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Endocrine-disrupting chemicals and fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a growing epidemic paralleling the increase in obesity and diabetes mellitus seen in Western diet-consuming countries. As NAFLD can lead to life-threatening conditions such as cirrhosis and hepatocellular carcinoma (HCC), an understanding of factors that trigger its development and pathological progression is needed. Although by definition this disease is not associated with alcohol consumption, exposure to environmental agents that have been linked to other diseases might have a role in the development of NAFLD. Here, we focus on one class of these agents, endocrine-disrupting chemicals (EDCs), and their potential to influence the initiation and progression of a cascade of pathological conditions associated with fatty liver. Experimental studies have revealed several potential mechanisms by which EDC exposures might contribute to disease pathogenesis, including modulation of nuclear hormone receptor (NR) function and alteration of the epigenome. However, many questions remain to be addressed about the causal link between acute and chronic EDC exposure and the development of NAFLD in humans. Future studies that address these questions hold promise not only for understanding the linkage between EDC exposure and liver disease, but for elucidating the molecular mechanisms underpinning NAFLD and the development of new prevention and treatment opportunities.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by simple, reversible hepatic steatosis (fatty liver) with or without additional macrophage infiltration and inflammation (steatohepatitis or nonalcoholic steatohepatitis (NASH), respectively), which can progress to

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Competing interests

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irreversible fibrosis and life-threatening cirrhosis and hepatocellular carcinoma (HCC) (FIG. 1)[1–6]. NAFLD is the most common chronic liver disease worldwide and is especially prevalent in high-fat diet (HFD) consuming countries such as the USA[7]. Since its first diagnosis in the 1980s[1], the incidence of NAFLD incidence has reached 30% in the USA[8], paralleling increases in obesity[9] (35% of adults in the USA have a body mass index (BMI) >30 kg/m^2), risk of HCC[6] and cardiovascular disease[10, 11]. Although NAFLD is most commonly spurred by overnutrition coupled with a lack of exercise, environmental factors might also contribute to the rapid rise in both obesity and NAFLD prevalence. Indeed, diets rich in fructose have now been implicated in the development of NAFLD development[1].

One class of environmental risk factors that might promote NAFLD is chemicals that can disrupt or alter the function of endocrine and metabolic organs such as the liver, which is the central organ controlling lipid homeostasis. These chemicals are termed endocrine-disrupting chemicals (EDCs)[12, 13], or more recently, metabolism-disrupting chemicals (MDCs)[12]. The timely interest in these compounds as potential stimulators of obesity and NAFLD, along with other risk factors such as a HFD and fructose, stems from the following: many EDCs have been mass produced over the past four decades, driven by their widespread use (for example, bisphenol A (BPA) and phthalates in plastic production)[12, 13]; animal ‘intervention’ studies have suggested that EDCs might cause increased adiposity or NAFLD in exposed animals[12, 13]; and direct measurement of EDC levels in human blood and urine has shown near ubiquitous exposures (for example, ≥95% of people in the USA have detectable levels of BPA in their urine[14]).

In this Review, we highlight the literature bridging the two ‘hot’ topics of NAFLD and EDCs, and posit that early-life exposure to EDCs might represent an unappreciated driver of NAFLD development and progression in adulthood. We describe basic liver physiology along with the molecular pathways that affect hepatic lipid homeostasis and how they impact NAFLD development. We then discuss various classes of EDCs that perturb hepatic lipid levels, bind to nuclear hormone receptors (NRs) and recruit transcriptional coregulators to alter the expression of lipid homeostasis genes and/or activate kinase signalling pathways, promote NAFLD in rodent models and are associated with human NAFLD, impact epigenetic modifications (that is, DNA methylation and histone modifications) and, in the setting of early-life exposures, increase susceptibility to obesity and NAFLD in adulthood.

Liver physiology

The liver is the largest glandular organ in the body. To perform its many and diverse functions, the liver relies on a compartmentalized structure. Liver architecture is composed of small hexagonal lobules, wherein each lobule is connected by a network of sinusoids formed by specialized sinusoidal endothelial cells (FIG. 1). Adjacent to the sinusoid resides hepatic stellate cells that function as a repository for lipids and vitamin A. The sinusoids traverse a collection of two other primary cell types: hepatocytes, which represent the parenchymal cell type of the liver, and Kupffer cells that represent the resident macrophage population[15].

Liver response to environmental cues

Although the many different cell types in the liver demonstrate considerable phenotypic and functional heterogeneity, their cooperative molecular contributions endow the liver with the ability to interpret and respond to a number of environmental stimuli. In some situations, these environmental responses have a beneficial effect on liver function and overall health. For example, in the setting of a starvation environment *in utero*, the liver develops in such a way that the physiological set points for several liver functions, including gluconeogenesis, are primed for an adult environment in which nutrients are in short supply. In this context, modulation of fetal development can confer a survival advantage on offspring exposed to an environment in which resources are likely to be limited, resulting in a thrifty phenotype[16]. However, individuals programmed with a thrifty phenotype *in utero* (for example as a result of famine or placental insufficiency), who go on to develop in a nutrient-rich environment instead of the 'anticipated' nutrient-poor environment, are more prone to metabolic disorders. For example, prenatal exposure to famine (especially in late gestation) during the Dutch Hunger Winter (1944–1945) was associated with decreased glucose tolerance in adults[17]. In the same cohort, prenatal exposure to famine was associated with a more atherogenic lipid profile than those who were not exposed to famine *in utero*[18]. Early-life exposure to famine during the Great Chinese Famine (1958–1961) was associated with a sex-specific increase in the prevalence of moderate–severe NAFLD in adulthood, providing direct evidence of the link between poor fetal nutrition and perturbed liver function[19].

Basis of NAFLD

As the epicenter for metabolic homeostasis, the liver performs a myriad of functions including haematopoiesis and turnover of red blood cells[20], production of enzymes for blood clotting, hormone biosynthesis and turnover, protein and bile synthesis, drug metabolism, lipid metabolism, glycogen storage and release, and gluconeogenesis[21]. Should any of these key functions become compromised (especially lipid metabolism), several disease sequelae can result. Initial excess lipid accumulation in the liver (steatosis; a reversible step) can progress to NASH (characterized by macrophage infiltration and inflammation) and then to fibrosis and/or cirrhosis (irreversible); a subset of the latter cases advance to HCC. Together, both the 'early' presenting conditions of hepatic steatosis and NASH constitute NAFLD (FIG. 1)[5, 10].

NAFLD is a growing problem in HFD-consuming countries such as the USA. For example, analysis of fatty liver (as assayed by ultrasound) in viral hepatitis negative patients in the U.S. National Health and Nutrition Examination Survey (NHANES) has suggested that NAFLD increased from 18% in 1998–1991 to 31% in 2011–2012[8]. At 30%, this prevalence represents ~75–100 million people in the USA[11]. The large increase in NAFLD incidence over the past two decades has been accompanied by an increased risk of HCC[6] and resultant deaths, as well as cardiovascular disease[10, 11]. Importantly, NASH, the inflammatory form of NAFLD, is currently the second leading cause of liver disease in adults scheduled for liver transplantation in the USA[10]. Thus, understanding the factors that trigger NAFLD is of the utmost importance to curbing the rising need for liver transplantation and later-stage lethal events such as HCC. Among the environmental factors that contribute to the development of NAFLD, early-life exposures to EDCs might represent an unappreciated risk factor to

consider in addition to obesity and type 2 diabetes mellitus (T2DM)[22], due to their potential to alter lipid homeostatic 'set points' that favour NAFLD.

Hepatic lipid homeostasis is maintained by hepatocyte uptake and *de novo* synthesis of free fatty acids (FFAs), FFA disposal by oxidation or *de novo* triglyceride synthesis, and export of triglycerides from hepatocytes as very low density lipoprotein (VLDL) (FIG. 2). Fatty liver or steatosis develops when hepatic uptake of FFAs exceeds their oxidation and secretion as triglycerides. The consequence of aberrant lipid accumulation in the liver imposes differential cellular effects on subpopulations of hepatic cell types. In hepatocytes, uptake and/or *de novo* synthesis of fatty acids are disproportionally increased relative to fatty acid oxidation. This imbalance stimulates triglyceride synthesis to dispose of the excess FFAs. As triglyceride synthesis outpaces the capacity for VLDL synthesis and export, triglycerides accumulate within hepatocytes, resulting in steatosis[23] (FIG. 2). Although triglycerides are not inherently hepatotoxic, aberrant hepatocyte processing of FFAs activates resident and infiltrated macrophages through Toll-like receptor 4 pathways to initiate a pro-inflammatory cascade that contributes to NAFLD[3, 24]. The chemokine and angiogenic signals produced from infiltrated macrophages leads to dysregulation of sinusoidal endothelial cells that form the fenestrated vasculature of the liver[25]. Excess hepatic lipid also serves as an activation signal for the normally quiescent stellate cells that initiates the fibrotic process that often accompanies more severe forms of liver disease such as NASH and HCC[3, 4, 23] (FIG. 1).

