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Review

Nuclear receptor-driven alterations in bile acid and lipid metabolic pathways during gestation ☆,☆☆

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ABSTRACT

Nuclear receptor signalling is essential for physiological processes such as metabolism, development, and reproduction. Alterations in the endocrine state that naturally occur during pregnancy result in maternal adaptations to support the feto-placental unit. A series of studies have shown that nuclear receptor signalling is involved in maternal adaptations of bile acid, cholesterol, and lipid homeostasis pathways to ensure maintenance of the nutritional demands of the fetus. We discuss regulation of hepatic nuclear receptors and their target genes in pregnancy and their impact on the development of disorders such as intrahepatic cholestasis of pregnancy and oestrogen-induced hepatotoxicity. This article is part of a Special Issue entitled: Translating nuclear receptors from health to disease.

1. General Introduction

There are profound alterations in the maternal hormonal milieu to support the feto-placental unit during pregnancy. Increased demand on the maternal hepatic functional activity is one of the major adaptations required to meet the nutritional development of the fetus. Any deficiencies in this process may have adverse outcomes for both the mother and the fetus, especially in the context of genetic predisposition to hepatic impairment. Recent studies have shown that hepatic nuclear receptor signalling is an important component of the mechanisms that coordinate metabolic processes during pregnancy. The scope of this review is to give an overview of what is known about the regulation and role of hepatic nuclear receptor function in bile acid, cholesterol, and lipid homeostasis during pregnancy.

2. Metabolic pathways are altered during pregnancy to support the developing feto-placental unit

Pregnancy is characterised by marked physiological adaptations of the mother that are essential for maintenance and growth of the feto-placental unit. Profound hormonal changes occur; the placenta synthesises large amounts of steroid hormones that are secreted to the maternal and fetal units. The mother, and to a lesser extent the fetus, is exposed to high levels of progestogens, oestrogens, mineralocorticoids, and glucocorticoids. Moreover, large quantities of prostaglandins, prolactin, and placental lactogen are released into the maternal compartment in the third trimester of pregnancy.

Whereas most attention has been given to the effects of pregnancy and steroid reproductive hormones on reproductive endocrine tissues, gestational effects on metabolic tissues such as the liver have not been studied extensively. It is well established that changes in gestational hormones coincide with dramatic maternal hepatic metabolic adaptations that serve to increase the liver's functional capacity to produce the nutritional and metabolic molecules needed for the developing placenta and fetus. Thus, there are alterations in uptake, storage, and distribution of nutrients and vitamins, homeostatic mechanisms for maintenance of blood sugar levels, regulation of circulating plasma lipids, the synthesis of circulating plasma proteins, and metabolism of nutrients, toxic compounds, and drugs [1–3]. This requires systemic alterations in hepatic cholesterol, lipid, glucose, and bile acid homeostasis [4–10] (Table 1). In a subgroup of pregnant women, pregnancy increases susceptibility to metabolic disorders of the liver, such as intrahepatic cholestasis of pregnancy (ICP), especially when they are genetically predisposed.

Hepatic nuclear receptors may be key signalling components that transduce the necessary changes in hepatic lipid metabolism during pregnancy. There are very limited studies of hepatic regulation of gestational metabolic profiles, and most of them are restricted to animal models as it is not ethically acceptable to take liver biopsies from healthy pregnant women.

Table 1
Biochemical metabolic features of human pregnancy.

Biochemical change	Direction	Reference
Free fatty acids	↑	[9,14]
Triglycerides	↑	[4]
Total cholesterol	↑	[13]
Apo-B	↑	[4]
LDL-C	↑	[4]
HDL-C	↓	[4]
Apo-A1	↓	[4]
Bile acids	↑	[10,95]
Insulin	↑	[9,14]
Glucose	↑	[5]

