Role of estrogen and endocrine disrupting chemicals in the development of non-alcoholic fatty liver disease in women

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Abstract

One of the most prevalent chronic liver diseases, non-alcoholic fatty liver disease (NAFLD), is characterized by accumulation of fat in the liver. Failures in the regulation of *de novo* lipogenesis, increased delivery of fatty acids to the liver, and decreased lipid clearance due to impaired fatty acid oxidation and lower lipid secretion are the main causes for fat to accumulate in the liver. However, the reason why some people accumulate fat in the liver while others do not is poorly understood.

A wide range of diseases and conditions are known to increase the risk of developing NAFLD. Furthermore, the role of estrogen in the development of NAFLD has been under investigation in the past years, however the exact mechanisms in which estrogen exerts this protection is still unknown. Another factor, next to steroid hormones, that has been described to play a role in inducing NAFLD in both men and women is found in the environment. More specifically, a group of chemicals known to disrupt or alter the function of endocrine and metabolic organs such as the liver; endocrine disrupting chemicals. In this review the role of estrogen and estrogen like endocrine disruptors in the development of NAFLD in women is explored.

Main results show that in males hepatic ERα plays a role in the development of liver steatosis after EEDC exposure. In females, EEDCs seem to directly influencing downstream regulators of hepatic lipid metabolism. Among other results, this concludes that the role of estrogen and EEDC exposure in the development of NAFLD in females is way more complex than in males. Multiple mechanisms of action that provide a link between EEDC exposure and NAFLD development are proposed in this review, however more research has to be performed to concluded if these mechanisms are true.

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Introduction

One of the most prevalent chronic liver diseases[1], non-alcoholic fatty liver disease (NAFLD), presents itself in approximately 25.24% of the world population[2]. Accumulation of fat in the liver is the main symptom present in NAFLD. Failures in the regulation of *de novo* lipogenesis, increased delivery of fatty acids to the liver, and decreased lipid clearance due to impaired fatty acid oxidation and lower lipid secretion are the main causes for fat to accumulate in the liver[3]. However, the reason why some people accumulate fat in the liver while others do not is poorly understood[4].

A wide range of diseases and conditions are known to increase the risk of developing NAFLD. A few examples of these diseases and conditions are: metabolic syndrome, obesity and type 2 diabetes; which are all characterized by defects in metabolic flexibility and signaling[5] Interestingly, several studies have shown that the risk of developing NAFLD is also higher in healthy post-menopausal women compared to healthy pre-menopausal women[6,7]. After a women experiences her last regular menstrual cycle, perimenopause starts. In this period estrogen levels drop significantly until menopause is reached, at this point estrogen levels are almost non-existent. This level of estrogen carries on in the post-menopausal phase[8]. Taking into account that estrogen levels significantly differ between pre- and post-menopausal women it can be suggested that estrogen could play a significant role in protection against development of NAFLD. The role of estrogen in the development of NAFLD has been under investigation in the past years, however the exact mechanisms in which estrogen exerts this protection is still unknown[9,10].

Another factor, next to female steroid hormones, that has been described to play a role in inducing NAFLD in both men and women is found in the environment[11,12]. These so-called environmental factors are defined by a group of chemicals known to disrupt or alter the function of endocrine and metabolic organs such as the liver[11–13]; also known as endocrine disrupting chemicals (EDCs). Specific chemicals disrupting endocrine and metabolic organs are found in daily used products such as processed food, plastics, make-up, perfume or household products[14]. EDCs can be divided into several categories; one of these categories being estrogen like endocrine disruptors (EEDCs)[15]. As the name already implies, EEDCs are known to interfere with estrogen signaling and the metabolism of estrogen. Interestingly, the effect that EEDCs have on endocrine function differs between males and females[16,17], suggesting that the mechanism how the disruptors affect liver function is gender-specific and is able to specifically targeting sex hormones. Most of the studies performed to date focus on estrogen receptor α (ERα), which is found to play a significant role in development of NAFLD in males[18]. In female subjects the evidence on the role of EEDCs in the development of NAFLD is scarce.

Therefore, this review aims to explore the role of estrogen and estrogen like endocrine disruptors in the development of NAFLD in women by (1) describing basic liver physiology along with the molecular pathways that affect hepatic lipid homeostasis and how they impact NAFLD development, (2) assessing the role of estrogen in hepatic lipid metabolism and protection against development of NAFLD, (3) reviewing the different mechanisms by which estrogen like endocrine disruptors induce NAFLD and (4) looking into exposure to estrogen like endocrine disruptors in different stages of life and their ability affect future generations by inducing epigenetic modifications.

Liver physiology and functions

The largest glandular organ in the body is the liver, characterized by its compartmentalized structure. So called lobules within the liver consist of hepatocytes with spaces between them called sinusoids, which are composed of specialized sinusoidal endothelial cells[19] (**Figure 1**). Blood from the portal triads is received by the sinusoids, bathes the hepatocytes and then exits the liver into the central vein.