Environmental exposures—Established risk factors for NAFLD in humans include obesity and insulin resistance or T2DM[22, 26, 27], as well as specific genetic mutations that result in increased lipid synthesis and uptake, and/or decreased FFA oxidation and triglyceride export[28]. However, such germ-line mutations are rare and would not explain the vast majority of NAFLD cases, which points to the involvement of environmental factors in the development of this disease. For example, dietary intake of saturated fat, trans-fatty acids, carbohydrate and simple sugars (fructose and sucrose) might contribute to aberrant hepatic lipid accumulation[26]. Interestingly, some polyunsaturated fatty acids (PUFAs), such as *n*-3 PUFAs like α-linolenic acid seem to reduce NAFLD[3]. However, true causal relationships between these nutrients and NAFLD remain to be fully determined. Of these dietary factors, fructose has been reported to be a risk factor for human NAFLD[1, 3, 29–31], but whether fructose alone (for example in the absence of obesity) can trigger or facilitate the progression of NAFLD is still debatable[26]. Finally, maternal diet might impact offspring susceptibility to NAFLD via changes in the neonatal or infant-gut microbiota, although again, causality remains to be firmly established[2].

Of the various environmental chemical exposures that might negatively affect the liver, the growing class of EDCs has gained attention for their ability to perturb hepatic function. EDCs are defined as compounds that exert adverse health effects secondary to disruption of the endocrine system. Structurally, EDCs comprise a wide range of both natural and manmade substances that are derived from persistent organic pollutants (POPs; that is, dioxins, benzo[a]pyrene and polychlorinated biphenyls (PCBs)), organochlorines (such as dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE)), plasticizers (such as bisphenol A (BPA)), phthalates (such as di-2-ethylhexyl

phthalate (DEHP)), organotins (such as tributyltin (TBT)), polyfluoroalkyls (such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS)) and other pesticides (such as cypermethrin (CYP), atrazine (ATZ), and carbendazim)[13, 32]. Mounting evidence suggests that these EDCs, many of which are also sequestered and metabolized in the liver, might contribute to the development of NAFLD.

Relevant to the NAFLD–obesity linkage, several of these EDCs including BPA, TBT, PCBs, phthalates, PFOA and PFOS are classified as 'obesogens', based on animal studies wherein early-life exposures promote obesity later in adulthood[33]. The original obesogen hypothesis proposed by Grun and Blumberg[34] primarily focused on the effects of these exposures on adipocytes (that is, fat storage) and pancreatic β cells (that is, insulin secretion)[34, 35]. In rodent models, perinatal exposure to obesogens increases fat mass in both male and female offspring[32, 34, 36]. Thus, one pathway by which EDCs might impact NAFLD is through peripheral effects of obesity on adipose dysfunction, deregulation of the satiety axis in the hypothalamus, as well as liver cell autonomous effects.

Liver fat metabolism and EDCs

Nuclear receptors and coregulators

The vast majority of EDCs exert their activity as endocrine disruptors via their ability to bind NRs and thus act as NR agonists or antagonists. The liver expresses an extensive repertoire of NRs that have important roles in hepatic lipid metabolism (FIG. 3). When activated by a ligand, the classic activity of NRs involves docking at response elements in the promoter or enhancer region of target genes followed by binding of steroid receptor coactivator (SRC) complexes that recruit additional coregulators with histone-modifying enzymatic activities, such as acetylases and methylases[37, 38]. The concerted action of these coregulators leads to transactivation of target gene programs[37]. Experience with selective oestrogen receptor (ER) modulators (SERMs)[39] as well as EDCs[40] showed that for agonists, NRs adopt an active conformation and interact with coactivators, whereas for antagonists, receptors adopt an inactive conformation that recruits corepressors. In addition to classic genomic activity, ligand-dependent NR signalling also occurs in the cytoplasm, so-called 'non-genomic' signalling[41, 42]. This extra-nuclear NR signalling results in the activation of kinases and downstream signalling pathways, such as protein kinase B (also called AKT) and mitogen-activated protein kinases (MAPKs) that mediate biological responses independent of NR nuclear localization. Non-genomic signalling is characterized by its rapid action, and occurs independent of RNA or protein synthesis.

Several NRs have roles in non-genomic signalling. Perhaps the most prominent of these are the steroid hormone receptors — oestrogen receptors (ERα and ERβ), androgen receptor (AR) and progesterone receptor (PR)[43, 44]. In addition to these steroid receptors, accumulating evidence suggests that other NRs (for example, peroxisome proliferator-activated receptor γ (PPARγ)[45], retinoid X receptor α (RXRα)[46], truncated thyroid receptor α (TRα) isoforms[44, 47] and retinoic acid receptors (RARα and RARγ[48])) might also signal via a similar mechanism. Non-genomic signalling does have the potential to affect the genome and alter transcription, as kinases activated in non-genomic signaling pathways can phosphorylate and regulate the activity of epigenomic programmers,

transcription factors and/or their associated coregulators, even in the absence of a liganded NR interacting with target genes.

Transcriptional profiling of all major isoforms of the 49 known NRs from isolated livers of 129/SvJ and C57BL/6J male mice revealed hepatic expression of 39 NRs[49]. A similar number of NRs (35) were identified by mass spectrometric profiling of NRs bound to their cognate DNA response elements in hepatocytes isolated from C57BL/6J male and female mice[50]. Of these, the NR1 subfamily that heterodimerize with retinoid X receptors (RXRα, RXRβ and RXRγ), which includes the peroxisome proliferator-activated receptors α, β and γ (PPARs), the liver X receptors α and β (LXRs), farnesoid X receptor α (FXRα), the constitutive androstane receptor (CAR), the pregnane X receptor (PXR) and thyroid receptors (TRs), have been implicated in modulation of NAFLD (FIG. 3)[51–53]. Activation of PPARα by its natural ligands (FFAs) increases the expression of genes encoding enzymes involved in FFA oxidation (such as *CPT1* and *ACOX1*) and hence leads to decreased hepatic steatosis[54]. Similarly, xenobiotics bind and activate CAR and PXR; however, species-specific differences clearly exist[55, 56]. For example, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) activates the mouse but not the human CAR[57,58], while 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (CITCO) activates the human, but not mouse, CAR[55, 56, 59]. Additionally, pregnenolone 16α-carbonitrile (PCN) preferentially activates mouse and rat PXRs over human PXR[57, 60–63], but rifampicin and SR12813 are specific ligands for human but not mouse PXR[57, 60–63]. CAR and PXR activation decreases and increases hepatic lipid accumulation, respectively[64, 65].

LXRs bind oxysterols and activate lipogenic gene programs (for example, *FAS* and *SREBP1*) that can lead to lipid accumulation in the liver[66]. FXR functionally responds to bile acids and induces the expression of bile acid exporter genes and *NR0B2*, which encodes the NR short heterodimer partner (SHP) that represses *SREBP1* expression[67], thereby decreasing hepatic steatosis. Due to this action, FXR agonists such as obeticholic acid and INT-767 are currently being tested in human NAFLD and NASH clinical trials[2]. Thyroid hormone T_3, as well as synthetic TR agonists, reduce hepatic steatosis in male Fischer 344 rats fed a diet deficient in choline and methionine[68] and in diabetic mice (for example, *ob/ob*)[69], suggestive of their potential clinical usefulness. Activation of a NR that can trigger increased steatosis does not always result in increased inflammation. For example, activation of PXR induces lipogenic gene expression (for example, *SCD1*) and suppresses FFA-oxidation enzyme gene expression (for example, *CPT1*)[70], yet PXR activation can also suppress the expression of inflammatory cytokines in hepatocytes treated with lipopolysaccharide[71].