3. Nuclear receptors—an overview

The nuclear receptor family comprises ligand-activated transcription factors that regulate genes with central roles in physiological processes such as metabolism, reproduction and development. In the human genome, at least 48 nuclear receptors have been identified with functional and structural similarities to nuclear receptors in *Caenorhabditis elegans*, indicating an ancient metazoan origin for this family [11]. To date, nuclear receptors have been divided in two groups; both share similar structural and functional characteristics. The first group comprises the classic endocrine nuclear receptors, i.e. thyroid, progesterone, oestrogen, androgen, mineralocorticoid, and glucocorticoid receptors (TR, PR, ER, AR, MR, and GR) that are activated by hormonal lipids that are bound with high-affinity to form homodimers in their active state and are regulated by negative feedback control of the hypothalamus–pituitary axis [12]. The second group includes the orphan nuclear receptors that encompass the so-called lipid sensors, activation of which is subject to binding of dietary lipids (typically with low affinity). Ligands include fatty acids, oxysterols, bile acids, and xenobiotics that activate the peroxisome proliferator-activated receptors (PPAR-α, β/δ, γ), liver x receptors (LXR-α, β), farnesoid x receptor (FXR), and the pregnane x receptor (PXR) and constitutive androstane receptor (CAR), respectively (hence the name adopted orphan nuclear receptors). The adopted orphan nuclear receptors form heterodimers with the retinoid x receptors (RXR-α, β, γ) when activated [13–15]. Upon activation, these receptors induce a feedforward metabolic cascade to maintain homeostasis by coordinating a network of genes with key roles in lipid metabolism, storage, transport, and clearance. The commonest gene families that are transcriptionally regulated by orphan nuclear receptors are the P450 cytochrome metabolising enzymes (CYPs), fatty acid binding proteins (FABPs), and ATP-binding cassette (ABC) proteins [16,17]. Other orphan nuclear receptors include the small heterodimer partner (SHP), an atypical nuclear receptor that acts as a transcriptional repressor, the liver receptor homologue-1 (LRH-1), and the hepatocyte nuclear factor-4 (HNF-4). Ligands of these receptors are not known; however, they are induced by other nuclear receptors [18,19]. REV-ERB-α is another orphan receptor that acts as a transcriptional repressor and exists as a monomer or homodimer. Heme has been identified as its physiological ligand [20–22]. All these receptors have been shown to play an essential role in lipid and bile acid homeostasis [23–27]. The main classic and orphan nuclear receptors along with their endogenous ligands are summarised in Table 2.

4. Hepatic nuclear receptor gene expression levels are decreased during pregnancy

Two independent studies in mouse and rat models have reported decreased expression of hepatic orphan nuclear receptors in late pregnancy [28,29]. Specifically, gene and protein expression studies in pregnant mouse livers showed reduced expression of all the Rxr-α, Rxr-β, and Rxr-γ that are essential partners for lipid sensor function as well as reduced Ppar-α, Ppar-β/δ and Ppar-γ, Lxr-α and Lxr-β and Fxr, Shp and Lrh-1 [28] (Table 2). Their target genes were also shown to have reduced expression, indicating that late pregnancy is associated with attenuated hepatic nuclear receptor signalling that in turn affects fatty acid and triglyceride synthesis, cholesterol elimination and storage, as well as bile acid homeostasis. Moreover, a microarray analysis in the liver of pregnant rats revealed decreased expression levels of xenobiotic receptors Pxr and Car [29] (Table 2). Intriguingly, besides the abovementioned nuclear receptors, the gene expression levels of the classic nuclear receptors, Gr and Mr, were reduced in late pregnancy (Papacleovoulou et al., unpublished data; Table 2).

Detailed mechanisms of these processes have not yet been identified. It is also not known whether reduced hepatic nuclear receptor levels are the cause or a consequence of altered hepatic metabolism in the mother. However, this process appears to be part of the maternal hepatic adaptations that secure energy storage and mobilisation towards the feto-placental unit to maintain fetal growth and development.

Table 2
Nuclear receptor expression in rodent pregnancy.

Classic nuclear receptors	Endogenous ligand	Expression in liver	Direction in rodent pregnancy	Reference
Tr-α, Tr-β	Tri-idothyronine	Not known	Not known	Not relevant
Er-α, Er-β	17β-Estradiol	Yes	no change	Papacleovoulou et al., unpublished[a]
Ar	5α-Dihydro-testosterone	No	Not relevant	Papacleovoulou et al., unpublished[a]
Pr	Progesterone	No	Not relevant	Papacleovoulou et al., unpublished[a]
Gr	Corticosterone	Yes	↓	Papacleovoulou et al., unpublished[a]
Mr	Aldosterone	Yes	↓	Papacleovoulou et al., unpublished[a]
Orphan nuclear receptors				
Ppar-α, Ppar-γ, Ppar-β/δ	Fatty acids	Yes	↓	[28]
Lxr-α, Lxr-β	Oxysterols	Yes	↓	[28]
Fxr	Hydrophobic bile acids; CDCA, DCA, CA, LCA	Yes	↓	[28,29]
Shp	Not known; transcriptionally activated by Fxr	Yes	↓	[28,29]
Pxr	Pregnenolone-16-carbonitrile, LCA, DCA, CA	Yes	↓	[29]
Car	5α-Androstan-3α-ol	Yes	↓ (Rat); no change (mice)	[28,29]
Hnf-4	Not known	Yes	No change	[28]
Lrh-1	Not known	Yes	↓	[28]
Rev-Erb-α	Heme	Yes	Not known	Not relevant
Rxr-α, Rxr-β, Rxr-γ	9-cis Retinoic acid	Yes	↓	[28]

CDCA: chenodeoxycholic acid, DCA: deoxycholic acid, CA: cholic acid, LCA: lithocholic acid.
[a] Our novel unpublished data.

5. Oestrogens are key regulators of hepatic function in pregnancy

As mentioned above, pregnancy is characterised by elevated serum progesterone and oestrogen levels that are at their highest in the third trimester when alterations in hepatic metabolism are also profound.

