediated *Cyp1a2* was significantly downregulated in high fat diet mice compared to controls. In addition, moderate induction of FXR mediated *Abcb4* was seen, suggesting a modification of bile acid biosynthesis and transport. Ingenuity pathway analysis based on RNA-seq results confirmed the modulation of nuclear receptors in mice with NAFLD compared to controls (Table 5).

Conclusions

Fatty liver disease involves the accumulation of hepatocellular fat from either alcoholic or non-alcoholic derived steatosis. Non-alcoholic fatty liver disease is a major cause of chronic liver disease in humans and is the predominantly the result of an increased supply of fat to the liver from dietary sources. A number of drugs and other chemicals have been shown to induce steatosis and subsequently steato fibrosis. Many of the same hepatic pathways, including metabolism, inflammation, and cell growth that are targeted by xenobiotic treatment are also modified in fatty liver disease. Based on the published literature and our preliminary results (Tables 4 and 5) it is apparent that nuclear receptor activation is definitely modify during the progression of the steatosis to steatohepatitis. Thus, the presence of fatty liver disease prior to exposure to drugs and other chemicals may modify the usual pattern of adaptive and adverse cellular and molecular effects of these xenobiotics on the liver. Conversely, exposure to xenobiotics may modify the progression and pathology of the non-alcoholic fatty liver disease resulting in either an enhancement or reduction of the toxicopathologic effects. Understanding the way that fatty liver changes modify hepatic pathways, in particular those dependent on nuclear receptor activation, are important in understanding how the presence of the fatty changes may modify the potential human risk of a drug or environmental chemical.

Conflicts of interest

There are no conflicts to declare for this manuscript.

References

1 S. Joshi-Barve, I. Kirpich, M. C. Cave, L. S. Marsano and C. J. McClain, Alcoholic, nonalcoholic, and toxicant-associated steatohepatitis: mechanistic similarities and differences, *Cell. Mol. Gastroenterol. Hepatol.*, 2015, **1**, 356–367.
2 M. E. Rinella and A. J. Sanyal, Management of NAFLD: a stage-based approach, *Nat. Rev. Gastroenterol. Hepatol.*, 2016, **13**, 196–205.
3 B. Wahlang, J. I. Beier, H. B. Clair, H. J. Bellis-Jones, K. C. Falkner, C. J. McClain and M. C. Cave, Toxicant-associated Steatohepatitis, *Toxicol. Pathol.*, 2013, **41**, 343–360.
4 L. Rabinowich and O. Shibolet, Drug induced steatohepatitis: an uncommon culprit of a common disease, *BioMed Res. Int.*, 2015, **2015**, 168905.
5 C. P. Day and O. F. James, *Gastroenterology*, 1998, **114**, 842–845.
6 A. Takaki, D. Kawai and K. Yamamoto, Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH), *Int. J. Mol. Sci.*, 2013, **14**, 20704–20728.
7 E. Buzzetti, M. Pinzani and E. A. Tsochatzis, The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD), *Metabolism*, 2016, **65**, 1038–1048.
8 M. Arrese, D. Cabrera, A. M. Kalergis and A. E. Feldstein, Innate immunity and inflammation in NAFLD/NASH, *Dig. Dis. Sci.*, 2016, **61**, 1294–1303.
9 C. Leung, L. Rivera, J. B. Furness and P. W. Angus, The role of the gut microbiota in NAFLD, *Nat. Rev. Gastroenterol. Hepatol.*, 2016, **13**, 412–425.
10 S. Bashiardes, H. Shapiro, S. Rozin, O. Shibolet and E. Elinav, Non-alcoholic fatty liver and the gut microbiota, *Mol. Metab.*, 2016, **5**, 782–794.