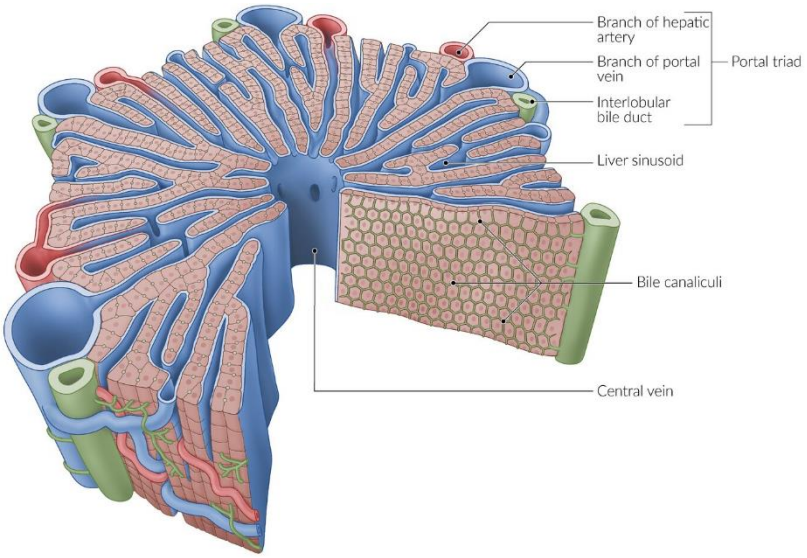

Figure 1: *Anatomy of a liver lobule, exhibiting hepatocytes separated by sinusoids*[19].

The liver can be considered as the epicenter for metabolic homeostasis. Some functions performed by the liver are: hormone biosynthesis and turnover, protein and bile synthesis, detoxification, lipid metabolism, glycogen storage and release, and gluconeogenesis[20]. As mentioned earlier, the development of NAFLD is characterized by accumulation of fat in the liver. Reports show that mainly hepatic lipid metabolism is altered in this disease[21,22]. Generally, lipid metabolism in liver hepatocytes can be summarized by the acquisition; storage and consumption of lipids (**Figure 2**)[3]. The acquisition of lipids includes uptake of lipids and fatty acids and *de novo* lipogenesis. Transport of hydrophobic fatty acids to the different cellular compartments within the cytoplasm is facilitated by fatty acid-binding protein-1 (FABP1) (**Figure 2**)[3]. Whereafter lipid storage takes place through synthesis of triglycerides and the formation of lipid droplets. Lastly, the consumption of lipids happens by fatty acid degradation or lipolysis, ß-oxidation and the secretion of very low density lipoproteins (VLDL).

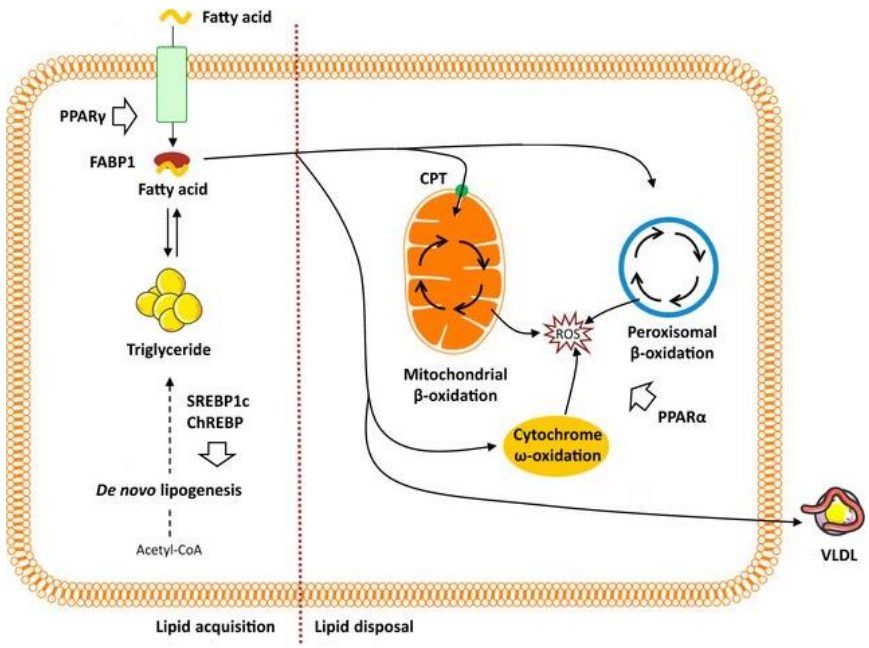

Figure 2: *Lipid metabolism in hepatocytes takes place through uptake and production of lipids, storage in the form of triglycerides and consumption via lipolysis; ß-oxidation and secretion of VLDL*[3].

All of these mechanisms are tightly regulated by so called nutrient sensors. Peroxisome proliferator-activated receptors (PPARs) contains several isotypes, which act in different compartments of lipid metabolism. PPARγ is identified as a nuclear receptor which regulates the uptake of circulating lipids by acting as a receptor for polyunsaturated fatty acids and their metabolites[23]. Next to this regulatory mechanism, another PPAR isotype; PPARα, plays a crucial role in the regulation of lipid consumption. This nutrient sensor reduces intrahepatic fat levels by utilizing lipids as an energy source through activation of ß-oxidation. More specifically, PPARα promotes ß-oxidation of acetyl-CoA by inducing the activity of enzymes such as medium chain coenzyme A dehydrogenase and cytochrome P450 fatty acid-hydroxylase[24].