Several studies have suggested that rising levels of oestrogens mediate metabolic adaptations in pregnancy. Early pregnancy is associated with an increase in fat mass to match fetal and post-partum nutrition. By 24 weeks of gestation, women gain an average of approximately 4 kg of fat without marked alterations in energy intake. Moreover, fat oxidation is suppressed as pregnancy progresses, whereas in post-menopausal women, fat oxidation is markedly increased. This implies that oestrogens work in favour of fat deposition required for reproduction and enables the shift from fat storage to fat mobilisation towards the feto-placental unit without the need for dietary changes [30].

Interestingly, of the two Er isoforms, Er-α and Er-β, Er-α is the main isoform that is expressed in the liver and participates in hepatic metabolism [31–33]. There is no evidence of nuclear progesterone receptor (Pr) expression in the liver. Therefore, oestrogen-mediated hepatic alterations in metabolism can be accomplished both directly and indirectly, whereas progestogen effects on liver capacity are likely to be exerted indirectly or through the membrane Pr, at least in rodents [34]. In agreement with this concept, oestrogen-induced hepatotoxicity in rodent models has been shown to be induced through hepatic Er-α [35] and also through cross-talk with thyroid tri-iodothyronine (T3) and pituitary growth hormone (Gh) [36,37]. Moreover, non-genomic effects of progesterone metabolites in hepatic metabolism have been demonstrated [38,39].

5.1. Role of oestrogens in hepatic bile acid homeostatic pathways in mice

As referred to above, oestrogens affect hepatic function. A series of studies have established that oestrogens impact the hepatic bile acid signalling pathways (Fig. 1) and alter bile acid and lipid homeostatic mechanisms, thereby causing hepatotoxicity and intrahepatic cholestasis. Treatments with supraphysiological doses of 17α-ethynylestradiol (EE2) in female mice altered the influx and efflux of bile acids and cholesterol [35,40–45]. Specifically, EE2 inhibited the expression of the bile salt export pump (Bsep), a high-affinity liver-specific transporter that exports conjugated bile acids into the bile canaliculus [40–42] as well as the Na+/taurocholate co-transporting polypeptide (Ntcp), the most important determinant of bile acid uptake [43–45]. Additionally, EE2 reduced the organic anion-transporting polypeptides (Oatp1/2) as well as the expression of the biliary cholesterol transporters, Abcg5 and

Abcg8 [35], all very important for clearance of the intrinsic toxic bile acids and maintenance of the enterohepatic circulation (Fig. 1). Altered gene expression of bile acid and cholesterol transporters was accompanied by a disruption of the bile acid pool as shown by an increase in the β-muricholic to cholic acid ratio. Mice lacking the Er-α were resistant to these changes and did not develop liver damage and intrahepatic cholestasis [35].

Both ethinylestradiol (EE2) and the glucuronidated oestrogen metabolite oestradiol-17β-D-glucuronide (E2-17βG) have been shown to cause cholestasis in rodent models [46–48]. EE2 administration reduced murine Bsep, Ntcp, Oatp1, Oatp2, and Oatp4 mRNA and protein expression [36,49,50], whereas E2-17βG has been shown to alter the subcellular localisation of rat canalicular transporters, specifically to induce internalisation of Bsep within submembrane vesicles in rats [47] and microtubule-dependent internalisation of Mrp2 [51]. Intriguingly, it was recently shown that phosphoinositide 3-kinase (PI3K) pathway is involved in E2-17βG-induced Bsep and Mrp2 subcellular internalisation [46].

6. Er signalling is associated with pregnancy-induced hepatotoxicity and cholestasis in mice

Recent evidence suggests that attenuated orphan nuclear receptor expression and function with accompanied alterations in hepatic metabolism is a consequence of a crosstalk between the Er and the orphan nuclear receptors, Fxr [52] and Ppar-α [53] at least in mice.

6.1. Attenuated Fxr function during mouse pregnancy

As discussed above, oestrogens affect a number of genes that are involved in maintenance of bile acid homeostasis. Transcriptional regulation of bile acid homeostasis-related genes is under the control of the nuclear receptor, FXR. Activation of hepatic FXR leads to detoxification of bile acids through suppression of bile acid biosynthesis and import. More precisely, FXR inhibits bile acid biosynthesis and uptake through induction of SHP that directly or indirectly downregulates CYP7A1 (in human and rodents), CYP8B1 (in rodents only) and NTCP (both human and rodents). Moreover, active FXR controls export and excretion through up-regulation of BSEP, MRP3, and MDR1 [45,54–58]. An alternative route of hepatic bile acid detoxification is through sulphation and excretion through the feces [59] (Fig. 1).