11 A. Takaki, D. Kawai and K. Yamamoto, Multiple Hits, Including Oxidative Stress, as Pathogenesis and Treatment Target in Non-Alcoholic Steatohepatitis (NASH), *Int. J. Mol. Sci.*, 2013, **14**, 20704–20728.
12 G. Baffy, E. M. Brunt and S. H. Caldwell, Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace, *J. Hepatol.*, 2012, **56**, 1384–1391.
13 E. J. Park, J. H. Lee, G. Y. Yu, G. He, S. R. Ali, R. G. Holzer, C. H. Osterreicher, H. Takahashi and M. Karin, Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression, *Cell*, 2010, **140**, 197–208.
14 R. A. Roberts, P. E. Ganey, C. Ju, L. M. Kamendulis, I. Rusyn and J. E. Klaunig, Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis, *Toxicol. Sci.*, 2006, **96**, 2–15.
15 P. Angulo, L. M. Alba, L. M. Petrovic, L. A. Adams, K. D. Lindor and M. D. Jensen, Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease, *J. Hepatol.*, 2004, **41**, 943–949.
16 S. Aleffi, I. Petrai, C. Bertolani, M. Parola, S. Colombatto, E. Novo, F. Vizzutti, F. A. Anania, S. Milani and K. Rombouts, Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells, *Hepatology*, 2005, **42**, 1339–1348.
17 V. Thakur, M. T. Pritchard, M. R. McMullen and L. E. Nagy, Adiponectin normalizes LPS-stimulated TNF-alpha production by rat Kupffer cells after chronic ethanol feeding, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2006, **290**, G998–1007.
18 S. Kaser, A. Moschen, A. Cayon, A. Kaser, J. Crespo, F. Pons-Romero, C. F. Ebenbichler, J. R. Patsch and H. Tilg, Adiponectin and its receptors in non-alcoholic steatohepatitis, *Gut*, 2005, **54**, 117–121.
19 E. M. Brunt, D. E. Kleiner, L. A. Wilson, P. Belt and B. A. Neuschwander-Tetri, Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings, *Hepatology*, 2011, **53**, 810–820.
20 P. Angulo, D. E. Kleiner, S. Dam-Larsen, L. A. Adams, E. S. Bjornsson, P. Charatcharoenwitthaya, P. R. Mills, J. C. Keach, H. D. Lafferty, A. Stahler, S. Haflidadottir and F. Bendtsen, Liver Fibrosis, but no Other Histologic Features, Associates with Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease, *Gastroenterology*, 2015, **149**, 389–397.
21 Z. M. Younossi, M. Stepanova, N. Rafiq, L. Henry, R. Loomba, H. Makhlouf and Z. Goodman, Nonalcoholic steatofibrosis independently predicts mortality in nonalcoholic fatty liver disease, *Hepatol. Commun.*, 2017, **1**, 421–428.
22 S. C. Yanguas, B. Cogliati, J. Willebrords, M. Maes, I. Colle, B. van den Bossche, C. de Oliveira, W. Andraus, V. A. F. Alves, I. Leclercq and M. Vinken, Experimental models of liver fibrosis, *Arch. Toxicol.*, 2016, **90**, 1025–1048.
23 T. J. Hsiao, J. D. Wang, P. M. Yang, P. C. Yang and T. J. Cheng, Liver fibrosis in asymptomatic polyvinyl chloride workers, *J. Occup. Environ. Med.*, 2004, **46**, 962–966.
24 H. I. Hsieh, P. C. Chen, R. H. Wong, J. D. Wang, P. M. Yang and T. J. Cheng, Effect of the CYP2E1 genotype on vinyl chloride monomer-induced liver fibrosis among polyvinyl chloride workers, *Toxicology*, 2007, **239**, 34–44.
25 L. W. Weber, M. Boll and A. Stampfl, Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, *Crit. Rev. Toxicol.*, 2003, **33**, 105–136.
26 K. Iwaisako, C. Jiang, M. Zhang, M. Cong, T. J. Moore-Morris, T. J. Park, X. Liu, J. Xu, P. Wang, Y. H. Paik, F. Meng, M. Asagiri, L. A. Murray, A. F. Hofmann, T. Iida, C. K. Glass, D. A. Brenner and T. Kisseleva, Origin of myofibroblasts in the fibrotic liver in mice, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, E3297–E3305.
27 I. S. Chen, Y. C. Chen, C. H. Chou, R. F. Chuang, L. Y. Sheen and C. H. Chiu, Hepatoprotection of silymarin against thioacetamide-induced chronic liver fibrosis, *J. Sci. Food Agric.*, 2012, **92**, 1441–1447.
28 D. Qin, Y. Nie and Z. Wen, Protection of rats from thioacetamide-induced hepatic fibrosis by the extracts of a traditional Uighur medicine *Cichorium glandulosum*, *Iran. J. Basic Med. Sci.*, 2014, **17**, 879–885.
29 H. R. Brown, S. Castellino, M. R. Groseclose, C. S. Elangbam, K. Mellon-Kusibab, L. W. Yoon, L. D. Gates, D. L. Krull, N. F. Cariello, L. Arrington-Brown, T. Tillman, S. Fowler, V. Shah, D. Bailey and R. T. Miller, Drug-induced Liver Fibrosis: Testing Nevirapine in a Viral-like Liver Setting Using Histopathology, MALDI IMS, and Gene Expression, *Toxicol. Pathol.*, 2016, **44**, 112–131.
30 S. Romeo, J. Kozlitina, C. Xing, A. Pertsemlidis, D. Cox, L. A. Pennacchio, E. Boerwinkle, J. C. Cohen and H. H. Hobbs, Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease, *Nat. Genet.*, 2008, **40**, 1461–1465.
31 M. Kumari, G. Schoiswohl, C. Chitraju, M. Paar, I. Cornaciu, A. Y. Rangrez, N. Wongsiriroj, H. M. Nagy, P. T. Ivanova, S. A. Scott, O. Knittelfelder, G. N. Rechberger, R. Birner-Gruenberger, S. Eder, H. A. Brown, G. Haemmerle, M. Oberer, A. Lass, E. E. Kershaw, R. Zimmermann and R. Zechner, Adiponutrin Functions as a Nutritionally Regulated Lysophosphatidic Acid Acyltransferase, *Cell Metab.*, 2012, **15**, 691–702.
32 J. W. Wu, H. Yang and G. A. Mitchell, Potential mechanism underlying the PNPLA3(I) (148) (M) -Hepatic steatosis connection, *Hepatology*, 2016, **63**, 676–677.
33 J. Z. Li, Y. Huang, R. Karaman, P. T. Ivanova, H. A. Brown, T. Roddy, J. Castro-Perez, J. C. Cohen and H. H. Hobbs, Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis, *J. Clin. Invest.*, 2012, **122**, 4130–4144.
34 F. V. Bruschi, T. Claudel, M. Tardelli, A. Caligiuri, T. M. Stulnig, F. Marra and M. Trauner, The PNPLA3

I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells, *Hepatology*, 2017, **65**, 1875–1890.

35 J. Kozlitina, E. Smagris, S. Stender, B. G. Nordestgaard, H. H. Zhou, A. Tybjærg-Hansen, T. F. Vogt, H. H. Hobbs and J. C. Cohen, Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease, *Nat. Genet.*, 2014, **46**, 352–356.

36 M. V. Machado, P. Ravasco, L. Jesus, P. Marques-Vidal, C. R. Oliveira, T. Proença, I. Baldeiras, M. E. Camilo and H. Cortez-Pinto, Blood oxidative stress markers in non-alcoholic steatohepatitis and how it correlates with diet, *Scand. J. Gastroenterol.*, 2008, **43**, 95–102.

37 M. Funaki, Saturated fatty acids and insulin resistance, *J. Med. Invest.*, 2009, **56**, 88–92.

38 X. Ouyang, P. Cirillo, Y. Sautin, S. McCall, J. L. Bruchette, A. M. Diehl, R. J. Johnson and M. F. Abdelmalek, Fructose consumption as a risk factor for non-alcoholic fatty liver disease, *J. Hepatol.*, 2008, **48**, 993–999.