Secondly, the enzyme carnitine palmitoyl transferase-I is up regulated by PPARα[25], which catalyzes the rate-limiting step in the translocation of acetyl-CoA across the mitochondrial membrane and is therefore crucial for mitochondrial ß-oxidation. Lastly, carbohydrate-responsive element-binding proteins (ChREBP) is a transcriptional inducer of glucose use and *de novo* lipogenesis by upregulating the expression of lipogenic genes[26,27].

Function of estrogen signaling and metabolism in hepatic lipid metabolism

Recently, sex hormones have been found to play a crucial role in regulation of lipid metabolism in the liver as well[28]. In particular the female sex hormone; estrogen regulates hepatic lipid metabolism through modulating uptake and metabolism of lipids in the liver[29]. Estrogens can be synthesized in non-reproductive tissue as liver, heart, muscle and brain[30]. There are three major endogenous estrogens that have estrogenic hormonal activity: estrone (E1), estradiol (E2), and estriol (E3)[30]. From the three estrogens, E2 is the most prevalent.

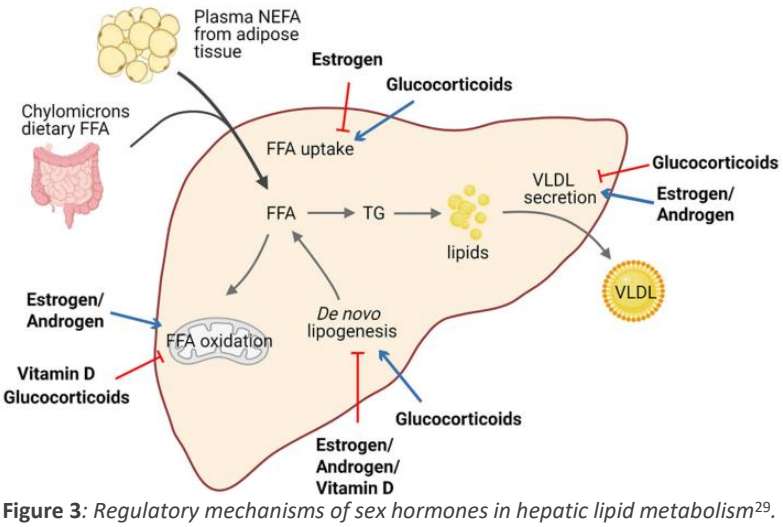

Figure 3: *Regulatory mechanisms of sex hormones in hepatic lipid metabolism*[29].

Estrogen signaling affects hepatic lipid metabolism at different sites[29]. In particular signaling through estrogen receptor α (ERα) has been shown to play a significant role in regulation of hepatic lipid metabolism[31]. Estrogen signaling through this receptor has been shown to limit liver fat accumulation by reducing *de novo* lipogenesis and the uptake of free fatty acids in the liver[32,33](**Figure 3**). Furthermore, fatty acid oxidation and very-low-density-lipoproteins (VLDL) secretion seems to be stimulated through ERα signaling[32,33](**Figure 3**).

Regulation of hepatic liver metabolism happens through action of estrogens, however the liver itself is able to regulate estrogen levels by metabolizing estrogen. In the liver estrogen is converted into inactive metabolites which leave the body through urine and feces[34]. Metabolism of estrogen takes place in three phases. It starts with hydroxylation, which is mediated by cytochrome P450 (CYP). This family contains a lot of isoforms of which mainly CYP1A1, CYP1A2 and CYP3A4 are active in the process of hydroxylation of estrogen[35]. Next, the hydroxylated metabolites are methylated by the enzyme catechol-O-methyltransferase (COMT)[36]. This step prevents the development of reactive oxygen species (ROS) capable of damaging cellular macromolecules such as DNA, lipids, and proteins. Lastly, the metabolites are conjugated with either glucuronic acid and sulfate by hepatic phase II enzymes including UDP-glucuronosyltransferases and sulfotransferases[37]. Conjugated metabolites are normally water soluble and excreted via the kidneys or bile. The final stage of estrogen metabolism is removing the metabolite from the cell, so it can then be excreted either through the bile or the urine.

Role of estrogen levels and ER signaling in development of NAFLD

As mentioned before chronic liver diseases are characterized by accumulation of fat in the liver. Furthermore, estrogen metabolism and signaling are proven to be active in maintenance of lipid homeostasis, suggesting that these mechanisms may play a pivotal role in preventing chronic liver disease[28]. Different types of chronic liver diseases are hepatic fibrosis, chronic hepatitis B and C infections and NAFLD[38]. Interestingly, the development of NAFLD occurs after impairment of the estrogen receptor α (ERα) in males[18]. This study used a mice model which contained a liver-specific knock out of ERα (LERKO mice). Activity of Acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) were measured, as these enzymes were suggested to be regulated by activity of ERα. These enzymes play important roles in *de novo* lipogenesis, as they catalyze the synthesis of

fatty acids from acetyl-CoA[39,40]. The results from the study in LERKO mice show an increase in lipogenic enzymes such as *FAS* and *ACC1*, indicating that regulation of lipid metabolism is impaired[18]. Similarly, global blocking ERα contributed to higher visceral fat accumulation and reduced energy expenditure in female and male mice[41]. This was found in a study using ERα-knockout (αERKO) mice, which display significant greater areas of epididymal and perirenal adipocytes in αERKO compared to wild-type (WT), additionally overall energy expenditure was significantly greater in WT males compared to αERKO males[41].