A recent study in our laboratory demonstrated reduction of Fxr function that may explain increased serum and hepatic bile acid levels along with pro-cholestatic hepatic gene expression in mouse pregnancy [52]. Specifically, it was established that late mouse
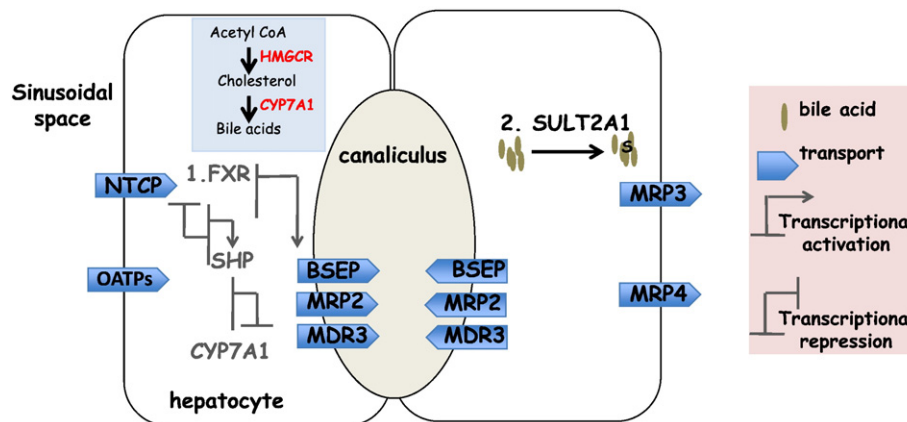
Fig. 1. Hepatic bile acid homeostatic mechanisms. Hepatic bile acid detoxification can be achieved through (1) FXR pathway activation and (2) sulphation of bile acids, so that they are committed to be excreted through feces and urine. Accumulation of bile acids induces the FXR pathway. As a result, SHP is activated to repress bile acid biosynthesis (↓ Cyp7a1) and bile acid import (↓ NTCP). FXR also activates bile acid export (↑ BSEP). 2. Alternative excretion of bile acids is also achieved by sulphation (↑ Sult2a1) and commitment of excretion through feces and urine (↑ MRP4).

pregnancy was characterised by reduced expression of bile acid transporter genes, Ntcp, Oatp2, Bsep, Mrp3, and Mdr1 mRNA along with increased expression of bile acid synthesis genes, Cyp7a1 and Cyp8b1. Moreover, pregnant mice had increased concentrations of hepatic and serum bile acids. This transcriptional and biochemical profile was comparable to non-pregnant *Fxr* null mice, implying that hepatic Fxr function is blunted at least in late pregnancy [52]. Consistent with this, hepatic Shp appeared to be down-regulated in the livers of mice on day 18 of gestation. In the light of these findings, *in vivo* studies in gonadectomised mice demonstrated that Shp mRNA expression was abrogated in response to subcutaneous insertion of slow-release silastic implants supplemented with 17β-oestradiol at levels and for a duration that mimic pregnancy. Furthermore, *in vitro* treatment of rat Fao liver cells with serum obtained from pregnant women suppressed Shp mRNA expression and this effect was reversed when the Er antagonist Fulvestrant was added. Failure to inhibit Shp induction in response to co-treatment of bile acids with pregnancy doses of 17β-oestradiol *in vitro* implied that oestrogen metabolites and not oestradiol on its own are more likely to be associated with loss of Fxr function [52]. Consistent with this, oestradiol 17β-D-glucuronide has been shown to impair Bsep function in *Xenopus laevis* oocytes expressing rat Bsep [38]. A schematic summary of hepatic gene profile in pregnant wild-type and non-pregnant *Fxr* null mice is depicted in Fig. 2.

As referred to above, pharmacological treatment with EE2 in female mice reduced mRNA expression of the bile acid biosynthetic genes Cyp7a1 and Cyp8b1. However, Shp mRNA expression was unaffected [35]. Moreover, in male rats [37] as well as in gonadecto-mised female mice [19], EE2 treatment resulted in up-regulation of Shp gene expression that in turn resulted in reduced Cyp7a1 transcript levels. In contrast, our studies showed that pregnant mice and gonadectomised mice treated with a pregnancy dose of 17β-oestradiol in the form of slow-release silastic implants for 18 days suppressed Shp mRNA expression levels [52]. The reason for these differences is not clear. However, differences in the treatment regimen (pharmacological versus physiological oestrogen treatment, injections versus slow-release silastic implants) might account for this discrepancy. Moreover, differences in oestrogen effects in the above-mentioned studies may originate from indirect effects of T3 and Gh on oestrogen-related hepatic bile acid and lipid homeostasis. In agreement with this, overlapping oestrogen effects through Er and T3 and

Gh on hepatic metabolism have been reported [37,60]. Remarkably, in late pregnancy, maternal pituitary GH is suppressed as a result of increased circulating oestrogens. As such, GH effects on hepatic metabolism that lead to reduced fatty acid oxidation and esterification to ensure free fatty acid transportation to the conceptus are replaced by placental lactogen and growth hormone [60]. Therefore, it is possible that a gestational signal originating from the feto-placental unit contributes to ER-dependent SHP suppression.