39 P. Jegatheesan and J. P. De Bandt, Fructose and NAFLD: The Multifaceted Aspects of Fructose Metabolism, *Nutrients*, 2017, **9**, 230.

40 M. F. Abdelmalek, A. Suzuki, C. Guy, A. Unalp-Arida, R. Colvin, R. J. Johnson and A. M. Diehl, Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease, *Hepatology*, 2010, **51**, 1961–1971.

41 R. Dentin, J. Girard and C. Postic, Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver, *Biochimie*, 2005, **87**, 81–86.

42 Q. M. Anstee and R. D. Goldin, Mouse models in non-alcoholic fatty liver disease and steatohepatitis research, *Int. J. Exp. Pathol.*, 2006, **87**, 1–16.

43 M. Sambasiva Rao and J. K. Reddy, PPARα in the pathogenesis of fatty liver disease, *Hepatology*, 2004, **40**, 783–786.

44 M. Rao, K. Papreddy, S. Musunuri and A. Okonkwo, Prevention/reversal of choline deficiency-induced steatohepatitis by a peroxisome proliferator-activated receptor alpha ligand in rats, *In Vivo*, 2001, **16**, 145–152.

45 Z. Chen, E. P. Newberry, J. Y. Norris, Y. Xie, J. Luo, S. M. Kennedy and N. O. Davidson, ApoB100 is required for increased VLDL-triglyceride secretion by microsomal triglyceride transfer protein in ob/ob mice, *J. Lipid Res.*, 2008, **49**, 2013–2022.

46 N. Berriot-Varoqueaux, L. Aggerbeck, M.-E. Samson-Bouma and J. Wetterau, The role of the microsomal triglygeride transfer protein in abetalipoproteinemia, *Annu. Rev. Nutr.*, 2000, **20**, 663–697.

47 E. Marcolin, L. F. Forgiarini, J. Tieppo, A. S. Dias, L. A. Rodrigues de Freitas and N. P. Marroni, Methionine- and choline-deficient diet induces hepatic changes characteristic of non-alcoholic steatohepatitis, *Arq. Gastroenterol.*, 2011, **48**, 72–79.

48 H. Itagaki, K. Shimizu, S. Morikawa, K. Ogawa and T. Ezaki, Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice, *Int. J. Clin. Exp. Pathol.*, 2013, **6**, 2683–2696.

49 M. J. Wolf, A. Adili, K. Piotrowitz, Z. Abdullah, Y. Boege, K. Stemmer, M. Ringelhan, N. Simonavicius, M. Egger, D. Wohlleber, A. Lorentzen, C. Einer, S. Schulz, T. Clavel, U. Protzer, C. Thiele, H. Zischka, H. Moch, M. Tschop, A. V. Tumanov, D. Haller, K. Unger, M. Karin, M. Kopf, P. Knolle, A. Weber and M. Heikenwalder, Metabolic activation of intrahepatic CD8+T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes, *Cancer Cell*, 2014, **26**, 549–564.

50 Y. Takahashi, Y. Soejima and T. Fukusato, Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, *World J. Gastroenterol.*, 2012, **18**, 2300–2308.

51 L. J. Dixon, C. A. Flask, B. G. Papouchado, A. E. Feldstein and L. E. Nagy, Caspase-1 as a Central Regulator of High Fat Diet-Induced Non-Alcoholic Steatohepatitis, *PLoS One*, 2013, **8**, 10.

52 M. N. VanSaun, I. K. Lee, M. K. Washington, L. Matrisian and D. L. Gorden, High Fat Diet Induced Hepatic Steatosis Establishes a Permissive Microenvironment for Colorectal Metastases and Promotes Primary Dysplasia in a Murine Model, *Am. J. Pathol.*, 2009, **175**, 355–364.

53 B. Wahlang, M. Song, J. I. Beier, K. Cameron Falkner, L. Al-Eryani, H. B. Clair, R. A. Prough, T. S. Osborne, D. E. Malarkey, J. Christopher States and M. C. Cave, Evaluation of Aroclor 1260 exposure in a mouse model of diet-induced obesity and non-alcoholic fatty liver disease, *Toxicol. Appl. Pharmacol.*, 2014, **279**, 380–390.

54 L. H. Tetri, M. Basaranoglu, E. M. Brunt, L. M. Yerian and B. A. Neuschwander-Tetri, Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2008, **295**, G987–G995.

55 M. Charlton, A. Krishnan, K. Viker, S. Sanderson, S. Cazanave, A. McConico, H. Masuoko and G. Gores, Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition (vol 301, pg G825, 2011), *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2015, **308**, G159–G159.

56 J. R. Clapper, M. D. Hendricks, G. Gu, C. Wittmer, C. S. Dolman, J. Herich, J. Athanacio, C. Villescaz, S. S. Ghosh, J. S. Heilig, C. Lowe and J. D. Roth, Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2013, **305**, G483–G495.

57 Z. Z. Li, M. Berk, T. M. McIntyre and A. E. Feldstein, Hepatic Lipid Partitioning and Liver Damage in Nonalcoholic Fatty Liver Disease role of stearoyl-CoA desaturase, *J. Biol. Chem.*, 2009, **284**, 5637–5644.

58 A. Asgharpour, S. C. Cazanave, T. Pacana, M. Seneshaw, R. Vincent, B. A. Banini, D. P. Kumar, K. Daita, H.-K. Min and F. Mirshahi, A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer, *J. Hepatol.*, 2016, **65**, 579–588.

59 L. Al-Eryani, B. Wahlang, K. Falkner, J. Guardiola, H. Clair, R. Prough and M. Cave, Identification of environmental chemicals associated with the development of toxicant-associated fatty liver disease in rodents, *Toxicol. Pathol.*, 2015, **43**, 482–497.