In addition to NRs that bind EDCs, coregulators that complex with liganded NRs might also have a role in NAFLD. The activity of NRs bound to endogenous ligands or EDCs is determined by the action of coregulator proteins that interact with these receptors. In the liver, a variety of coregulators have key functional roles in hepatic lipid metabolism via recruitment to ligand-activated NRs bound at genes involved in lipid homeostasis (FIG. 4). Examples of critical coactivators are the three SRC family proteins (SRC1, SRC2 and SRC3), PPARγ coactivators (PGC1α and PGC1β) and the Mediator complex subunit

MED1. The key corepressors are the nuclear receptor corepressor (NCoR)–silencing mediator of retinoic acid and thyroid hormone receptor (SMRT)–histone deacetylase 3 (HDAC3) complexes and receptor-interacting protein 140 (RIP140)[72].

Studies in knockout mice clearly show that NR coactivators have an important role in the development of fatty liver. Genetic ablation of *Src1* increased acylcarnitine levels in the fed-to-fasting transition, which is suggestive of an important role for SRC1 in regulating hepatic FFA-oxidation[73]. Both whole-body and liver-specific ablation of *Src2* phenocopies a Von Gierke–like disease that is characterized by fasting hypoglycaemia, hepatic steatosis and increased circulating levels of triglycerides, cholesterol and FFAs[74]. Hepatic SRC3 mRNA and protein levels increase upon HFD feeding, and genetic ablation of *Src3* protects against HFD-induced hepatic steatosis by reducing lipid accumulation and the accompanying inflammatory response[75,76,77]. Whole-body genetic ablation of *Pgc1a/b* (encoding *PGC1α/β)* results in increased hepatic steatosis, although liver-specific deletion is needed to confirm whether these effects are intrinsic to the liver[78–80]. Interestingly, PGC1α mediates the recruitment of the BAF60a subunit of the SWI/SNF chromatin-remodelling complex to PPARα-binding sites, which leads to transcriptional activation of FFA-oxidation genes; mice overexpressing BAF60a fed a HFD display reduced fatty liver[81]. Finally, MED1 is the key subunit of the Mediator complex that interacts with liganded NRs via its LxxLL motifs[82]. MED1 is required for HFD-induced hepatic steatosis as well as PPARγ-stimulated hepatic steatosis, as revealed by analysis of liver-specific *Med1* knockout mice and *Pparg* hepatic overexpression by tail vein adenoviral injection[83].

Studies in knockout mice highlight the importance of specific NR corepressors for modulating hepatic steatosis. For example, NCoR or SMRT associate with HDAC3 as part of a multi-subunit protein complex that functions generally as a NR corepressor[84–86]. Liver-specific ablation of *Hdac3* or *Ncor*, but not *Smrt*, in mice results in hepatic steatosis[87, 88]. Whole-body *Rip140* knockout mice are lean and resistant to HFD-induced obesity and hepatic steatosis[89]. Liver-specific deletion of *Rip140* also reduced hepatic lipid levels in mice, which suggests that RIP140 serves as a corepressor of LXR-activated lipogenic genes such as *FAS* and *SREBP1*[90]. Other studies have identified additional NR corepressors that regulate hepatic steatosis, such as ligand-dependent corepressor (LCOR) suppression of TRβ-induced lipogenic gene expression and hepatic steatosis in obese mice[91], and small heterodimer partner interacting leucine zipper protein (SMILE) repression of LXRα-mediated *SREBP1* gene expression and hepatic lipid accumulation[92]. Although the above mentioned animal ablation studies emphasize the importance of coregulators in modulating hepatic lipid homeostasis, information on which cell types in the liver are critical to the development of fatty liver is lacking, emphasizing the need for liver cell-type specific genetic ablation studies.

Like endogenous hormones, EDCs can activate both genomic and non-genomic actions of NRs, and induce posttranslational modifications that modulate NR and/or coregulator activity. For example, SRC3 is initially phosphorylated by oestradiol-activated kinases in the cytoplasm on a subset of conserved phosphorylation sites that subsequently leads to enhanced NR–SRC3 target gene transcription[93,94]. Mice harboring loss-of-function mutations in four of these conserved phosphorylation sites in SRC3 develop insulin

resistance, dyslipidaemia, liver steatosis and accelerated hepatic tumorigenesis[95]. Furthermore, insulin (a mimic of the fed state) activates AKT resulting in phosphorylation of NCoR on Ser1460, which enhances its interaction with PPARα over LXRα, and results in repression of PPARα, decreased FFA-oxidation and enhanced hepatic lipogenesis[96] (FIG. 4). Different structural classes of EDCs have been shown to modulate the activity of NRs expressed in the liver and associated with NAFLD, such as the NR1 subfamily NRs (PPARs, RXRs, PXR, CAR and TRs), steroid receptors (glucocorticoid receptor (GR), ERs and AR) and the aryl hydrocarbon receptor (AhR) (FIG. 3)[32, 97, 98]. POPs such as dioxins, benzo[a]pyrene and some PCBs bind and activate AhR (some also bind PPARγ and ERs); organochlorines such as DDT bind and activate CAR and ERα; the plasticizer BPA binds and activates ERs and GR, yet represses TR; organotins such as TBT bind and activate RXRs and PPARγ; polyfluoroalkyls such as PFOA and PFOS bind and activate ERs and PPARs; and phthalates such as DEHP bind and activate PPARs, CAR, PXR and GR[32]. Some of the EDC–NR interactions are of low affinity (for example, the equilibrium dissociation constant K_d of BPA:ERα is 0.2 μM[99]), whereas other interactions are much stronger (for example, the K_d of TBT:RXR or TBT:PPARγ are 12.5–20 nM[100]).

EDC–NAFLD link

EDC activation of NRs has been proposed to be an initiating event in the development of steatosis[101], as well as promoting the transition of steatosis to steatohepatitis[102]. Several EDCs that function as ligands for the NRs described earlier have been shown to impact the liver and the development of NAFLD[12,101,102]. A 2015 review of 371 studies in federal databases suggested that 123 unique environmental chemicals are associated with NAFLD in rodents, with pesticides representing the majority (44%) and PCBs and dioxins the most potent based on lowest effect level[103]. To extend these data from ‘associations’ to ‘cause-and-effect’ relationships, we performed an extensive analysis of the literature with a focus on ‘interventional’ studies wherein rodents were exposed to an EDC (or mixture that might better represent environmental exposures) or vehicle control and a NAFLD phenotype scored (see Supplementary information S1 (table). These studies used several different strains of rats (for example, Sprague-Dawley, Wister, Fischer 344, Han/Wistar and Obese JCR (LA)-Leprcp (cp/cp)) and mice (for example, CD1, C57BL/6J, KM, *Ldlr* knockout, *Apoe* knockout, Std:ddY, BALB/c and ICR) exposed to different EDCs (for example, BPA, TBT, benzo[a]pyrene, dioxins (2,3,7,8-tetrachlorodibenzodioxin (TCDD) and hexachlorodibenzo-*p*-dioxin (HxCDD)), PCBs (77, 105, 126, 153, 126 + 118, 126 + 153, Aroclor 1260 or Aroclor 1254 (a mixture of up to 60 PCBs)), DDE, PFOA, PFOS, DEHP, pesticides (CYP, ATZ or carbendazim), or a ‘Northern contaminant mixture’ (22 compounds including 11 PCBs, DDE and PFOS)). Liver endpoints following exposure revealed increased hepatic lipid accumulation (assessed by histological analysis, Oil Red O staining or hepatic triglyceride measurements). Importantly, both perinatal (*in utero*) and adult animal EDC exposures showed signs of fatty liver development with different doses (see Supplementary information S1 (table)). Interestingly, combined treatment of rodents with one type of EDC followed by another class of EDC can modify the NAFLD phenotype. For example, pre-treatment of rats with TCDD led to the appearance of NAFLD, whilst DEHP reduced the TCDD-induced phenotype[104]. Similarly, the combination of TCDD and Aroclor 1254 seemed to enhance NAFLD in mice, compared with treatment with either EDC

alone[105]. Although this approach has been applied in only a few experimental settings, the data are extremely relevant to humans, where exposure to more than one EDC during development and over the course of a lifetime may occur. Additional animal studies examining EDC mixtures are certainly warranted.