6.2. Reduced hepatic Ppar-α function in the mouse appears to be oestrogen-dependent

The nuclear receptor Ppar-α targets the trans-repression of the oxysterol 7α-hydroxylase (Cyp7b1), the enzyme that catalyses the clearance of 27-hydroxycholesterol, a competitive antagonist of oestrogen receptor action [61]. Specifically, Er-α activation positively regulates Cyp7b1 expression, which promotes oestrogen-induced hepatotoxicity and intrahepatic cholestasis through elimination of 27-hydroxycholesetrol and its conversion to oxysterol 7α-hydroxylase that is a precursor of chenodeoxycholic acid (CDCA) in the alternative/acidic bile acid biosynthetic pathway [35,62]. It was recently shown that sumoylation of the ligand binding domain of Ppar-α induced interaction of the receptor with Ga-binding protein α (Gabpα) that was bound to the promoter of the *Cyp7b1* gene. This interaction triggered recruitment of the nuclear receptor co-repressor (Ncor), histone deacetylases (Hdacs), and DNA methyl transferase 3 (Dnmt3) that in turn silenced Sp1 expression, thus leading to down-regulation of Cyp7b1 and protection of the female liver from inflammation and hepatic toxicity [53]. Protective effects of Ppar-α-induced Cyp7b1 trans-repression were further demonstrated in *Ppar-α* null female mice that displayed increased Cyp7b1 mRNA expression levels, increased serum bilirubin, and increased liver weight in response to the oestrogen analogue 17-α-ethynylestradiol (EE2) [53].

In support of this, we showed that the down-regulation of hepatic Ppar-α mRNA in pregnant mice (day 18 of gestation) was accompa-nied by a ~2-fold mRNA up-regulation of Cyp7b1 (unpublished data; Fig. 3). These findings agree with the EE2-induced alterations in bile acid and cholesterol homeostasis discussed above [35] as well as the EE2-related inhibition of Ppar-α that has been reported in hypophy-sectomised male rats [37].
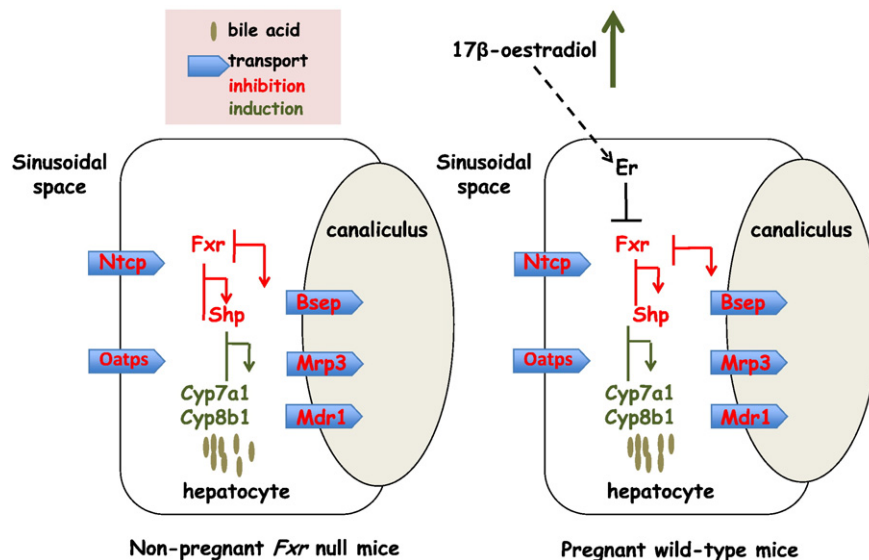

**Fig. 2.** Impaired hepatic Fxr function in mouse pregnancy. In mouse pregnancy, the FXR pathway appears blunted: ↓ Shp results in ↑ Cyp7a1 and ↑ Cyp8b1, maintaining hepatic bile acid biosynthesis; ↓ Bsep, ↓ Mrp3, ↓ Mdr1 block BA efflux to the bile. Therefore, bile acids are accumulated in the liver. Er signalling is a strong candidate that appears to contribute to these alterations, represented by the dashed line in the figure.
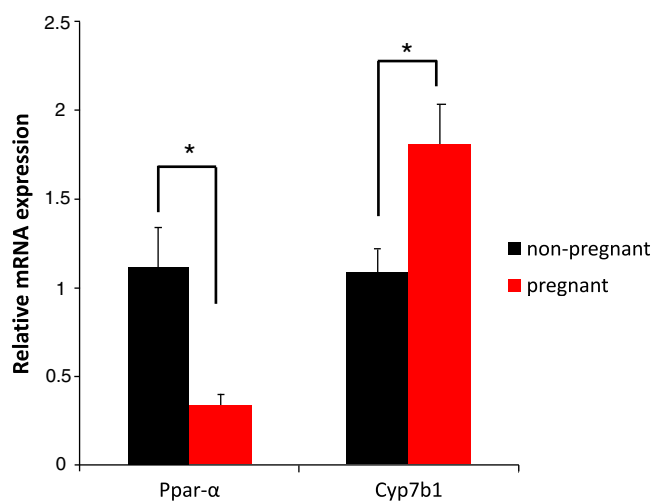
Fig. 3. Hepatic Ppar-α and Cyp7b1 mRNA expression in mouse pregnancy. Combined data of 5 independent animals. Data are presented as means and standard errors. Livers collected from pregnant (d18) and non-pregnant mice (5 mice/group) were assessed for Ppar-α (left panel) and Cyp7b1 (right panel) mRNA ($*p < 0.05$).