60 J. J. Heindel, B. Blumberg, M. Cave, R. Machtinger, A. Mantovani, M. A. Mendez, A. Nadal, P. Palanza, G. Panzica and R. Sargis, Metabolism disrupting chemicals and metabolic disorders, *Reprod. Toxicol.*, 2017, **68**, 3–33.

61 J. M. Kneeman, J. Misdraji and K. E. Corey, Secondary causes of nonalcoholic fatty liver disease, *Ther. Adv. Gastroenterol.*, 2012, **5**, 199–207.

62 E. Farinelli, D. Giampaoli, A. Cenciarini, E. Cercado and A. Verrotti, Valproic acid and nonalcoholic fatty liver disease: A possible association?, *World J. Hepatol.*, 2015, **7**, 1251–1257.

63 L. F. Zhang, L. S. Liu, X. M. Chu, H. Xie, L. J. Cao, C. Guo, J. Y. A, B. Cao, M. J. Li, G. J. Wang and H. P. Hao, Combined effects of a high-fat diet and chronic valproic acid treatment on hepatic steatosis and hepatotoxicity in rats, *Acta Pharmacol. Sin.*, 2014, **35**, 363–372.

64 S. Antherieu, A. Rogue, B. Fromenty, A. Guillouzo and M. A. Robin, Induction of vesicular steatosis by amiodarone and tetracycline is associated with up-regulation of lipogenic genes in HepaRG cells, *Hepatology*, 2011, **53**, 1895–1905.

65 M. López-Riera, I. Conde, L. Tolosa, Á. Zaragoza, J. V. Castell, M. J. Gómez-Lechón and R. Jover, New microRNA Biomarkers for Drug-Induced Steatosis and Their Potential to Predict the Contribution of Drugs to Non-alcoholic Fatty Liver Disease, *Front. Pharmacol.*, 2017, **8**, 3.

66 R. Sakthiswary, G. Y. L. Chan, E. T. Koh, K. P. Leong and B. Y. H. Thong, Methotrexate-associated nonalcoholic fatty liver disease with transaminitis in rheumatoid arthritis, *Sci. World J.*, 2014, **2014**, 823763.

67 H.-J. Pan, H.-T. Chang and C.-H. Lee, Association between tamoxifen treatment and the development of different stages of nonalcoholic fatty liver disease among breast cancer patients, *J. Formosan Med. Assoc.*, 2016, **115**, 411–417.

68 M. Cave, K. C. Falkner, M. Ray, S. Joshi-Barve, G. Brock, R. Khan, M. Bon Homme and C. J. McClain, Toxicant-associated steatohepatitis in vinyl chloride workers, *Hepatology*, 2010, **51**, 474–481.

69 J. J. Guardiola, J. I. Beier, K. C. Falkner, B. Wheeler, C. J. McClain and M. Cave, Occupational exposures at a polyvinyl chloride production facility are associated with significant changes to the plasma metabolome, *Toxicol. Appl. Pharmacol.*, 2016, **313**, 47–56.

70 Y. Igarashi, N. Nakatsu, T. Yamashita, A. Ono, Y. Ohno, T. Urushidani and H. Yamada, Open TG-GATEs: a large-scale toxicogenomics database, *Nucleic Acids Res.*, 2014, **43**, D921–D927.

71 N. Sahini, S. Selvaraj and J. Borlak, Whole genome transcript profiling of drug induced steatosis in rats reveals a gene signature predictive of outcome, *PLoS One*, 2014, **9**, e114085.

72 J. P. Kaiser, J. C. Lipscomb and S. C. Wesselkamper, Putative Mechanisms of Environmental Chemical–Induced Steatosis, *Int. J. Toxicol.*, 2012, **31**, 551–563.

73 L. Al-Eryani, B. Wahlang, K. Falkner, J. J. Guardiola, H. Clair, R. Prough and M. Cave, Identification of environmental chemicals associated with the development of toxicant associated fatty liver disease in rodents, *Toxicol. Pathol.*, 2015, **43**, 482–497.

74 L. S. Treviño and T. A. Katz, Endocrine Disruptors and Developmental Origins of Nonalcoholic Fatty Liver Disease, *Endocrinology*, 2017, **159**, 20–31.

75 M. C. Cave, H. B. Clair, J. E. Hardesty, K. C. Falkner, W. Feng, B. J. Clark, J. Sidey, H. Shi, B. A. Aqel, C. J. McClain and R. A. Prough, Nuclear receptors and nonalcoholic fatty liver disease, *Biochim. Biophys. Acta*, 2016, **1859**, 1083–1099.

76 C. Handschin and U. A. Meyer, Induction of drug metabolism: the role of nuclear receptors, *Pharmacol. Rev.*, 2003, **55**, 649–673.

77 C. Prakash, B. Zuniga, C. S. Song, S. Jiang, J. Cropper, S. Park and B. Chatterjee, Nuclear Receptors in Drug Metabolism, Drug Response and Drug Interactions, *Nucl. Recept. Res.*, 2015, **2**, 101178.

78 J. H. Lee, T. Wada, M. Febbraio, J. He, T. Matsubara, M. J. Lee, F. J. Gonzalez and W. Xie, A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis, *Gastroenterology*, 2010, **139**, 653–663.

79 J. C. Corton, M. L. Cunningham, B. T. Hummer, C. Lau, B. Meek, J. M. Peters, J. A. Popp, L. Rhomberg, J. Seed and J. E. Klaunig, Mode of action framework analysis for receptor-mediated toxicity: The peroxisome proliferator-activated receptor alpha (PPARalpha) as a case study, *Crit. Rev. Toxicol.*, 2014, **44**, 1–49.

80 C. Lau, K. Anitole, C. Hodes, D. Lai, A. Pfahles-Hutchens and J. Seed, Perfluoroalkyl acids: a review of monitoring and toxicological findings, *Toxicol. Sci.*, 2007, **99**, 366–394.

81 E. T. Chang, H. O. Adami, P. Boffetta, P. Cole, T. B. Starr and J. S. Mandel, A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and cancer risk in humans, *Crit. Rev. Toxicol.*, 2014, **44**(Suppl 1), 1–81.