When combined with other risk factors for NAFLD (such as a HFD), EDCs generally exacerbate the NAFLD phenotype in exposed rodents. Two seminal studies reported that perinatal exposures to BPA (50–100 μg/kg per day) combined with a HFD after weaning (at postnatal day 21) led to male, but not female, offspring displaying more severe hepatic steatosis[106, 107] and increased inflammation as well as mild fibrosis in the liver[106]. These data indicate that in addition to increasing hepatic lipid accumulation in the liver, EDC exposure might also trigger macrophage infiltration that can further contribute to the development of NASH (analogous to that already proposed for fructose[1]), although additional mechanistic studies are needed to adequately test this hypothesis. In terms of altered gene expression, the increased hepatic lipid accumulation observed with BPA treatment could be due to an imbalance of FFA uptake, synthesis or β-oxidation, and/or triglyceride export via secretion as VLDL (FIG. 2). Indeed, livers of BPA-exposed animals exhibit increased expression of a key gene involved in FFA uptake (*Cd36*; also known as *Fat*), decreased expression of genes related to triglyceride synthesis and FFA oxidation (*Dgat*, *Agpat6*, *Cebpα*, *Cebpβ*, *Pck1*, *Acox1*, *Cpt1a* and *Cybb*)[107].

Relevant to the ability of EDCs to induce developmental reprogramming of the epigenome, BPA exposure altered DNA methylation and histone modifications associated with active transcription (for example, acetylation of histones H3 and H4, and trimethylation of histone H3 at lysine 36) and decreased occupancy of RNA polymerase II and critical transcription factors (C/EBPβ and SREBP1) within the *Cpt1a* gene[107]. Understanding how these epigenetic alterations are modulated by environmental exposures holds promise for understanding the increased NAFLD susceptibility caused by EDC exposures as well as the gender bias underlying the observation that female rats are refractory to BPA-induced steatosis. Some gender-bias might be EDC-specific, as in other studies both male and female mice and rats exposed to EDCs, such as TBT, polycyclic aromatic hydrocarbons (PAHs) and PCBs, displayed an observable NAFLD-like phenotype (see Supplementary information S1 (table)).

In addition to BPA, other EDCs can enhance NAFLD when promoted by a HFD (for example, treatment of mice with PFOA[108] or the pesticides CYP or ATZ[109]). Importantly, some EDC exposures alone failed to trigger disease, with NAFLD observed only when EDC exposure was combined with a HFD. For example, treatment of male mice with PCB153 alone did not lead to NAFLD, whereas NAFLD was observed in HFD-fed animals exposed to PCB153[110]. Finally, some EDC exposures might not increase HFD-induced hepatic steatosis, but rather induce a NASH-like phenotype instead. An example of this phenomenon was reported for adult male mice treated with the PCB mixture Aroclor 1260[111].

Taken together, the existing animal data suggest that EDC exposures might promote NAFLD, and in some cases, NASH and fibrosis as well. However, critical unsettled questions still remain with regard to exactly how an EDC exposure can promote NAFLD.

Whether EDCs affect mainly hepatocytes or influence the activity and expansion and/or recruitment of hepatic macrophages, sinusoidal endothelial cells or stellate cells remains to be clarified. Although studies with the aforementioned EDCs certainly support a causal link between EDC exposure and NAFLD (see Supplementary information S1 (table)), they do not inform as to which NRs, coregulator complexes or epigenetic marks are involved in the increased disease susceptibility. Future studies should assay the appearance or loss of NAFLD in liver-specific NR knockout mice to define the NR signalling axis activated by a given EDC exposure. This knowledge could guide targeting the correct NR with a selective ligand for therapeutic purposes. In addition, as NAFLD is observed most often in experimental animal studies using a combination of EDC exposure (especially during early life) and a HFD, from a prevention standpoint, defining the interaction between EDC exposure and diets that promote this disease could be important, in addition to efforts to improve dietary habits by encouraging consumption of a low-fat diet.

In human epidemiological studies, several inherent challenges exist in comparing exposed with non-exposed populations, which makes drawing causal inferences from such studies exceedingly difficult. For example, human exposure to some EDCs, such as short-lived BPA, is nearly ubiquitous, with up to 95% of all people in the USA having detectable levels of BPA in their urine[14]. In the case of POPs such as dioxins and PCBs, the EDCs are very long-lived, which can result in continual exposure to animals and watersheds that humans consume. The available literature contains cross-sectional epidemiological studies which, by nature, lack the power of causal prediction. In these studies, several EDCs have been associated with either disrupted liver function (measured by levels of liver enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), or γ-glutamyl transpeptidase (GGT)) or fatty liver (assayed rarely by biopsy or more frequently by ultrasound): BPA, TCDD, polychlorinated dibenzo-*p*-dioxins, dibenzofurans (polychlorinated dibenzodioxins and polychlorinated dibenzofurans), POPs (17 dioxins or furans and 18 PCB congeners) and PCBs (see Supplementary information S2 (table)). Importantly, one of these human association studies found that one-third of 55 men exposed to TCDD during a 10-year period had liver biopsy histologies revealing not only steatosis, but also fibrosis or macrophage infiltration[112]. Of note, altered enzymatic markers of liver function more correctly represent ‘liver damage’ rather than NAFLD; although elevated serum ALT and AST levels are the primary abnormality observed in patients with NAFLD, liver enzyme levels can be normal in up to 78% of patients with NAFLD[113]. Overall, the limited epidemiological human data to date suggest an association, but are insufficient to conclude a cause–effect relationship for EDC exposure and NAFLD in humans.

Mechanisms of EDC action—A central mechanism by which EDCs are thought to exert long-term adverse health effects is by inducing alterations in the epigenome, which due to the heritable nature of epigenetic programs, can persist across many cell generations and throughout the lifecourse. The term ‘epigenetics’ was coined to describe a process in which variations in gene expression give rise to distinct patterns of differentiation[114]. A more modern definition of epigenetics is the heritable alterations that regulate gene expression in the absence of changes in DNA sequence. While every cell in the human body shares essentially the same DNA sequence, epigenetic processes (sometimes refered to as

"programs") determine the phenotypic heterogeneity observed in different cell types of various tissues and organs throughout the body, and both normal and abnormal physiological function.

DNA methylation was the first identified molecular mechanism for epigenetic regulation of gene expression[115, 116] and occurs by enzymatic transfer of a methyl group to cytosine bases of DNA, giving rise to 5-methylcytosine. The addition of methyl groups is the function of DNA methyltransferases (DNMTs), whereas removal of these methyl groups, and formation of oxidized derivatives of 5-methylcytosine, is the function of ten-eleven translocation (TET) enzymes (FIG. 5 and 6). DNA methylation alters the conformation of DNA via the action of methyl-binding proteins that inhibit transactivation of gene expression by preventing binding of transcription factors to promoters[117–119] and via recruitment of chromatin-remodelling complexes that lock DNA in a closed chromatin structure[120].

Histone proteins are stably associated with DNA and form the basic scaffolding structure for DNA — the nucleosome[121]. The four core histone proteins (H2A, H2B, H3 and H4) are subject to post-translational modification of their N-terminal region (known as the histone 'tail'), which protrudes out from the nucleosome and provides a platform for the assembly of protein complexes and proteins that 'read' epigenetics marks on these tails. Post-translational modification of histones modulates chromatin conformation and gene expression by altering the binding sites for proteins that regulate gene expression, or by facilitating the formation of secondary chromatin structure that controls chromatin accessibility. Combinations of post-translational modifications are both variable and dynamic, generating a 'histone code' or complex language for transcriptional regulation[122, 123]. Like DNA methylation, methylation of histones is a stable epigenetic modification and patterns of histone methylation can be epigenetically inherited across cell divisions and the lifecourse.

Histone methylation is regulated by the action of histone methyltransferases (HMTs) and histone demethylases (HDMs) that add or remove methyl groups, respectively (FIG. 6). Histone methylation is associated with both transcriptional activation and repression depending on the specific residue modified. Histones can also be acetylated (a transient modification compared to methylation) by histone acetyltransferase (HAT) enzymes; acetylation is associated with open chromatin conformation and activation of gene expression[124]. Conversely, deacetylation of histones by histone deacetylases (HDACs) promotes condensation of chromatin and repression of transcription[125, 126].