In conclusion, it appears that the physiological alteration in the sex steroid hormonal environment that maintains pregnancy is a factor that impacts upon liver physiology. Overall activity of the orphan nuclear receptors such as PPAR-α and FXR, as well as their downstream targets, appears to be primarily affected by these changes. This, in turn, leads to disruption of hepatic lipid and bile acid metabolism during gestation that may be exacerbated in high risk individuals. Elucidating the mechanisms that mediate alterations of hepatic nuclear receptor activity during pregnancy will expedite the development of therapeutic strategies that target metabolism-related complications in pregnancy and metabolic disease.

7. Functional Fxr is required for normal gestational liver growth in mouse pregnancy

Liver enlargement is one of the physiological maternal metabolic adaptations that occur during rodent pregnancy and is essential to support developmental nutrient demands of the fetus. Reports of gestational liver growth go back to 1958 [63]. Other studies have also reported pregnancy and reproductive steroid hormone-induced liver growth [7,35], although detailed mechanisms have not yet been identified. Bile acid nuclear receptors appear to exert key roles in the process of liver mass reconstitution, since FXR, CAR, and PXR have been shown to be essential for liver regeneration after partial hepatectomy [64,65]. Moreover, it has been shown that increased bile acids accelerate liver regeneration after hepatectomy, and conversely, a decrease in bile acid levels inhibits liver growth [65]. Therefore, decreased levels of hepatic bile acid nuclear receptors during pregnancy [28,29,52] may result in a net accumulation of bile acids that, in turn, drive liver enlargement to meet increasing demands for hepatic metabolic activity. This is in agreement with a recent study where elevated serum bile acid levels, resulting from a diet supplemented with 0.5% cholic acid during gestation, caused a further increase of liver weight relative to wild-type pregnant mice. Experiments in Pxr and Fxr null mice demonstrated that these receptors were not involved in bile acid-induced liver enlargement [6], indicative of another gestational signal that drives liver growth. However, Fxr null mice did undergo gestational liver growth that was caused by different processes compared to wild-type and Pxr null mice. Specifically, the mechanisms that were studied as potential drivers for hepatomegaly were the same in Pxr null mice when compared to wild-type mice, whereas in the Fxr null mice, liver enlargement was associated with increased hepatocyte mitosis (hyperplasia) as well as increased hepatocyte apoptosis. These findings demonstrated that Fxr has an essential role in the progression of liver mass expansion during a period of increasing liver function such as in pregnancy [6]. On the other hand, it has been proposed that pregnancy-induced hyperplasia, as shown in Fxr null pregnant mice, is associated with delayed restoration of liver function [66]. The switch from hepatocyte hyperplasia to hepatocyte hypertrophy as seen in wild-type pregnant mice appears to be a physiological response. This results in a rapid expansion of liver mass in transient situations where there is time-limited demand for increasing liver function such as during pregnancy. Interestingly, it was shown that pregnancy-induced liver hypertrophy requires activation of the Akt/mammalian target of rapamycin c1 (mTorc1) pathway [66]. It would be of interest to investigate whether this pathway is impaired in Fxr null pregnant mice.

No studies have investigated the effects of pregnancy on liver growth in humans. It is plausible that gestational maternal liver growth occurs to compensate for the increasing metabolic and nutrient developmental demands of the feto-placental unit. Nevertheless, potential pregnancy-induced liver growth should be less marked in humans compared to mice, as the feto-placental unit weight-to-maternal body weight ratio in the former is lower than in the latter [6]. This could be physiologically important, as failure of human maternal liver function to proportionately reflect the feto-placental unit weight (or metabolic load) could be relevant to the reduced ability of the pregnant woman to sustain hepatic metabolic homeostatic mechanisms, thereby increasing susceptibility to gestational liver disease.

8. Altered bile acid homeostasis in pregnancy can result in intrahepatic cholestasis of pregnancy (ICP) in predisposed women

Intrahepatic cholestasis of pregnancy (ICP), also called obstetric cholestasis, is the commonest liver-specific disease of pregnancy. It has a varying incidence globally; while it affects 0.7% of pregnancies in the UK, it affects up to 4% of pregnant women in Chile in recent studies [67]. The prevalence of ICP has decreased in certain countries in recent years, e.g., up to 10% of the Chilean population was reported to have ICP in older studies, with higher rates in the native Araucanean population [68]. ICP typically presents with pruritus in association with increased maternal serum bile acids and abnormal liver function tests. Maternal clinical and biochemical features of the condition usually resolve rapidly after delivery, indicating that the feto-placental unit plays a role in the aetiology of ICP. It can be complicated by fetal distress, preterm labour, and intrauterine death [69,70]. The largest published series of fetal complications in ICP demonstrated that pregnancies with higher maternal serum bile acids are more likely to have adverse fetal outcomes [70].