82 J. E. Klaunig, M. A. Babich, K. P. Baetcke, J. C. Cook, J. C. Corton, R. M. David, J. G. DeLuca, D. Y. Lai, R. H. McKee and J. M. Peters, PPARα agonist-induced rodent tumors: modes of action and human relevance, *CRC Crit. Rev. Toxicol.*, 2003, **33**, 655–780.

83 M. L. Takacs and B. D. Abbott, Activation of mouse and human peroxisome proliferator-activated receptors (alpha,

beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate, *Toxicol. Sci.*, 2007, **95**, 108–117.

84 B. Haughom and Ø. Spydevold, The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid, *Biochim. Biophys. Acta, Lipids Lipid Metab.*, 1992, **1128**, 65–72.

85 N. Kudo and Y. Kawashima, Fish oil-feeding prevents perfluorooctanoic acid-induced fatty liver in mice, *Toxicol. Appl. Pharmacol.*, 1997, **145**, 285–293.

86 M. T. Martin, R. J. Brennan, W. Hu, E. Ayanoglu, C. Lau, H. Ren, C. R. Wood, J. C. Corton, R. J. Kavlock and D. J. Dix, Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity, *Toxicol. Sci.*, 2007, **97**, 595–613.

87 K. P. Das, C. R. Wood, M. T. Lin, A. A. Starkov, C. Lau, K. B. Wallace, J. C. Corton and B. D. Abbott, Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis, *Toxicology*, 2017, **378**, 37–52.

88 H. Wan, Y. Zhao, X. Wei, K. Hui, J. Giesy and C. K. Wong, PFOS-induced hepatic steatosis, the mechanistic actions on β-oxidation and lipid transport, *Biochim. Biophys. Acta, Gen. Subj.*, 2012, **1820**, 1092–1101.

89 X. Tan, G. Xie, X. Sun, Q. Li, W. Zhong, P. Qiao, X. Sun, W. Jia and Z. Zhou, High fat diet feeding exaggerates perfluorooctanoic acid-induced liver injury in mice via modulating multiple metabolic pathways, *PLoS One*, 2013, **8**, e61409.

90 J. Bjork, J. Butenhoff and K. Wallace, Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes, *Toxicology*, 2011, **288**, 8–17.

91 C. R. Elcombe, B. M. Elcombe, J. R. Foster, D. G. Farrar, R. Jung, S. C. Chang, G. L. Kennedy and J. L. Butenhoff, Hepatocellular hypertrophy and cell proliferation in Sprague-Dawley rats following dietary exposure to ammonium perfluorooctanoate occurs through increased activation of the xenosensor nuclear receptors PPARalpha and CAR/PXR, *Arch. Toxicol.*, 2010, **84**, 787–798.

92 E. Ip, G. Farrell, P. Hall, G. Robertson and I. Leclercq, Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice, *Hepatology*, 2004, **39**, 1286–1296.

93 F. Lalloyer, K. Wouters, M. Baron, S. Caron, E. Vallez, J. Vanhoutte, E. Baugé, R. Shiri-Sverdlov, M. Hofker and B. Staels, Peroxisome Proliferator–Activated Receptor-α Gene Level Differently Affects Lipid Metabolism and Inflammation in Apolipoprotein E2 Knock-In Mice, *Arterioscler., Thromb., Vasc. Biol.*, 2011, **31**, 1573–1579.

94 G. Latini, Monitoring phthalate exposure in humans, *Clin. Chim. Acta*, 2005, **361**, 20–29.

95 I. Rusyn, J. M. Peters and M. L. Cunningham, Effects of DEHP in the Liver: Modes of Action and Species-Specific Differences, *Crit. Rev. Toxicol.*, 2006, **36**, 459–479.

96 N. Kambia, N. Renault, S. Dilly, A. Farce, T. Dine, B. Gressier, M. Luyckx, C. Brunet and P. Chavatte, Molecular modelling of phthalates–PPARs interactions, *J. Enzyme Inhib. Med. Chem.*, 2008, **23**, 611–616.

97 H. Chen, W. Zhang, B. B. Rui, S. M. Yang, W. P. Xu and W. Wei, Di(2-ethylhexyl) phthalate exacerbates non-alcoholic fatty liver in rats and its potential mechanisms, *Environ. Toxicol. Pharmacol.*, 2016, **42**, 38–44.

98 R. B. Remillard and N. J. Bunce, Linking dioxins to diabetes: epidemiology and biologic plausibility, *Environ. Health Perspect.*, 2002, **110**, 853–858.

99 M. M. Angrish, C. Y. Dominici and T. R. Zacharewski, TCDD-Elicited Effects on Liver, Serum, and Adipose Lipid Composition in C57BL/6 Mice, *Toxicol. Sci.*, 2013, **131**, 108–115.

100 J. H. Lee, T. Wada, M. Febbraio, J. He, T. Matsubara, M. J. Lee, F. J. Gonzalez and W. Xie, A novel role for the dioxin receptor in fatty acid metabolism and hepatic steatosis, *Gastroenterology*, 2010, **139**, 653–663.

101 M. M. Angrish, A. D. Jones, J. R. Harkema and T. R. Zacharewski, Aryl Hydrocarbon Receptor–Mediated Induction of Stearoyl-CoA Desaturase 1 Alters Hepatic Fatty Acid Composition in TCDD-Elicited Steatosis, *Toxicol. Sci.*, 2011, **124**, 299–310.

102 Z. Shaban, S. El-Shazly, S. Abdelhady, I. Fattouh, K. Muzandu, M. Ishizuka, K. Kimura, A. Kazusaka and S. Fujita, Down regulation of hepatic PPARalpha function by AhR ligand, *J. Vet. Med. Sci.*, 2004, **66**, 1377–1386.