Methyl groups for both DNA and histone methylation are derived from one-carbon metabolism and utilize the same methyl donor, *S*-Adenosyl methionine (SAM). Both DNMTs and HMTs transfer methyl groups from SAM to cytosine in DNA and lysine or arginine residues on histone tails, respectively, forming the byproduct *S*-Adenosyl-L-homocysteine (SAH; FIG. 5). The liver has a major role in SAM metabolism, with SAM biosynthesis and degradation regulated by the enzymes methionine adenosyltransferase (MAT) and glycine-*N*-methyltransferase (GNMT), respectively[127]. Maintenance of SAM homeostasis is necessary for liver health and to prevent injury and HCC[4, 128]. For example, *Mat1a* knockout (chronic SAM deficiency) in mice results in increased susceptibility to

steatosis in response to a choline-deficient diet and spontaneous development of NASH[129]. *Gnmt* knockout mice (chronic SAM excess) also develop liver steatosis, fibrosis and HCC concordant with increased DNA and histone methylation[130]. Interestingly, the observed liver phenotype and DNA hypermethylation in *Gnmt* knockout mice can be reversed upon treatment with nicotinamide, which markedly reduces SAM levels[131]. Furthermore, children with mutations in *GNMT* exhibit mild to moderate liver disease[132, 133]. Collectively, these data support a role for disruption of SAM homeostasis in the development of liver disease.

In adult rodents, exposure to a methyl-deficient diet (MDD) results in increased hepatic steatosis and alteration of DNA methylation[134–137]. For example, MDD causes hypermethylation of *Ahcy*, the gene that encodes the enzyme responsible for hydrolysis of SAH, and thus increased SAH levels[137]. Interestingly, individual mouse strains exhibit differential susceptibility to MDD-induced liver disease, and this difference might be due to inter-strain epigenetic differences. For example, in the WSB/EiJ strain that exhibits severe NASH-like liver injury compared to the A/J strain that exhibits mild NAFLD-like liver injury in response to MDD[135, 136], increased DNA methylation at gene promoters and increased *Dnmt1* and *Dnmt3a* expression[136] have been reported in the more susceptible strain, consistent with the hypothesis that epigenetic alterations might have a role in modulating NAFLD susceptibility.

Few studies to date have examined the role of histone modifications in NAFLD, but evidence exists that an imbalance between HATs and HDACs might have a role in the progression of NAFLD[138]. For example, liver-specific knockout of the HDAC gene *Sirt1*, increases susceptibility to HFD-induced hepatic steatosis[139]. In addition, HDAC3 has been shown to control hepatic lipogenesis in a circadian fashion and deletion of *Hdac3* causes hepatic steatosis[140]. Furthermore, adult mice fed a HFD exhibit altered histone acetylation at genes involved in the inflammatory response[141]. Studies in primates have examined the epigenetic effects of maternal diet on liver disease in offspring. Maternal HFD alters histone acetylation in the livers of offspring, with a concomitant increase in lipogenic gene expression and a decrease in HDAC1 and SIRT1 expression and activity[142, 143]. Interestingly, these effects can be abrogated with diet reversal[143].

A similar lack of data exists for alterations in DNA methylation that are associated with NAFLD, although nutrient modulation of DNA methylation in the context of obesity has been demonstrated in the *agouti* mouse model[144, 145]. Constitutive, ectopic *agouti* transcription (due to altered DNA methylation) results in a yellow coat phenotype, as well as increased susceptibility to diabetes mellitus, obesity and tumorigenesis[146, 147]. Maternal nutrient supplementation with the phyto-oestrogen (and EDC) genistein alters coat color and protects offspring from obesity by modifying the fetal epigenome[148]. Supplementation with genistein (or folic acid) also counteracts BPA-mediated DNA hypomethylation in early development in this mouse model[149]. These studies support the use of the *agouti* mouse as a biosensor for the study of epigenomic modulation by the environment[144], including future studies aimed at examining the link between DNA methylation and the development of NAFLD. In humans, only a handful of studies have reported gene-specific alterations in DNA methylation in patients with advanced NAFLD compared with mild NAFLD[150, 151], highlighting the need for additional research in this area.

Liver disease and environmental exposures across the lifecourse—Although adverse environmental exposures can occur at any time along the lifecourse to increase the risk of disease, the perinatal period might represent a window of particular vulnerability[152]. For example, in the context of rodent models of nutritional modulation, a maternal energy-rich diet is associated with the development of NAFLD in offspring[153–161]. In addition, as mentioned earlier, studies in humans have shown that fetal exposure to famine 'mis-matched' with a nutrient-rich adult environment[19, 162] is associated with the development of hepatic steatosis. Similarly, perinatal exposure to EDCs can result in adult susceptibility to development of NAFLD in rodent models (see Supplementary information S1 (table)), although only a limited number of these studies examined epigenetic alterations that could be responsible for NAFLD susceptibility. To date, most studies have focused on epigenetic alterations associated with the disease itself, rather than a change in susceptibility of the liver that precedes disease onset. Therefore it remains an attractive, but untested, hypothesis that early-life exposure to EDCs might increase the risk of liver disease by altering patterns of DNA and/or histone methylation, and thereby changing physiological 'set-points' in the liver to reprogram hepatic gene expression programs to promote NAFLD (FIG. 6).

An intriguing, but underexplored aspect of the EDC–NAFLD linkage is the interplay between EDC exposure, obesity and NAFLD. As obesity is a known risk factor for NAFLD, early-life exposure to EDCs that act as 'obesogens' could increase susceptibility to NAFLD via increasing susceptibility to obesity. Early-life EDC exposures could also deliver a 'double hit', by altering both liver physiological set-points in concert with other physiological changes that increase the propensity for obesity. Alternatively, susceptibility to the NAFLD-promoting effects of later-life EDC exposure could be enhanced in obese individuals. Just as obesity combined with alcohol increases the risk of fatty liver disease, so could the physiological effects of other risk factors such as obesity, a HFD and T2DM combined with EDC exposure. Investigating the impact of early-life EDC exposures, as well as later-life exposures in the setting of other risk factors such as obesity, are therefore important future areas of study for understanding the potential contribution of EDCs to the development and progression of NAFLD (FIG. 7).

Conclusions

NAFLD, the fastest growing and most prevalent liver disease worldwide, represents a spectrum of diseases from simple steatosis to steatohepatitis that can progress to fatal cirrhosis and HCC. In addition to obesity and fructose as risk factors for NAFLD development and progression, certain environmental exposures to chemicals such as EDCs might increase susceptibility to NAFLD and/or co-operate with a high-fat Western diet to promote development of this disease. One mechanism of EDC action involves physical binding to NRs, which then can recruit coregulator proteins (either coactivators or corepressors) to modulate transcription of hepatic lipid homeostasis gene expression programs to favour NAFLD. In addition, early-life EDC exposures can impact the epigenome, altering DNA methylation and/or histone modifications, to affect metabolic reprogramming via altered expression of hepatic lipid pathway genes. Such reprogramming of the epigenome during development in response to nutrient availability is well established; EDC exposure in early-life might similarly reprogram hepatic lipid homeostasis gene

programs toward a metabolic ‘set point’ that promotes NAFLD. Additionally, EDC exposure in adulthood might also contribute to NAFLD in combination with other prevalent predisposing factors, such as diets rich in fat, a BMI >30 kg/m^2 and T2DM. We hope this Review will encourage more mechanistic studies aimed at better understanding how EDC exposures impact the epigenome to alter the expression of genes associated with hepatic lipid metabolism, which in turn promote the development of NAFLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary terms

NAFLD	nonalcoholic fatty liver disease
HCC	hepatocellular carcinoma
EDCs	endocrine-disrupting chemicals
NR	nuclear hormone receptor
NASH	nonalcoholic steatohepatitis
HFD	high-fat diet
BMI	body mass index
MDCs	metabolism-disrupting chemicals
BPA	bisphenol A
NRs	nuclear hormone receptors
NHANES	National Health and Nutrition Examination Survey
T2DM	type 2 diabetes mellitus
FFAs	free fatty acids
VLDL	very low density lipoprotein
PUFAs	polyunsaturated fatty acids
POPs	persistent organic pollutants
PCBs	polychlorinated biphenyls
DDT	dichlorodiphenyltrichloroethane