Both studies point out the importance of estrogen signaling in prevention of fat accumulation in both sexes. Furthermore, estrogens itself are known for their anti-inflammatory effects in different organs including the liver[42,43], suggesting that changes in estrogen levels are also able to influence a person's susceptibility to developing chronic liver disease. There is however some controversy present in the literature around what specifically drives the development of NAFLD in the light of estrogen levels. Although, some papers show that lower estrogen levels contribute to the development of NAFLD[18,30]. Others, have found contradicting evidence and show that an excess of estrogen contributes to the development of the disease[43–45]. Most of the studies that found a correlation between lower estrogen levels and development of NAFLD have been performed in small vertebrates such as mice and zebrafish, whereas the studies finding a correlation between higher estrogen levels and chronic liver disease are performed in humans and primary cell culture.

For instance, a study by Gutierrez-Grobe et al. showed that the lack of regulatory activity of estrogens in hepatocytes favors the development and progression of NAFLD[46]. This study investigated serum levels of estrogens in pre- and post-menopausal women. Results show a prevalence of NAFLD in nearly 60% in post-menopausal patients, whereas in 30% of the pre-menopausal women NAFLD was present. Furthermore, results indicated a higher probability of developing NAFLD with decreasing serum levels of estrogen[46]. Secondly, estrogen deficiency has been shown to promote non-alcoholic steatohepatitis (NASH) progression in female mice fed cholesterol-rich hyper lipidemic diet[47]. Estrogen treatment seems to improves NASH progression in these mice. In this study estrogen deficiency was induced in mice through ovariectomy, which is a technique where the ovaries are operationally removed. These mice were fed a cholesterol-rich hyper lipidemic diet, which is a dietary model NASH. Hypercholesterolemia is an important risk factor for NASH progression, and is next to NAFLD often observed after menopause[48]. Lastly, ovarian senescence; which is defined by a reduce number and quality of ovarian follicles, is proven to facilitate both the development of hepatic steatosis and the fibrotic progression of liver disease[49]. This study used overfed zebrafishes of both sexes in different age categories. Results from the study showed that old overfed female zebrafishes developed hepatic steatosis and fibrosis in a manner similar to overfed age-independent males. Oppositely, young female zebrafish developed less steatosis and were completely protected from the development of fibrosis. This occurred despite a high increase in BMI measured in these female zebrafish, which suggest that the presence of estrogen plays a key role in maintaining lipid homeostasis and protection of the liver against fat accumulation resulting in the development of chronic liver disease.

In contrast, the prevalence of chronic liver disease shows a correlation with increased estrogen levels[30,44]. In postmenopausal women suffering from primary biliary cirrhosis (PBC) significantly elevated serum concentrations of estrone (E1), androstenedione and sex hormone binding globulin (SHBG) were found compared to healthy controls[44]. Secondly, a correlation of excess estrogen levels in chronic liver disease with NF-κB mediated induction of steroid sulfatase was found in a study using human serum from patients with alcoholic cirrhosis and cell culture of primary hepatocytes[45]. Steroid sulfatase facilitates the conversion of inactive estrogen sulfates to active estrogen sulfates, thereby increasing the amount of active estrogen sulfates in the circulation[50]. Interestingly, these results are all found in patients who were already suffering from primary liver damage. This suggests that liver damage weakens the liver's ability to metabolically deactivate estrogens, thereby contributing to further development of chronic liver diseases.

Gender bias in NAFLD development

Impairments in estrogen signaling and abnormal levels of active estrogens have been shown to induce the development of chronic liver diseases such as NAFLD in males and females. Interestingly, overall NAFLD prevalence is higher in men (38%) than in women (21%) [51–53]. Therefore, it can be suggested that maintenance of proper estrogen levels primarily serves in the prevention against the development of NAFLD in women. The primary female sex steroid hormones are estrogen and progesterone; androgens are responsible for the development of secondary sexual characteristics in males[54]. Although estrogens are responsible for female sexual characteristics, they are also synthesized in males. Bone, breast, brain and adipose tissue express aromatase[55], which facilitates the conversion of circulating testosterone to E2 and androstenedione to E1[56]. The same mechanisms are applicable for androgens, which are shown to play important physiologic roles in females as well[56].

Since estrogen is synthesized in females as well as in males, lipid metabolism could be differently affected by estrogens in males than in females. This concept could serve as causative explanation for the difference in NAFLD prevalence between men and women. Interestingly, ERα-mediated transcription in non-hepatic tissues is essential for estrogen-mediated protection against hepatic steatosis in females[57]. This was found in a study using female ERα mutant mice fed high-fat diets. The study used a global ERα knockout mouse (αERKO), ERα DNA-binding domain mutant mouse (KIKO) and liver-specific ERα knockout mouse (LERKO). Results from the study show that liver fat accumulation was greater in αERKO and KIKO female mice than in LERKO female mice fed a high fat diet[57]. Additionally, a study looking into the effect of selective estrogen receptor modulators (SERMs) showed that SERM treatment significantly increases the risk for development of a fatty liver in Asian breast cancer patients[58]. SERMs are able to selectively inhibit or stimulate estrogen-like action via ER in various tissues[59]. Both studies show a great significance of proper estrogen receptor functioning in protecting females against fatty liver disease.