103 A. Schecter, J. Colacino, D. Haffner, K. Patel, M. Opel, O. Päpke and L. Birnbaum, Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA, *Environ. Health Perspect.*, 2010, **118**, 796.

104 D.-H. Lee, I.-K. Lee, K. Song, M. Steffes, W. Toscano, B. A. Baker and D. R. Jacobs, A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes, *Diabetes Care*, 2006, **29**, 1638–1644.

105 D.-H. Lee, I.-K. Lee, M. Porta, M. Steffes and D. Jacobs, Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002, *Diabetologia*, 2007, **50**, 1841–1851.

106 M.-P. Boucher, C. Lefebvre and N. A. Chapados, The effects of PCB126 on intra-hepatic mechanisms associated with non alcoholic fatty liver disease, *J. Diabetes Metab. Disord.*, 2015, **14**, 88.

107 G. S. Gadupudi, W. D. Klaren, A. K. Olivier, A. J. Klingelhutz and L. W. Robertson, PCB126-induced disruption in gluconeogenesis and fatty acid oxidation precedes fatty liver in male rats, *Toxicol. Sci.*, 2016, **149**, 98–110.

108 B. Wahlang, K. C. Falkner, B. Gregory, D. Ansert, D. Young, D. J. Conklin, A. Bhatnagar, C. J. McClain and M. Cave, Polychlorinated biphenyl 153 is a diet-dependent

obesogen that worsens nonalcoholic fatty liver disease in male C57BL6/J mice, *J. Nutr. Biochem.*, 2013, **24**, 1587–1595.

109 P. S. Larson, J. L. Egle, G. R. Hennigar, R. W. Lane and J. F. Borzelleca, Acute, subchronic, and chronic toxicity of chlordecone, *Toxicol. Appl. Pharmacol.*, 1979, **48**, 29–41.

110 A. M. Khasawinah and J. F. Grutsch, Chlordane: 24-month tumorigenicity and chronic toxicity test in mice, *Regul. Toxicol. Pharmacol.*, 1989, **10**, 244–254.

111 J. Ross, S. M. Plummer, A. Rode, N. Scheer, C. C. Bower, O. Vogel, C. J. Henderson, C. R. Wolf and C. R. Elcombe, Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo, *Toxicol. Sci.*, 2010, **116**, 452–466.

112 J. Lee, R. C. Scheri, Y. Zhang and L. R. Curtis, Chlordecone, a mixed pregnane X receptor (PXR) and estrogen receptor alpha (ERalpha) agonist, alters cholesterol homeostasis and lipoprotein metabolism in C57BL/6 mice, *Toxicol. Appl. Pharmacol.*, 2008, **233**, 193–202.

113 J. L. Ray, A. K. Kopec, N. Joshi, H. Cline-Fedewa, L. H. Lash, K. J. Williams, P. S. Leung, M. E. Gershwin and J. P. Luyendyk, Trichloroethylene Exposure Reduces Liver Injury in a Mouse Model of Primary Biliary Cholangitis, *Toxicol. Sci.*, 2017, **156**, 428–437.

114 A. R. Laughter, C. S. Dunn, C. L. Swanson, P. Howroyd, R. C. Cattley and J. C. Corton, Role of the peroxisome proliferator-activated receptor alpha (PPARalpha) in responses to trichloroethylene and metabolites, trichloroacetate and dichloroacetate in mouse liver, *Toxicology*, 2004, **203**, 83–98.

115 D. H. Ramdhan, M. Kamijima, D. Wang, Y. Ito, H. Naito, Y. Yanagiba, Y. Hayashi, N. Tanaka, T. Aoyama, F. J. Gonzalez and T. Nakajima, Differential Response to Trichloroethylene-Induced Hepatosteatosis in Wild-Type and PPARα-Humanized Mice, *Environ. Health Perspect.*, 2010, **118**, 1557–1563.

116 K. Z. Guyton, K. A. Hogan, C. S. Scott, G. S. Cooper, A. S. Bale, L. Kopylev, S. Barone, S. L. Makris, B. Glenn, R. P. Subramaniam, M. R. Gwinn, R. C. Dzubow and W. A. Chiu, Human Health Effects of Tetrachloroethylene: Key Findings and Scientific Issues, *Environ. Health Perspect.*, 2014, **122**, 325–334.

117 J. A. Cichocki, S. Furuya, A. Venkatratnam, T. J. McDonald, A. H. Knap, T. Wade, S. Sweet, W. A. Chiu, D. W. Threadgill and I. Rusyn, Characterization of Variability in Toxicokinetics and Toxicodynamics of Tetrachloroethylene Using the Collaborative Cross Mouse Population, *Environ. Health Perspect.*, 2017, **125**, 057006.

118 J. A. Cichocki, S. Furuya, Y. S. Luo, Y. Iwata, K. Konganti, W. A. Chiu, D. W. Threadgill, I. P. Pogribny and I. Rusyn, Nonalcoholic Fatty Liver Disease Is a Susceptibility Factor for Perchloroethylene-Induced Liver Effects in Mice, *Toxicol. Sci.*, 2017, **159**, 102–113.

119 M. D. Merrell and N. J. Cherrington, Drug metabolism alterations in nonalcoholic fatty liver disease, *Drug Metab. Rev.*, 2011, **43**, 317–334.

120 J. E. Klaunig, X. Li and Z. Wang, Xenobiotic Nuclear Receptors Expression in High Fat Diet-Induced Non-Alcoholic Fatty Liver Disease: A Time-Course Study, in *The Toxicologist*, 2018, vol. 162, abstract no. 2051.

121 R. M. Evans and D. J. Mangelsdorf, Nuclear receptors, RXR, and the big bang, *Cell*, 2014, **157**, 255–266.

122 S. Song, R. R. Attia, S. Connaughton, M. I. Niesen, G. C. Ness, M. B. Elam, R. T. Hori, G. A. Cook and E. A. Park, Peroxisome proliferator activated receptor alpha (PPARalpha) and PPAR gamma coactivator (PGC-1alpha) induce carnitine palmitoyltransferase IA (CPT-1A) via independent gene elements, *Mol. Cell. Endocrinol.*, 2010, **325**, 54–63.