DDE	dichlorodiphenyldichloroethylene
DEHP	di-2-ethylhexyl phthalate
TBT	tributyltin
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
CYP	cypermethrin
ATZ	atrazine
SRC	steroid receptor coactivator
SERMs	selective oestrogen receptor modulators
MAPKs	mitogen-activated protein kinases
ER	oestrogen receptor
AR	androgen receptor
PR	progesterone receptor
PPAR	peroxisome proliferator-activated receptor
RXR	retinoid X receptor
TR	thyroid receptor
RAR	retinoic acid receptor
LXR	liver X receptor
FXR	farnesoid X receptor
CAR	constitutive androstane receptor
PXR	pregnane X receptor
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)]benzene
CITCO	6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime
PCN	pregnenolone 16α-carbonitrile
SHP	short heterodimer partner
PGC1	PPARγ coactivator 1
MED1	Mediator complex subunit MED1
NCoR	nuclear receptor corepressor

SMRT	silencing mediator of retinoic acid and thyroid hormone receptor
HDAC	histone deacetylase
RIP140	receptor-interacting protein 140
LCOR	ligand-dependent corepressor
SMILE	SHP interacting leucine zipper protein
GR	glucocorticoid receptor
AhR	aryl hydrocarbon receptor
K_d	equilibrium dissociation constant
TCDD	2,3,7,8-tetrachlorodibenzodioxin
HxCDD	hexachlorodibenzo-*p*-dioxin
PAHs	polycyclic aromatic hydrocarbons
AST	aspartate transaminase
ALT	alanine transaminase
GGT	γ-glutamyl transpeptidase
DNMTs	DNA methyltransferases
TET	ten-eleven translocation
HMTs	histone methyltransferases
HDMs	histone demethylases
HAT	histone acetyltransferase
HDACs	histone deacetylases
SAM	*S*-Adenosyl methionine
SAH	*S*-Adenosyl-L-homocysteine
MAT	methionine adenosyltransferase
GNMT	glycine-*N*-methyltransferase
MDD	methyl-deficient diet

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Biographies

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Brian York is an Associate Professor in the Department of Molecular and Cellular Biology and the Dan L. Duncan Cancer Center at Baylor College of Medicine, Houston, Texas, USA. He received his PhD in biochemistry from the University of Kentucky, Lexington, Kentucky, USA, and completed postdoctoral training with Bert W. O'Malley. The research focus of the York laboratory is to elucidate the metabolic events involved in transcriptional reprogramming of the liver during the spectrum of hepatic pathology that begins with benign hepatic steatosis but progresses to nonalcoholic fatty liver disease, nonalcoholic steatohepatitis and ultimately to hepatocellular carcinoma.

Cheryl L. Walker is Director of the Center for Precision Environmental Health and Professor in the Departments of Molecular and Cellular Biology and Medicine at Baylor College of Medicine, Houston, Texas, USA. She received her PhD from the University of Texas Southwestern Medical School, Dallas, Texas, USA and completed her postdoctoral training at the National Institute of Environmental Health Sciences, North Carolina, USA. A major research focus of the Walker laboratory is on gene–environment interactions that contribute to disease pathogenesis, and on elucidating how epigenomic plasticity creates a vulnerability to environmental exposures that cause metabolic disease, obesity and cancer.

Review criteria

Full-text papers written in English cited in this review were selected based on Pubmed searches using the following search terms: “endocrine disruptor or EDC”, “fatty liver or NAFLD”, “epigenetic”, “mice”, “rat”, “human”, “NASH”, “lipogenesis”, “reprogramming” without a publication year cutoff. Additional references found in these publications provided additional papers to search and cite. Many of cited studies on nuclear receptors, coregulators and epigenetics came from our collective background knowledge.

Key points

- Nonalcoholic fatty liver disease (NAFLD) is a growing epidemic in countries consuming a Western diet, and can lead to irreversible cirrhosis and hepatocellular carcinoma
- Exposure to endocrine-disrupting chemicals (EDCs) in early life could represent a ‘new’ risk factor for the development of NAFLD later in life
- EDCs mechanism of action involves both modulation of nuclear hormone receptor (NR) function via coregulator proteins and alteration of the epigenome (DNA methylation and histone modification)
- Animal model studies suggest causality between certain early-life EDC exposures and NAFLD presentation later in life
- Studies are needed to define causality of an EDC exposure in humans with development of NAFLD, as well as to develop new prevention and treatment regimes

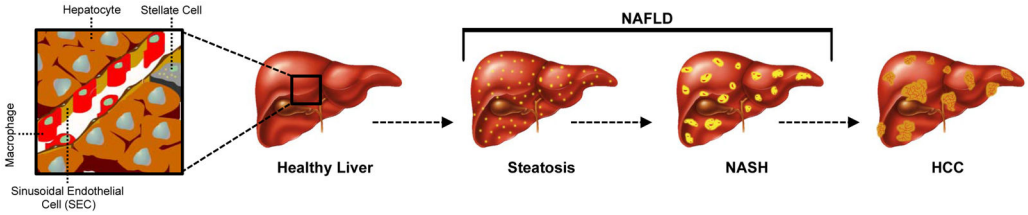

Figure 1. Pathophysiology of NAFLD progression

From left to right: a healthy liver is presented, that upon presentation of 'risk factors' such as obesity, fructose consumption and/or exposure to endocrine-disrupting chemicals, leads to lipid accumulation (depicted as small yellow dots) in the liver (steatosis), which is the first (reversible) stage of nonalcoholic fatty liver disease (NAFLD). Activation and/or recruitment of macrophages to the liver leads to nonalcoholic steatohepatitis (NASH) and eventual fibrosis. Importantly, progression to NASH and the development of fibrotic and/or cirrhotic lesions (not pictured) represent an irreversible stage of liver disease. Left untreated, a subset of cases culminates in the development of neoplastic events that give rise to hepatocellular carcinoma (HCC), with or without cirrhosis, as the final endpoint of hepatic disease progression.

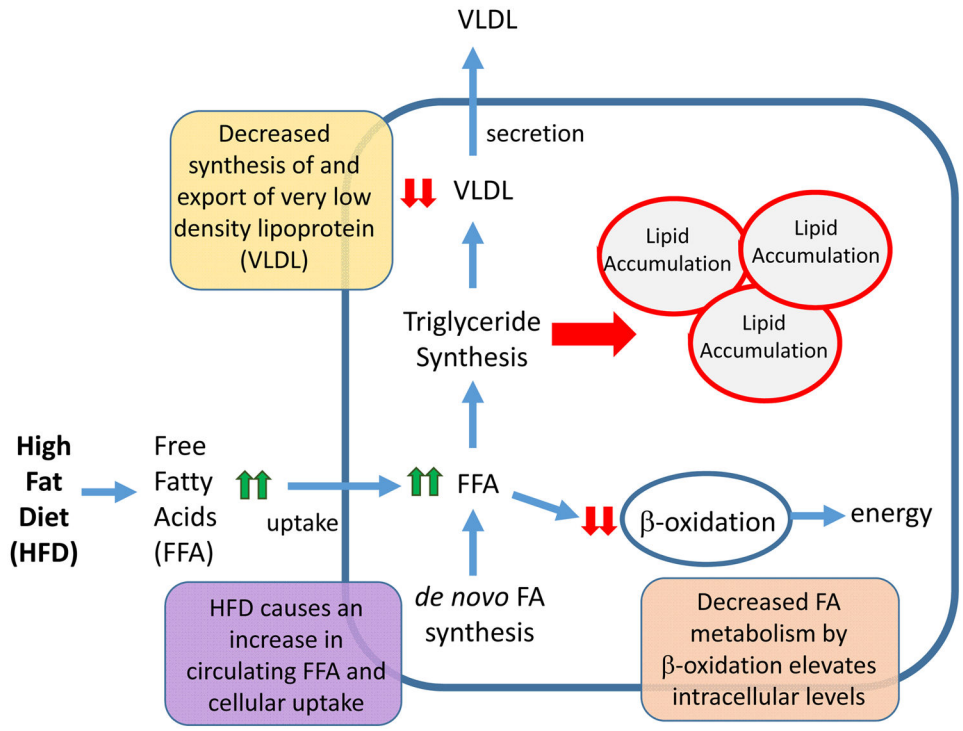

Figure 2. Altered hepatic metabolic pathways leading to NAFLD

The liver is central to the maintenance of whole-body lipid homeostasis. Mechanistically, uptake of dietary fats is facilitated by release of bile acids that are synthesized in the liver and secreted by the gall bladder into the intestine. Bile salts emulsify fat, creating free fatty acids (FFAs) and monoglycerides, which are rapidly absorbed by enterocytes of the intestine. In the intestine, FFAs and monoglycerides are resynthesized into triglycerides, which are packaged into chylomicrons and are taken up by the liver via receptor-mediated endocytosis. The liver is also is responsible for converting carbohydrates and protein into FFAs, which are packaged into triglycerides and exported from the liver as VLDL. The liver is also the primary source of β-oxidation that serves to metabolize FFAs to produce energy in the form of ATP, as well as to generate ketone bodies that are used as an alternative fuel source during periods of fasting. Altogether the balance between lipid uptake and release, triglyceride synthesis and β-oxidation helps to preserve energy homeostasis in the liver. Disruption of these processes by a high-fat diet (HFD) is accompanied by aberrant lipid accumulation in the liver, which leads to a cascade of pathologies ranging from steatosis to hepatocellular carcinoma. Endocrine-disrupting chemicals (EDCs) can also promote nonalcoholic fatty liver disease (NAFLD), either alone or with a HFD, by increasing FFA uptake, increasing *de novo* lipogenesis, decreasing triglyceride export via VLDL, and/or decreasing FFA β-oxidation.