Hepatic ERα is also found to be critical for regulation of lipid metabolism in males. In contrast to results found in female mice, male liver-specific ERα knockout mouse (LERKO) showed increased hepatic lipogenesis as reflected by increased mRNA levels of fatty acid synthase and acetyl-CoA carboxylase resulting in increased liver lipid deposits and triglyceride levels in these mice[18]. Both enzymes are active in *de novo* lipogenesis and repressed after activation of ERα[39,40], indicating that hepatic E2 binding with ERα plays a key role in the maintenance of lipid metabolism in males.

Additionally, hepatic lipid metabolism in male mice is altered when the circulating estrogen to androgen ratio is increased, suggesting that maintenance of estrogen levels plays a pivotal role in male hepatic lipid metabolism as well. This was reported in a study using male mice which are universally expressing human aromatase enzyme (AROM+ mice)[60]. These mice have an increased conversion of androgens to estrogens, leading to increased circulating estrogens[61,62]. Using AROM+ mice it was demonstrated that high circulating estrogen to androgen ratio in males indicate a PPARα-mediated increase in the peroxisomal β-oxidation[60], thereby preventing fat accumulation in the liver. Another study used a female aromatase knockout (ArKO) mouse model[63]. This model displays a decreased conversion of androgens to estrogens through disruption of the CYP19 gene, resulting in a decrease in circulating estrogen levels[64]. Results found in female ArKO mice showed impaired fatty acid oxidation and elevated fatty acid synthase in the liver resulting in hepatic steatosis[63]. Taking the results from both studies together, it can be suggested that fat accumulation is dependent on circulating estrogen levels in a sex-independent matter. However, liver-specific ERα seems to play a greater role in activation of liver steatosis in males than in females[57,65].

Estrogen like endocrine disrupting chemicals in the onset of NAFLD

Next to estrogen, EDCs are known to play a pivotal role in the development of NAFLD[66]. A specific category of EDCs are chemicals that pose as, or exert the same functions as estrogens. These chemicals are known under the name of estrogen like endocrine disrupting chemicals (EEDCs). As mentioned before daily used products contain different types of EEDCs, of which the most prevalent ones and their sources are presented in **Table 1**. Interestingly, the mechanism of action depends on the specific type of EEDC. Most of the mentioned EEDCs (**Table 1**) exert their activity as endocrine disruptors via their ability to bind to nuclear receptors (NRs), and thus act as NR agonists or antagonists. More specifically, NR agonist substances are the kind of EEDCs that can be defined as compounds that produce effects that are mediated through the NR, initiating similar effects as when the receptor is bound by E2[67]. These receptors interact with an estrogen response element (ERE), and estrogen receptors (ERα and -β)[68,69].

There are some EEDCs which exert estrogenic functions or even disrupt the production of estrogen[70–72]. These estrogenic substances are compounds that produce effects resembling those of estrogen but are not mediated by the NR. These specific types of EEDCs are reported to act through other receptors and signal transduction pathways, thereby modulating production and catabolism of active steroid hormones[73]. Reviewing the effect estrogenic EEDCs on estrogen signaling and metabolism could help in understanding the complex role that endocrine disruptors play in the development of NAFLD, and identify if males and females are differently affected by EEDCs.

Table 1*: Most prevalent EEDCs in current day environment and products: Biological activities and sources.*

	EEDC	Sources	Mode of action
Additives	Parabens	Preservative in cosmetic and pharmaceutical products	ER agonists[74]
	Butylated hydroxy anisole (BHA)	Food additive with the E number E320	Estrogenic[72]
Industrial chemicals	Nonylphenol	Metabolite of commercial detergents	ER agonist, estrogenic[72,75]
	Bisphenol A (BPA)	Transparent plastic, coating of canned food and drinks, sales receipts and building materials	ER agonist[71,72,76]
	Dibutyl phthalate (DBP)	Flexible plastics	ER agonist[70,77]
Organic pollutants	Polychlorinated dibenzodioxin (PCDD)	Insulators and lubricants in electrical equipment	Anti-estrogenic[78]
	Polycyclic aromatic hydrocarbon (PAH)	Coal, crude oil, and gasoline	ER agonists, estrogenic, anti-estrogenic[78–80]
	6-hydroxy chrysene	Natural constituent of coal tar	Anti-estrogenic[81]
	2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	By-product of incomplete combustion	Anti-estrogenic[82]