123 M. Rakhshandehroo, B. Knoch, M. Müller and S. Kersten, Peroxisome proliferator-activated receptor alpha target genes, *PPAR Res.*, 2010, **2010**.

124 S. Francque, A. Verrijken, S. Caron, J. Prawitt, R. Paumelle, B. Derudas, P. Lefebvre, M.-R. Taskinen, W. Van Hul and I. Mertens, PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis, *J. Hepatol.*, 2015, **63**, 164–173.

125 D. Patsouris, J. K. Reddy, M. Müller and S. Kersten, Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression, *Endocrinology*, 2006, **147**, 1508–1516.

126 Y. Zou, H. Du, M. Yin, L. Zhang, L. Mao, N. Xiao, G. Ren, C. Zhang and J. Pan, Effects of high dietary fat and cholesterol on expression of PPAR alpha, LXR alpha, and their responsive genes in the liver of apoE and LDLR double deficient mice, *Mol. Cell. Biochem.*, 2009, **323**, 195–205.

127 V. de Fourmestraux, H. Neubauer, C. Poussin, P. Farmer, L. Falquet, R. Burcelin, M. Delorenzi and B. Thorens, Transcript profiling suggests that differential metabolic adaptation of mice to a high fat diet is associated with changes in liver to muscle lipid fluxes, *J. Biol. Chem.*, 2004, **279**, 50743–50753.

128 M. Pawlak, P. Lefebvre and B. Staels, Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease, *J. Hepatol.*, 2015, **62**, 720–733.

129 T. Varga, Z. Czimmerer and L. Nagy, PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation(), *Biochim. Biophys. Acta*, 2011, **1812**, 1007–1022.

130 M. V. Chakravarthy, I. J. Lodhi, L. Yin, R. R. Malapaka, H. E. Xu, J. Turk and C. F. Semenkovich, Identification of a physiologically relevant endogenous ligand for PPARalpha in liver, *Cell*, 2009, **138**, 476–488.

131 C. Desmarchelier, C. Dahlhoff, S. Keller, M. Sailer, G. Jahreis and H. Daniel, C57Bl/6 N mice on a western diet display reduced intestinal and hepatic cholesterol

levels despite a plasma hypercholesterolemia, *BMC Genomics*, 2012, **13**, 84.

132 O. Gavrilova, M. Haluzik, K. Matsusue, J. J. Cutson, L. Johnson, K. R. Dietz, C. J. Nicol, C. Vinson, F. J. Gonzalez and M. L. Reitman, Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass, *J. Biol. Chem.*, 2003, **278**, 34268–34276.

133 M. Inoue, T. Ohtake, W. Motomura, N. Takahashi, Y. Hosoki, S. Miyoshi, Y. Suzuki, H. Saito, Y. Kohgo and T. Okumura, Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice, *Biochem. Biophys. Res. Commun.*, 2005, **336**, 215–222.

134 V. Souza-Mello, Peroxisome proliferator-activated receptors as targets to treat non-alcoholic fatty liver disease, *World J. Hepatol.*, 2015, **7**, 1012.

135 C. H. Lee, P. Olson, A. Hevener, I. Mehl, L. W. Chong, J. M. Olefsky, F. J. Gonzalez, J. Ham, H. Kang, J. M. Peters and R. M. Evans, PPARdelta regulates glucose metabolism and insulin sensitivity, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 3444–3449.

136 B. Staels, A. Rubenstrunk, B. Noel, G. Rigou, P. Delataille, L. J. Millatt, M. Baron, A. Lucas, A. Tailleux, D. W. Hum, V. Ratziu, B. Cariou and R. Hanf, 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**, 1941–1952.

137 K. Iwaisako, M. Haimerl, Y.-H. Paik, K. Taura, Y. Kodama, C. Sirlin, E. Yu, T. Y. Ruth, M. Downes and R. M. Evans, Protection from liver fibrosis by a peroxisome proliferator-activated receptor δ agonist, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, E1369–E1376.

138 T. Nagasawa, Y. Inada, S. Nakano, T. Tamura, T. Takahashi, K. Maruyama, Y. Yamazaki, J. Kuroda and N. Shibata, 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**, 182–191.

139 T. Wada, J. Gao and W. Xie, PXR and CAR in energy metabolism, *Trends Endocrinol. Metab.*, 2009, **20**, 273–279.

140 A. Roth, R. Looser, M. Kaufmann, S. M. Blattler, F. Rencurel, W. Huang, D. D. Moore and U. A. Meyer, Regulatory cross-talk between drug metabolism and lipid homeostasis: constitutive androstane receptor and pregnane X receptor increase Insig-1 expression, *Mol. Pharmacol.*, 2008, **73**, 1282–1289.

141 D. Kolwankar, R. Vuppalanchi, B. Ethell, D. R. Jones, S. A. Wrighton, S. D. Hall and N. Chalasani, Association between nonalcoholic hepatic steatosis and hepatic cytochrome P-450 3A activity, *Clin. Gastroenterol. Hepatol.*, 2007, **5**, 388–393.

142 C. D. Fisher, A. J. Lickteig, L. M. Augustine, J. Ranger-Moore, J. P. Jackson, S. S. Ferguson and N. J. Cherrington, Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease, *Drug Metab. Dispos.*, 2009, **37**, 2087–2094.

143 S. J. Woolsey, S. E. Mansell, R. B. Kim, R. G. Tirona and M. D. Beaton, CYP3A activity and expression in nonalcoholic fatty liver disease, *Drug Metab. Dispos.*, 2015, **43**, 1484–1490.

144 M. Hanagama, H. Inoue, M. Kamiya, K. Shinone and M. Nata, Gene expression on liver toxicity induced by administration of haloperidol in rats with severe fatty liver, *Legal Med.*, 2008, **10**, 177–184.