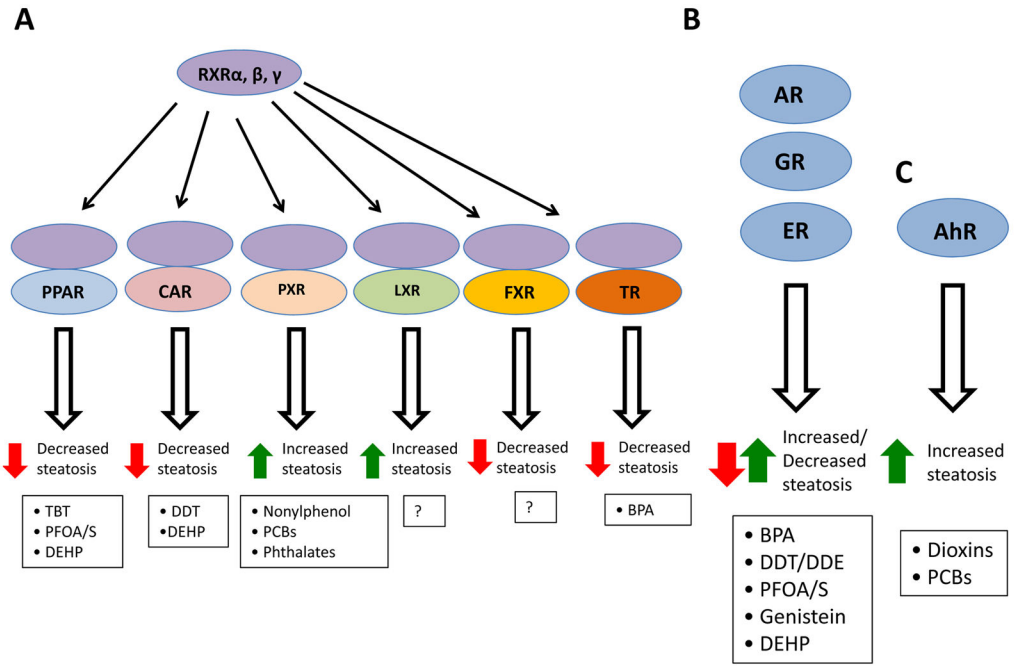

Figure 3. NR-mediated effects of EDCs on fatty liver development

a The NR1 subfamily of nuclear hormone receptors (NRs) heterodimerize with retinoid X receptors (RXRs) to either promote (pregnane X receptor (PXR) or liver X receptor (LXR)) or inhibit (peroxisome proliferator-activated receptors (PPARs), constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and thyroid receptors (TRs)) hepatic steatosis upon binding their naturally occurring agonist ligands. Select endocrine-disrupting chemicals (EDCs) known to bind these NRs and affect their activity are depicted at the bottom of the figure. For example, tributyltin (TBT) binding RXR–PPAR enhances steatosis, unlike natural free fatty acid ligands. LXR activates lipogenic genes upon binding its natural ligands (oxysterols) and promotes steatosis, but whether its activity is modulated by specific EDCs is currently unclear. **b** Another major class of NRs that bind EDCs is the steroid receptors such as the androgen receptor (AR), glucocorticoid receptor (GR) and oestrogen receptor (ER). Steroid hormones can either increase (glucocorticoid) or decrease (oestrogen and androgen) hepatic steatosis. Select EDCs known to bind these NRs and affect their activity are depicted at the bottom of the figure. **c** Aryl hydrocarbon receptor (AhR) represents the third major NR effector of EDC action in the liver. AhR binds EDCs, such as dioxins and polychlorinated biphenyls (PCBs), leading to enhanced steatosis. BPA, bisphenol A; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DEHP, di-2-ethylhexyl phthalate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

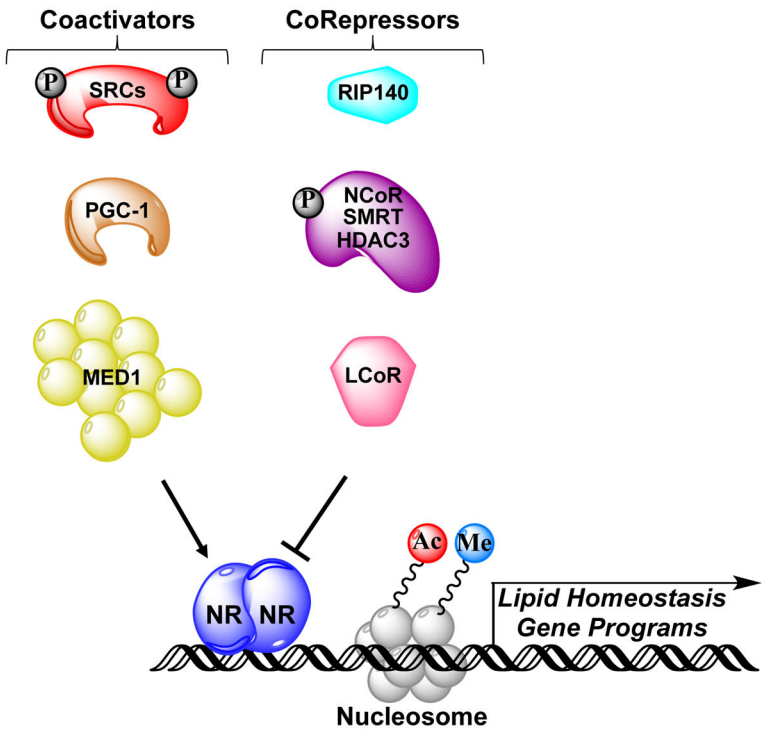

Figure 4. Potential genomic mechanism of EDC action

Once an endocrine-disrupting chemical (EDC) enters the liver, it is bound by specific nuclear hormone receptors (NRs). This action can either positively or negatively affect transcription of lipid homeostasis genes via specific EDC–NR complexes that recruit coactivators or corepressors to target genes. Key coactivators that modulate fatty liver progression include steroid receptor coactivators (SRCs), peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) and mediator of RNA polymerase II transcription subunit 1 (MED1), whereas nuclear receptor corepressor (NCoR)–silencing mediator of retinoic acid and thyroid hormone receptor (SMRT)–histone deacetylase 3 (HDAC3) complexes, receptor-interacting protein 140 (RIP140) and ligand-dependent corepressor (LCOR) act as corepressors. Coactivator complexes induce histone modifications associated with active gene transcription, such as acetylation (Ac) and methylation (Me), whereas corepressors generally utilize associated histone deacetylases or demethylases to remove these marks. SRCs and NCoR are subject to regulatory phosphorylation (P) events. Engagement of coregulators by EDC-bound NRs results in modulation of lipid homeostasis gene cassettes and/or reprogramming of the epigenome, which ultimately promotes NAFLD: for example, via enhanced lipogenesis gene expression and/or inhibition of free fatty acid-oxidation gene expression.