A few studies explain that mainly activator protein-1 (AP-1) and the aryl hydrocarbon receptor (AhR) are affected by estrogenic EEDCs. These transcription factors are activated through protein phosphorylation[83–85]. The process of phosphorylation can be mediated through EEDCs. Through activation of these receptors estrogenic EEDCs are able to affect homeostasis through alterations in steroid hormone synthesis or metabolism. For instance, CYP1A1 transcription can be induced by action of AP-1, suggesting that metabolism of estrogen via oxidative mechanisms is induced through EEDC activation of AP-1[86]. Therefore a drop in circulating estrogens is expected when exposed to estrogenic EEDCs. Secondly, AhR has been described to have an anti-estrogenic effect; meaning that activation of this receptor leads to reduced production of estrogen[87]. Multiple mechanisms are described to explain the anti-estrogenic activities that are mediated by AhR. More specifically, these mechanisms are proposed to induce alterations in steroid metabolism or alterations in ER levels and ligand binding potential[87]. A number of genes have been shown to be induced by

the AhR including CYP1A1 and CYP1A2[87–89]. As mentioned earlier, these proteins are involved in the oxidative metabolism of E2. Therefore, decreased E2 bioavailability due to increased E2 metabolism may be one way to explain the anti-estrogenic activities observed with AhR activation through EEDCs.

A link between development of NAFLD and altered estrogen signaling or metabolism due to EEDC exposure can be suggested as fat accumulation in the liver occurs after exposure to EEDCs[90,91]. The studies that showed hepatic lipid accumulation after exposure to EEDCs have been performed in male mice and female rats. Using adult male CD1 mice the effect of low doses of BPA on lipid accumulation in the liver was examined. Lipid droplets were larger and more present in the livers of mice exposed to BPA compared to mice who were not exposed to BPA[90]. Interestingly, expression of ERα was lowest in control mice and was significantly increased in mice exposed to BPA in concentrations of 5 and 50 µg/kg/day, suggesting a relation between altered estrogen signaling due to exposure to BPA and lipid accumulation in the liver of male mice.

Additionally, BPA exposure in fructose-fed female Fischer 344 rats induced fat accumulation in the liver[91], but did not increase the amount of adipose tissue. Furthermore, the increase in liver fat accumulation appeared at a low dose, but was not further increased when the BPA dose was increased. Lastly, high doses of BPA significantly induced the level of plasma apolipoprotein (apo A-I), which results in an increase in reverse cholesterol transport. This mechanism is known to be a cause of fatty liver disease as induced reverse cholesterol transport results in increased transport of high-density lipids (HDL) to the liver[92]. Interestingly, E2 is also found to increase the expression of apo A-I[93], suggesting that BPA acts in an estrogen-like way in females. As both rodent studies show accumulation of fat in the liver after exposure to BPA, it can be suggested that EEDC exposure influences hepatic lipid metabolism by estrogen related mechanisms in both sexes.

Early-life EEDC exposure

With the knowledge that EEDC exposure affects hepatic lipid metabolism in both sexes, it can be argued if the timing of EEDC exposure in the life-course influences males and females differently. Early-life seems to be a critical period when it comes to EDC exposure. In this period of development exposure seems to be more likely to be permanent, yet, in mature individuals, the effects of EDC exposure can be alleviated when the causative agent is removed[94]. This was found in adult barbel which were exposed to polychlorinated biphenyls (PCBs)[94]. In the adult fish exposed to PCB a significant increase in cytochrome P450 was observed together with changes is liver ultrastructure. As described earlier cytochrome P450 mediates the hydroxylation of estrogen[35], therefore increase in cytochrome P450 activity may result in a higher clearance of estrogen in the liver. Interestingly, all of the effects of PCB exposure on hepatic lipid metabolism were reversed after a 1-year detoxication period.

Additionally, perinatal exposure of BPA in rats results in the downregulation of *Cpt1a* mRNA expression in male, but not female offspring at birth[95]. The *Cpt1a* gene is a key ß-oxidation gene[96], therefore downregulation of this gene will result in reduced consumption of lipids in the liver[3]. Measurements performed a week postnatal showed that in female rats BPA only increased the expression of genes involved in free fatty acid uptake and triglyceride synthesis[95], thereby stimulating acquisition and storage of lipids in the liver. However, no effect of early-life BPA exposure on body composition (fat/lean mass ratio) was measured in these females[95]. Taking together the results describing the short term effects of early-life EEDC exposure it can be suggested that when exposed to EEDCs early in life, females are less susceptive to developing symptoms of fatty liver disease than males.

Maternal EEDC exposure and future generations

Another mechanism by which EDCs are known to exert damage in an individual is by inducing alterations in the epigenome[97], thereby affecting the offspring of the exposed individual. It can be questioned if the same differences, as found in direct exposure to EEDCs, are also present in the next generation. Interestingly, EEDC exposure during pregnancy is found to affect lipid metabolism differently in males and female offspring. In a study using C57BL/6J mice exposed to BPA during pregnancy it was shown that both male and female offspring exhibited lipid metabolism disorders, however hepatic lipid accumulation was significantly different in the male offspring only[98]. The male offspring expressed induced liver weight gain and increased hepatic triglyceride levels when reaching adulthood, accumulation of hepatic triglyceride levels worsened when exposed to a high-fat diet. These results suggest that mainly male offspring is susceptible to development of NAFLD after maternal EEDC exposure. As maternal EEDC exposure causes development of NAFLD in the offspring it can be suggested that alterations in the epigenome can be found in the offspring. Modifications that are initiated by EEDC exposure during fetal development mainly occur through histone modifications and DNA methylation[99]. Interestingly, epigenetic modifications play a role in the development of NAFLD as well, which suggests a link between EEDC induced epigenetic reprogramming and NAFLD development in future generations.