145 M. Osabe, J. Sugatani, T. Fukuyama, S. Ikushiro, A. Ikari and M. Miwa, Expression of hepatic UDP-glucuronosyltransferase 1A1 and 1A6 correlated with increased expression of the nuclear constitutive androstane receptor and peroxisome proliferator-activated receptor alpha in male rats fed a high-fat and high-sucrose diet, *Drug Metab. Dispos.*, 2008, **36**, 294–302.

146 M. Ning and H. Jeong, High-Fat Diet Feeding Alters Expression of Hepatic Drug-metabolizing Enzymes in Mice, *Drug Metab. Dispos.*, 2017, **45**, 707–711.

147 I. A. Kirpich, L. N. Gobejishvili, M. B. Homme, S. Waigel, M. Cave, G. Arteel, S. S. Barve, C. J. McClain and I. V. Deaciuc, Integrated hepatic transcriptome and proteome analysis of mice with high-fat diet-induced nonalcoholic fatty liver disease, *J. Nutr. Biochem.*, 2011, **22**, 38–45.

148 J. Sonoda, L. W. Chong, M. Downes, G. D. Barish, S. Coulter, C. Liddle, C.-H. Lee and R. M. Evans, Pregnane X receptor prevents hepatorenal toxicity from cholesterol metabolites, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 2198–2203.

149 J. Sugatani, T. Wada, M. Osabe, K. Yamakawa, K. Yoshinari and M. Miwa, Dietary inulin alleviates hepatic steatosis and xenobiotics-induced liver injury in rats fed a high-fat and high-sucrose diet: association with the suppression of hepatic cytochrome P450 and hepatocyte nuclear factor 4alpha expression, *Drug Metab. Dispos.*, 2006, **34**, 1677–1687.

150 S. J. Cho, S. B. Kim, H. J. Cho, S. Chong, S. J. Chung, I. M. Kang, J. I. Lee, I. S. Yoon and D. D. Kim, Effects of Nonalcoholic Fatty Liver Disease on Hepatic CYP2B1 and in Vivo Bupropion Disposition in Rats Fed a High-Fat or Methionine/Choline-Deficient Diet, *J. Agric. Food Chem.*, 2016, **64**, 5598–5606.

151 Y. Ma and D. Liu, Activation of pregnane X receptor by pregnenolone 16 α-carbonitrile prevents high-fat diet-induced obesity in AKR/J mice, *PLoS One*, 2012, **7**, e38734.

152 J. Hernandez, L. Mota and W. Baldwin, Activation of CAR and PXR by Dietary, Environmental and Occupational Chemicals Alters Drug Metabolism, Intermediary Metabolism, and Cell Proliferation, *Curr. Pharmacogenomics Pers. Med.*, 2009, **7**, 81–105.

153 H. Wang and L. M. Tompkins, CYP2B6: new insights into a historically overlooked cytochrome P450 isozyme, *Curr. Drug Metab.*, 2008, **9**, 598–610.

154 U. M. Zanger and M. Schwab, Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation, *Pharmacol. Ther.*, 2013, **138**, 103–141.

155 A. Gunes and M.-L. Dahl, *Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms*, 2008.

156 J. George, M. Murray, K. Byth and G. C. Farrell, Differential alterations of cytochrome P450 proteins in livers from patients with severe chronic liver disease, *Hepatology*, 1995, **21**, 120–128.

157 B. J. Moyer, I. Y. Rojas, J. S. Kerley-Hamilton, K. V. Nemani, H. W. Trask, C. S. Ringelberg, B. Gimi, E. Demidenko and C. R. Tomlinson, Obesity and fatty liver are prevented by inhibition of the aryl hydrocarbon receptor in both female and male mice, *Nutr. Res.*, 2017, **44**, 38–50.

158 M. Teboul, E. Enmark, Q. Li, A. Wikström, M. Pelto-Huikko and J.-A. Gustafsson, OR-1, a member of the nuclear receptor superfamily that interacts with the 9-cis-retinoic acid receptor, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 2096–2100.

159 S. Ducheix, A. Montagner, V. Theodorou, L. Ferrier and H. Guillou, 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.

160 S. B. Ahn, K. Jang, D. W. Jun, B. H. Lee and K. J. Shin, Expression of liver X receptor correlates with intrahepatic inflammation and fibrosis in patients with nonalcoholic fatty liver disease, *Dig. Dis. Sci.*, 2014, **59**, 2975–2982.

161 R. H. Ghoneim, E. T. Ngo Sock, J. M. Lavoie and M. Piquette-Miller, Effect of a high-fat diet on the hepatic expression of nuclear receptors and their target genes: relevance to drug disposition, *Br. J. Nutr.*, 2015, **113**, 507–516.

162 Z.-H. Yang, H. Miyahara, J. Takeo and M. Katayama, Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice, *Diabetol. Metab. Syndr.*, 2012, **4**, 32.

163 H. Basciano, A. E. Miller, M. Naples, C. Baker, R. Kohen, E. Xu, Q. Su, E. M. Allister, M. B. Wheeler and K. Adeli, Metabolic effects of dietary cholesterol in an animal model of insulin resistance and hepatic steatosis, *Am. J. Physiol.: Endocrinol. Metab.*, 2009, **297**, E462–E473.

164 Y.-D. Wang, W.-D. Chen, D. D. Moore and W. Huang, FXR: a metabolic regulator and cell protector, *Cell Res.*, 2008, **18**, 1087.

165 A. H. Ali, E. J. Carey and K. D. Lindor, Recent advances in the development of farnesoid X receptor agonists, *Ann. Transl. Med.*, 2015, **3**, 5.

166 X. Li, Z. Wang and J. E. Klaunig, Role of microRNAs in Nonalcoholic Fatty Liver Disease. The Toxicologists: 150 (1), *Soc. Toxicol.*, 2017, 1082.