ICP has a complex aetiology with genetic and endocrine components. Heterozygous mutations in the biliary transporters BSEP (ABCB11) and MDR3 (ABCB4) have been demonstrated in a small proportion of ICP cases [71,72] and a common single nucleotide polymorphism (SNP) in BSEP has been shown to be a susceptibility allele for ICP [73]. Both transporters are transcriptionally regulated by the nuclear receptor FXR and genetic variation in FXR (NR1H4) has also been shown in ICP cases [74]. There have also been SNPs reported in other biliary transporters, e.g., ABCC2 [75] and ATP8B1 [72]. Thus, the majority of genetic studies have implicated genes that are under the transcriptional influence of FXR. It is noteworthy that normal human and mouse pregnancy are relatively cholestatic states.

Observations to support this include the fact that asymptomatic hypercholanaemia is a relatively common phenomenon found in pregnant women [10,76], and raised serum and hepatic bile acids have been reported in mouse pregnancy [52]. Therefore, it is likely that women that harbour genetic variants of bile acid homeostasis-

related genes such as the central bile acid sensor (i.e., FXR) and the primary determinant of bile flow (i.e., BSEP) are more susceptible to the development of ICP [73,74].

The nuclear receptor PXR transcriptionally regulates genes that influence xenobiotic metabolism. One study that sequenced the coding sequence of *PXR* in 121 Caucasian ICP cases did not demonstrate any SNPs that were associated with the disease [77]. However, a study of four tagged SNPs in 101 South American ICP cases demonstrated a positive association between one SNP (rs2461823) and alanine aminotransferases (ALT), aspartate aminotransferase (AST), and bilirubin concentrations in affected women [78]. Larger studies will establish whether genetic variation in PXR plays a role in the aetiology of ICP.

9. The impact of pregnancy-specific endocrine signals on nuclear receptor function

There are hepatic nuclear receptors that are not faithful to a single ligand in the liver. Thus, it is not surprising that several molecules that have been shown to be elevated in pregnancy (e.g., steroid hormones and their metabolites) have the ability to modulate the activity of CAR [79] and PXR [80].

Pregnenolone, progesterone, and their 17α hydroxylated derivatives have been shown to activate human and rodent PXR, Pxr, respectively [81]. Additionally, it has been demonstrated that oestrone and 17β-oestradiol are able to activate rodent Car [79]. The concentrations of these reproductive hormones shown to activate PXR and CAR fall within levels observed during the final trimester of pregnancy [82,83]. Both PXR and CAR have been shown to induce phase I and II xenobiotic metabolising enzymes [84,85]. This is intriguing as this may link pregnancy levels of reproductive hormones to the activation of PXR and CAR as a protective mechanism against potentially harmful xenobiotics, offering increased protection to the mother and fetus.

10. Expression of hepatic nuclear receptors in extrahepatic reproductive tissues during pregnancy

Several lines of evidence have shown a role of orphan nuclear receptors in placentation and uterine contractility. LXRs appear to have a primary role in these processes. Recent studies have demonstrated that deficiencies in LXR signalling are associated with impaired trophoblast invasion, lipid transport towards the fetus, and uterine contractility [86–91].

In placenta and trophoblast, Lxr transcripts are expressed throughout gestation in mice and are detectable from early placentation steps (7 days post-coitum). In humans, both LXR-α and LXR-β transcripts are present from the 6th week of gestation. However, their expression pattern differs. LXR-α is abundant in the yolk sac membranes in mouse and in amniotic membranes in human, whereas LXR-β is ubiquitously and homogenously expressed in both human and rodents. This pattern implies distinct roles of each isoform during choriovitellite and chorioallantoic placentation [87]. Moreover, LXR agonists have been shown to inhibit human trophoblast invasiveness *in vitro*, suggesting a role for them in implantation that might be relevant to the pathogenesis of preeclampsia and spontaneous abortion [88].

Placental LXR may also coordinate cholesterol transport from the maternal to the fetal unit. This is particularly important in cases of Smith–Lemli–Opitz syndrome where the fetus cannot synthesise cholesterol *de novo*. It has been recently shown that endogenous oxysterol-induced LXR activation triggers ABCA1- and ABCG1-mediated cholesterol efflux to apolipoprotein (Apo)A-1 and high-density lipoprotein (HDL) from human placental endothelial cells to the fetal circulation [89]. Moreover, enhanced cholesterol transpor-

ters have been shown to protect the placenta against oxidative stress and this appeared to be LXR-regulated [86].

A role of LXR-β in the uterus has also been established. Remarkably, in mouse uterus, Lxr-β has been shown to be a cholesterol sensor as *Lxr-β* null mice accumulated cholesteryl esters in the uterine smooth muscle cells, resulting in reduced contractile capacity. Therefore, Lxr-β appears to be a master sensor of lipid regulation of uterine myocytes, at least in rodents, thereby securing the ability of the uterus to properly deliver the pups [91].