There are several studies that have examined the role of histone modifications in NAFLD. Histone modifications are characterized by the modulation of chromatin structures. There are many types of histone modifications, including acetylation, methylation, phosphorylation of specific amino acids within the histone protein[100]. Acetylation of histones increases gene expression in general, whereas methylation of histones can either repress or activate gene expression depending on which residue is methylated[101]. Histone phosphorylation mainly results in cell cycle arrest or apoptosis, serving as a protective mechanism against cell damage[102].

Studies examining histone modification in the onset of NAFLD mainly suggest that the modifications are induced by activation of gene transcription catalyzed by histone acetyltransferase (HAT), and repression of genes catalyzed by histone deacetylase (HDAC). An imbalance between HAT and HDAC might play a role in the progression of NAFLD[103]. More specifically, studies performed in mice show SIRT1 and SIRT3 to be affected in the development of liver steatosis[104,105]. SIRT1 is a known NAD^+-dependent protein deacetylase, whereas SIRT3 acts in mitochondrial protein deacetylation[106,107]. These studies show that deficient SIRT3 activity and deletion of SIRT1 results in acceleration of the development of NAFLD[104,105], suggesting that repression of genes catalyzed by deacetylation is decreased. Additionally, results found in SIRT3 knock out mice show that lipogenic enzyme stearoyl-CoA desaturase 1 is highly induced[107], which is an enzyme known to induce hepatic steatosis[108]. Lastly, liver-specific SIRT1 knockout mice show impaired activity of PPARα, resulting in decreased fatty acid oxidation, eventually leading to the development of hepatic steatosis[105].

Epigenetic modulation through DNA methylation has been shown to play an important role in the onset of NAFLD as well. Addition of methyl groups to the DNA molecule occurs during DNA methylation. This process can change the activity of a DNA segment without changing the sequence. When methylation takes place in gene promoter region, DNA methylation typically acts to repress gene transcription[109]. Hepatic methylation of the mitochondrially encoded NADH dehydrogenase 6 (MT-ND6) is positively correlated with the histological severity of NAFLD[110]. This was found in a study where liver biopsies obtained from patients with NAFLD were examined. Methylation of MT-ND6 results in mitochondrial dysfunction, which has been proven to be closely related to the development of NAFLD[111].

Above described studies show that histone modifications and DNA methylation play a pivotal role in the development of NAFLD. However, there is not a lot of evidence yet that supports the theory that epigenetic modifications caused by EEDC exposure play any role in the development of NAFLD. One study examining EDC exposure in initiating epigenetic modifications showed that maternal exposure to BPA disrupts DNA methylation at several imprinting control regions in mice. Imprinting often occurs at genes that play important

roles in placental development, fetal growth, and normal cell and tissue function In the fetus, increased DNA methylation at the *Igf2* methylated region occurred with maternal BPA exposure[112]. Interestingly, elevated *Igf2* in the F1 fetus is linked to disruptions in metabolic health during adulthood in male, but not in female offspring of BPA-exposed mice[112]. More specifically, male offspring displayed impairments in glucose homeostasis. Furthermore, males were more susceptible to developing obesity later in life than the female offspring of BPA-exposed mice. These results suggest that metabolic health in the offspring of BPA-exposed mice is affected in a sex-dependent way. These impairments in metabolic health could be suggested to lead to the development of NAFLD, as many patients with NAFLD display impairments in glucose regulation as well[113]. Unfortunately, hepatic liver metabolism in the offspring of EEDC exposed individuals has not been examined yet, therefore only indirect causes of NAFLD in EEDC exposed offspring can be suggested for now.

Discussion

This review explored the role of estrogen and estrogen like endocrine disruptors in the development of NAFLD in women. This was performed by (1) describing basic liver physiology along with the molecular pathways that affect hepatic lipid homeostasis and how they impact NAFLD development, (2) assessing the role of estrogen in hepatic lipid metabolism and protection against development of NAFLD, (3) reviewing the different mechanisms by which estrogen like endocrine disruptors induce NAFLD and (4) looking into exposure to estrogen like endocrine disruptors in different stages of life and their ability to affect future generations by inducing epigenetic modifications.

In a healthy situation it was found that the steroid hormone estrogen affects hepatic lipid metabolism at different sites. Main results in this review show that the development of NAFLD due to fat accumulation is dependent on circulating estrogen levels and signaling through ERα in a sex-independent matter[29]. However, the downstream effects after activation of the estrogen receptor are sex-specific. In males liver-specific ERα seems to play a greater role in the activation of liver steatosis than in females[57,65]. This conclusion is supported by a study that showed that in males, hepatic ERα action contributes to liver lipid accumulation mainly by stimulating lipid uptake and synthesis[114]. This same study found contrasting functions of hepatic ERα signaling in females. In contrast to the results found in males, the receptor inhibits lipid synthesis, which resulted in the prevention of hepatic lipid deposition. In females it seems that impairments in circulating estrogens are the main cause for developing NAFLD. This conclusion is supported by the fact that a great number of studies performed in both sexes have found that the lack of regulatory activity of estrogens in hepatocytes favors the development of NAFLD. More specifically, ArKO and AROM+ mice models displayed that fat accumulation in the liver occurs in a sex-independent matter when circulating estrogen levels are reduced[60,63], indicating that specifically a reduction in circulating estrogen levels is one of the causative mechanisms for development of NAFLD in females.