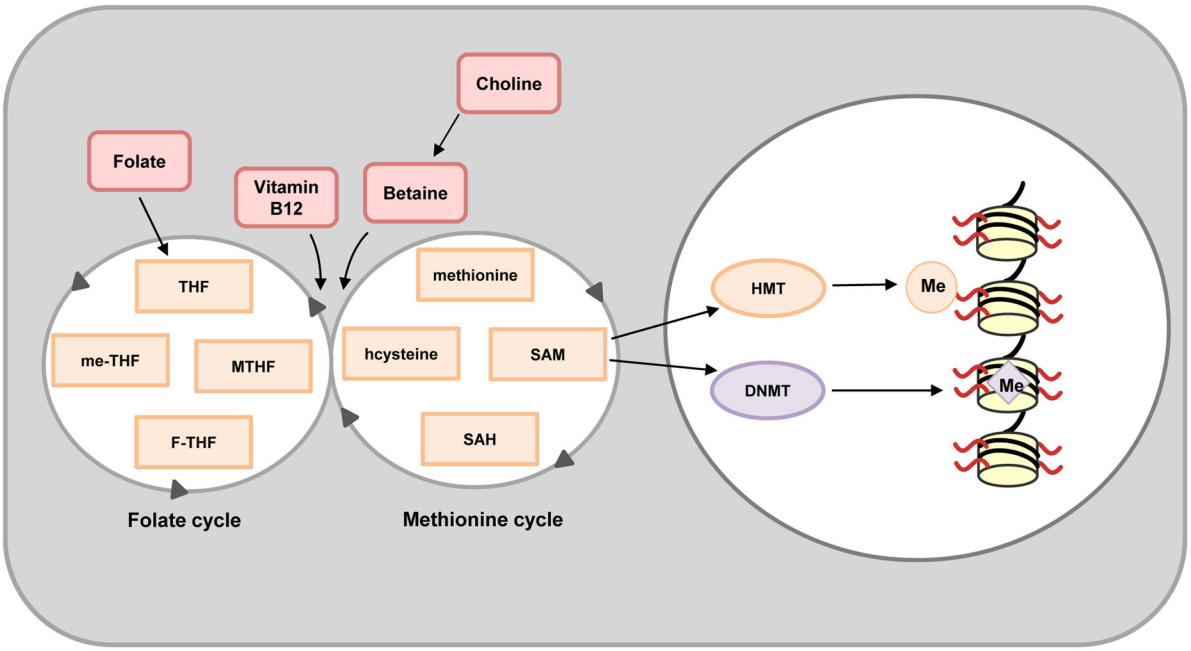

Figure 5. Epigenomic action of 'writers' of DNA or histone methylation

Specific arginine and lysine residues on histone tails are methylated by distinct histone methyltransferases (HMTs), whereas DNA methylation occurs via the action of DNA methyltransferases (DNMTs). Both HMTs and DNMTs utilize *S*-Adenosyl methionine (SAM) as their methyl (Me) donor. SAM is created from methionine and its levels are influenced by methionine and interconnected folate cycles. Importantly, high folate maternal diets have been shown to affect DNA methylation patterns in rodent offspring[163–165]. F-THF, 10-formyltetrahydrofolate; me-THF, 5,10-methylene-THF; MTHF, 5-methyltetrahydrofolate; SAH, *S*-Adenosyl-L-homocysteine; THF, tetrahydrofolate.

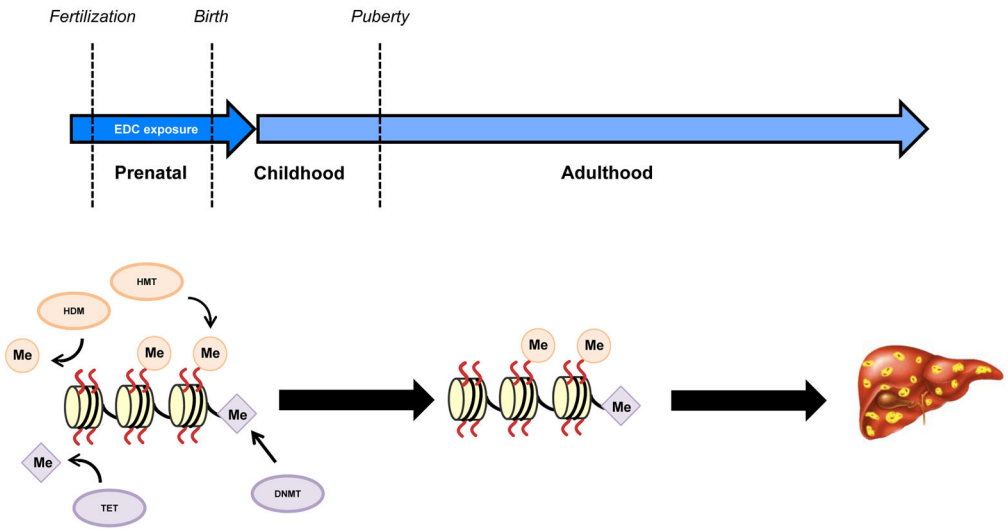

Figure 6. Early-life exposure to EDCs trigger the development of NAFLD

Exposure to endocrine-disrupting chemicals (EDCs) during the prenatal period (a critical 'window of susceptibility') can result in changes to the liver epigenome that influence susceptibility to liver disease in adulthood. The activity of epigenetic 'writers' of DNA (DNA methyltransferases; DNMTs) or histone (histone methyltransferases; HMTs) methyl marks (Me) or 'erasers' of these heritable marks (ten-eleven translocation (TET) or histone demethylases (HDMs), respectively) can be influenced by a prenatal EDC exposure, which changes their activity and alters the epigenome. Such epigenetic reprogramming could confer a propensity to develop nonalcoholic fatty liver disease (NAFLD) in adulthood via reprogrammed expression of genes involved in lipid homeostasis.

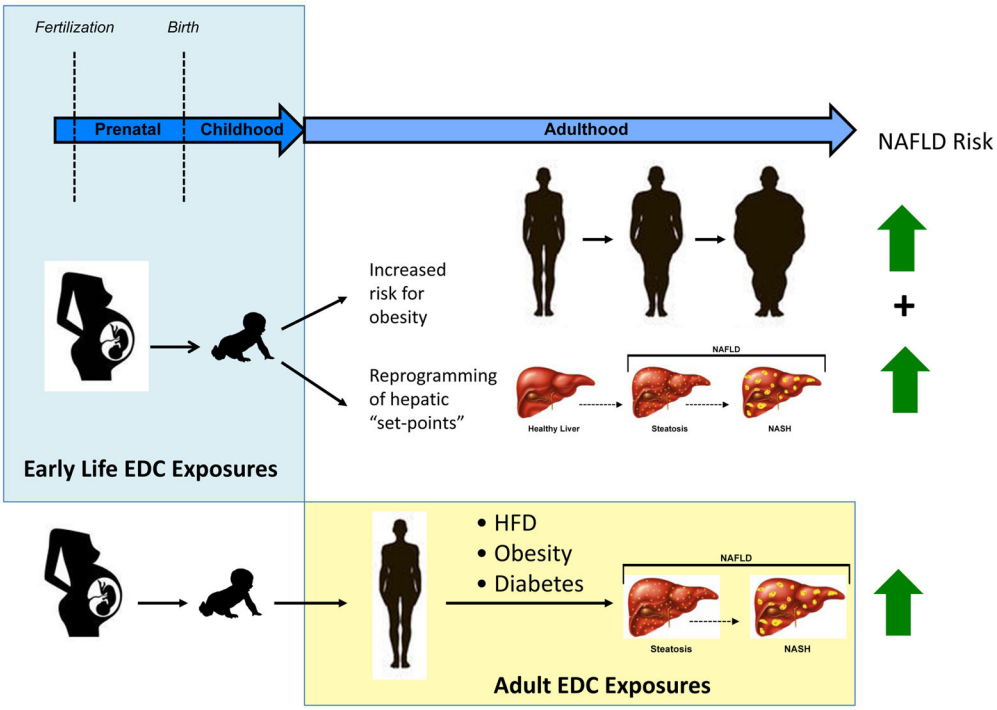

Figure 7. EDCs and NAFLD risk across the life-course

Endocrine-disrupting chemicals (EDCs) can alter susceptibility to develop nonalcoholic fatty liver disease (NAFLD) via early-life effects that increase susceptibility to obesity and alter hepatic 'set-points that favour the development of fatty liver, and later-life effects that contribute to the development of liver disease alone or in combination with other NAFLD risk factors such as diet, diabetes mellitus and/or obesity. HFD, high-fat diet; NASH, nonalcoholic steatohepatitis.