It is noteworthy that the expression and functionality of other orphan nuclear receptors such as PXR, CAR, and FXR have been investigated in human term placenta of healthy and diseased pregnancy. However, data from these studies are not all consistent so no conclusions can be made at present. Briefly, trophoblast cells extracted by whole placenta showed relatively high expression of FXR, CAR, and PXR along with their target genes [92], whereas villous trophoblast tissue showed very low or no expression of these receptors (Geenes et al., unpublished data). It is important to elucidate the extent to which there is signalling of these receptors in placenta as this will give insights into the mechanisms that secure fetal development and maintenance in addition to the prevention of adverse fetal outcomes in complicated pregnancies such as in ICP.

11. Disrupted bile acid homeostasis during pregnancy could be a result of altered circadian rhythms

Hepatic pro-cholestatic changes that take place during pregnancy contribute to accumulation of maternal hepatic and serum bile acids and this might be relevant to the development of ICP in genetically predisposed women, as discussed above. Pregnancy presents a phenotype that resembles Fxr deficiency, as Shp is down-regulated, resulting in Cyp7α1 induction [52]. It is noteworthy that the bile acid homeostasis gene profile that we have observed in mouse pregnancy also resembles mice over-expressing the nuclear receptor gene, *Rev-erb-α* [27]. Rev-erb-α has been shown to be a positive regulator of bile acid synthesis, since it directly represses Shp expression in favour of Cyp7a1, thus maintaining bile acid synthesis. Moreover, Rev-erb-α deficiency disrupts circadian rhythms of bile acid biosynthesis in mouse [26,27]. Intriguingly, hepatic Fxr, Shp, and Rev-erb-α circadian regulation was altered in mice that were not fed between Zeitgeber time (ZT) 2 and ZT10 (ZT0 is at 7:00 am, when light cycle begins) for 5 days. Restricted feeding also increased the amplitude of diurnal serum and hepatic bile acids. This phenotype was accompanied by an up-regulation of Cyp7a1 and Ntcp mRNA expression, while Bsep mRNA was down-regulated during this period of feeding restriction [93]. Steroid reproductive hormones appear to impact upon circadian rhythms as oestrogens have been shown to alter hepatic circadian expression of the peripheral clock gene Per1 [94]. Furthermore, circadian regulation of sleep and core body temperature differs between the follicular and luteal phases of the menstrual cycle [95] and increased bile acids during the mid-follicular phase of the menstrual cycle have been also reported in women [96]. These findings indicate a direct role of the gestational hormonal environment in hepatic metabolism and hepatic circadian regulation.

Thus, changes in sleep and eating patterns as a result of the altered hormonal milieu that typifies pregnancy might well impact upon nuclear receptor functionality that in turn may disrupt circadian regulation of hepatic bile acid and lipid homeostasis during gestation. Identification of gestational circadian regulation of liver metabolism may provide further insights for the development of strategies to prevent and treat complicated pregnancies, especially in predisposed women.

12. Conclusions and future perspectives

It is clear that nuclear receptors are differentially regulated in pregnancy and comprise a fundamental component of hepatic

maternal adaptations. However, the precise mechanisms that signal the metabolic homeostatic mechanisms that are essential to maintain and support the feto-placental unit are yet to be identified. Oestrogens and oestrogen metabolites appear to be strong candidates, although the potential contribution of other endocrine components such as progestogens and their metabolites as well as placental lactogen or environmental factors deserves further investigation.

Attenuated functionality of nuclear receptor signalling in the liver is a marked adaptation during pregnancy. Elucidation of the molecular mechanisms that regulate these alterations will give insights into the homeostatic processes that the liver undergoes under stress conditions, such as pregnancy. It would be interesting to investigate whether reduced activity of all hepatic nuclear receptors is governed by the same signals, e.g. if oestrogens or oestrogen metabolites affect other receptor pathways besides the PPAR-α and FXR-α pathways. Therefore, development of agonists to target these receptors could be of therapeutic benefit. Moreover, gestational effects on nuclear receptor pathways in energy expenditure tissues such adipose and skeletal muscle will allow us to gain knowledge about how energy is stored and distributed within the body of the pregnant woman to fulfil nutritional developmental demands of the conceptus. Disruption of circadian rhythms of nuclear receptors is potentially a key process of gestational metabolic adaptations and is an exciting field for future study. Expression of hepatic nuclear receptors in placenta and uterus is indicative of a major role of these receptors in placentation and support of the fetus.

In conclusion, the field of nuclear receptor biology in the metabolic state of pregnancy is still at an early stage. Pregnancy is an exceptional model to study hepatic bile acid and lipid homeostatic pathways, as physiological gestational signals cause alterations in bile acid and lipid homeostatic pathways. Elucidation of the molecular mechanisms that govern these alterations as well as the time window of pregnancy when these metabolic changes take place will allow the development of therapeutic strategies targeted not only to the treatment of pregnancy-induced metabolic complications but also to metabolic disease outside pregnancy.

Acknowledgments

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