Interestingly, during the course of liver disease circulating estrogen levels seem to rise[30,44]. One way the rise in estrogen levels can be explained is by the fact that liver damage weakens the liver's ability to metabolically deactivate estrogens, thereby contributing to further accumulation of estrogen. This theory is supported by Jiang et al., they proposed steroid sulfatase (STS) as potential target for regulation of estrogen levels. Their study showed that inflammatory activation of NF-κB induces STS gene expression, thereby activating the conversion of inactive estrogen sulfates to active estrogen[45]. This process reverses the metabolism of estrogen, thereby contributing to accumulation of estrogen in the liver. As the rise in estrogen levels occurs later on in the disease it can be concluded that heightened circulating estrogen don’t contribute to the development of NAFLD. Therefore, estrogen can be seen as a key player in maintaining lipid homeostasis and protection of the liver against fat accumulation in both sexes.

Coming back to the conclusion that hepatic ERα regulation in males mainly causes NAFLD development, it can be suspected that males are more susceptible to action of ERα agonists or antagonists than females. Interestingly, exposure to BPA leads to an increase in the expression of ERα in males together with accumulation of hepatic lipids[90]. This result concludes a relation between altered estrogen signaling via ERα due to exposure to BPA and lipid accumulation in the liver in males. In females, BPA contributes to the development of fatty liver disease by posing as estrogen without affecting ERα signaling, concluding that the mechanism in which EEDC exposure contributes to liver disease is sex-dependent. More specifically, mechanisms which are normally controlled by estrogen are affected through BPA in females, as an increase in the expression of apo A-I was found in fructose-fed female Fischer 344 rats[91]. Furthermore, EEDC exposure seems to interfere with clearance mechanisms of estrogen in the liver as well. A few studies explain that mainly AP-1 and the AhR are affected by estrogenic EEDCs[86,87]. Through activation of these receptors estrogenic EEDCs are able to affect homeostasis by inducing alterations in estrogen metabolism. Using these results it can be concluded that EEDC exposure indirectly leads to decreased E2 bioavailability due to increased E2 metabolism. A link between development of liver disease and altered estrogen metabolism due to EEDC exposure can be suggested as fat accumulation in the liver occurs after exposure to EEDCs[90,91]. For now this link can't be concluded yet, because there are no studies to date that looked into the activation of AP-1 and AhR in relation with fat accumulation in the liver.

The above mentioned studies concerning the effects of BPA exposure were performed in adult rats, nevertheless the effects of EEDC exposure occurring early in life seems to be more likely to cause permanent effects. Interestingly, longer exposure to EEDCs during the perinatal phase is necessary to find similar effects in females compared to males[95]. These results conclude that short term early-life EEDC exposure is more detrimental in terms of metabolic health in males than in females. Furthermore, it can be concluded that long term exposure to EEDCs is detrimental in both sexes in terms of NAFLD development. In contrast, EEDC exposure during pregnancy leads to disordered lipid metabolism in both male and female offspring[98]. Male offspring expressed significant differences in fat accumulation in the liver of EEDC exposed individuals compared to the controls. Similar results were found in female offspring, however the effects were less pronounced than what was observed in males. Again, concluding that females seem to be more protected to the detrimental effects of EEDC exposure on metabolic health.

The exact mechanism of how EEDC exposure could potentially lead to development of NAFLD during fetal development of the offspring is not known. One way which was proposed in this review was through epigenetic modifications, these modifications were suggested to play a role in the development of NAFLD in future generations. Despite the fact that a great number of studies prove that epigenetic modifications play a role in the development of NAFLD[103–105,110,111], no studies are present to date that link epigenetic modifications due to EEDC exposure to the development of NAFLD. However, in the present studies an indirect cause of NAFLD development was proposed, as maternal EEDC exposure is related to impairments in glucose metabolism. This conclusion can be supported by the fact that impairments in glucose metabolism are proposed to increase the risk of developing NAFLD[5].

In summary, this review showed that mainly in males hepatic ERα plays a role in the development of liver steatosis after EEDC exposure. In females, the role of estrogen and EEDC exposure in the development of NAFLD is way more complex than in males. Therefore, more research has to be performed using female model organisms to explore the exact mechanisms in which estrogens and EEDCs act in females. Secondly, the link between development of liver disease and altered estrogen metabolism due to EEDC exposure can be examined by using AP-1 and AhR induced models and measurements of fat accumulation in the liver need to be performed. Lastly, a link between epigenetic modifications due to EEDC exposure and the development of NAFLD is now only suggested via indirect mechanisms. Looking into the direct effects of epigenetic modifications on liver health could provide a more detailed picture of the lasting effects of EEDC exposure during fetal development.

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