



# FXR in liver physiology: Multiple faces to regulate liver metabolism

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## ABSTRACT

The liver is the central metabolic hub which coordinates nutritional inputs and metabolic outputs. Food intake releases bile acids which can be sensed by the bile acid receptor FXR in the liver and the intestine. Hepatic and intestinal FXR coordinately regulate postprandial nutrient disposal in a network of interacting metabolic nuclear receptors. In this review we summarize and update the “classical roles” of FXR as a central integrator of the feeding state response, which orchestrates the metabolic processing of carbohydrates, lipids, proteins and bile acids. We also discuss more recent and less well studied FXR effects on amino acid, protein metabolism, autophagic turnover and inflammation. In addition, we summarize the recent understanding of how FXR signaling is affected by posttranslational modifications and by different FXR isoforms. These modifications and variations in FXR signaling might be considered when FXR is targeted pharmaceutically in clinical applications.

## 1. Introduction

Nuclear Receptors as metabolic integrators in liver physiology:

Metabolically active nuclear receptors (NRs) are anatomically enriched within the gastroenterohepatic axis and in key metabolic peripheral tissues such as adipose and muscle tissue [1]. Among metabolically active tissues the liver is the central metabolic hub which connects metabolic organs and coordinates nutritional inputs and metabolic outputs [2]. Likewise, metabolically active NRs are highly expressed in the liver to orchestrate the transcriptional requirements during nutrient-rich/feeding and nutrient-low/fasting transitions. In its simplest understanding NRs translate chemical changes into physiological effects.

There are 48 NRs in humans and 49 in mice. The additional NR in mice is FXR $\beta$ , which encodes a pseudogene in humans and primates (see also below) [3]. All NRs are classified according to structural homology into 7 subfamilies, NR0–NR6 [4]. Metabolic NRs belong in most cases to the NR1 subfamily (such as farnesoid X receptor (FXR $\alpha$ /NR1H4), liver X receptor  $\alpha,\beta$  (LXR $\alpha,\beta$ /NR1H3,2), peroxisome proliferator-activated receptor  $\alpha,\beta/\delta,\gamma$  (PPAR $\alpha,\beta/\delta,\gamma$ /NR1C1,2,3) which heterodimerize with retinoid X receptors  $\alpha,\beta,\gamma$  (RXR $\alpha,\beta,\gamma$ /NR2B1,2,3). When a metabolic ligand is bound to the NRs and/or the RXR heterodimer partner, corepressors are released and co-activators are recruited to ignite the transcriptional process [5]. That way ligand activation allows an amplified transcriptional activity even to small variations of single

metabolic ligands and robust physiological responses (4). These primary metabolic NRs are supported by additional metabolically active NRs such as short heterodimeric partner (SHP/NR0B2) and liver receptor homologue-1 (LRH-1/NR5A2) which act as downstream targets or as competence factors for the primary metabolic NR, respectively, and by the hormonal activity of fibroblast growth factors (FGF)19 and FGF21. Feeding responses for nutrient uptake, storage and distribution are primarily conducted by the FXR-LXR-PPAR $\beta/\delta,\gamma$  axis and supported by FGF19, while the fasting response for metabolite mobilization and break-down is centrally organized by PPAR $\alpha$  and FGF21 [6–8].

## 2. FXR, a metabolic nuclear receptor for bile acids

FXR $\alpha$ /NR1H4 (further on referred to as “FXR”) has been cloned in 1995 and was initially described as a receptor recognizing farnesol [9]. In 2003 a second form of FXR, FXR $\beta$ /NR1H5, has been identified which only constitutes a functional NR in mice, rats, rabbits and dogs [3]. Both FXRs only share 50% amino acid identity, and also differ in their ligand specificity. FXR $\beta$  is transactivated only by the cholesterol precursor lanosterol, but a functional role of FXR $\beta$  is not known [3]. In 1999 bile acids were found to be the natural ligands of FXR [10–12]. Interestingly, bile acids differ in their affinity and transactivation capacity to FXR with chenodeoxycholic acid (CDCA) and the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA) as the most potent natural ligands. In mice CDCA is transformed by the enzyme Cyp2c70 [13] to  $\alpha$ - and

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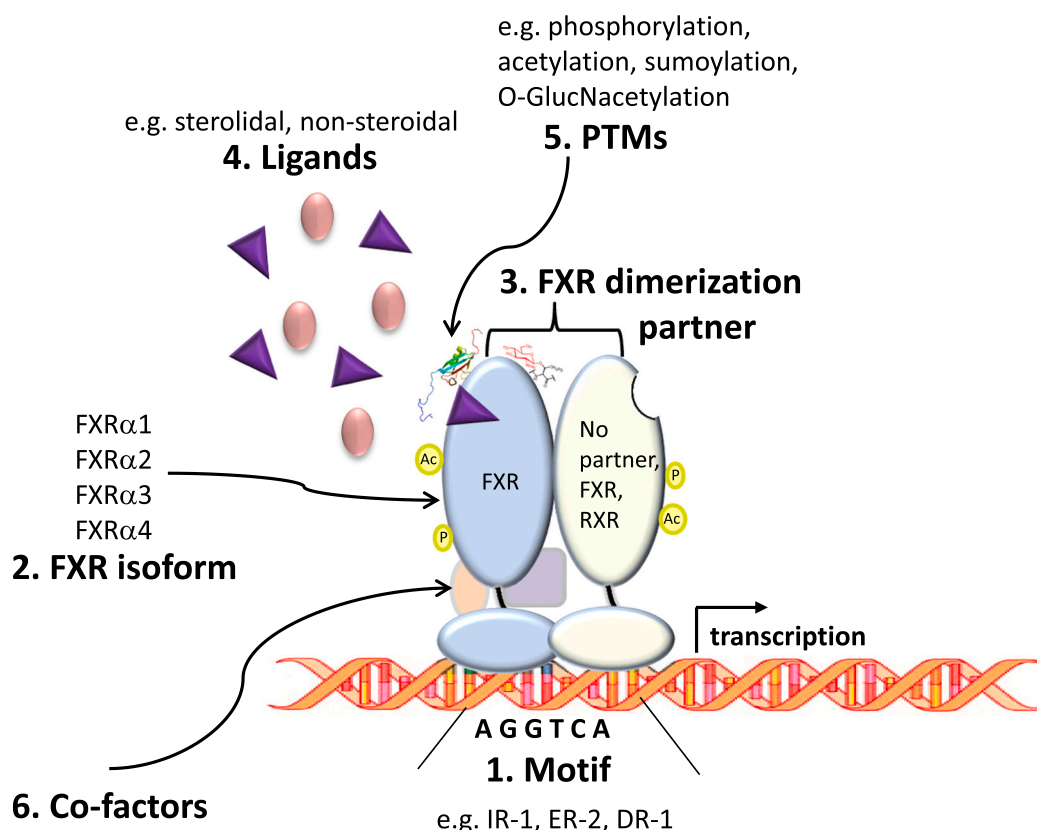
$\beta$ -muricholic acid (MCA), which are the dominating murine bile acids but antagonists to FXR [14]. Thus, the murine bile acid pool dramatically differs from the human bile acid pool but can be humanized by deletion of *Cyp2c70*. [15]. FXR exists in 4 different isoforms (FXR $\alpha$ 1-4), which arise from differential promotor usage and alternative splicing of the same gene [16,17]. Importantly, the isoforms are species-specifically and differentially expressed in different organs and (gene specific) activity depends on bile acid composition [18]. In human liver FXR $\alpha$ 1/2 dominates, with FXR $\alpha$ 2 being the transcriptionally most active form [19], but feeding and fasting cycles (at least in mice) dynamically regulate FXR splicing [20]. These are important species-relevant variables to consider when studying and comparing metabolic models and bile acid signaling.

FXR is enriched in the gastroenterohepatic axis, with the highest expression levels in the liver and along the intestine. The positioning of FXR in the liver and the ileum allows for sensing and controlling bile acid concentrations at the gates of the enterohepatic circulation. Additionally, FXR is also expressed in the adrenal glands [21] and highest in the kidney, although the role of FXR in these tissues is less well studied. In the adrenal glands supraphysiological concentrations of bile acids may stimulate adrenal steroidogenesis, but this appears to be independent of FXR [22]. In the kidney FXR is involved in the regulation of urine volume. Activation of FXR increases the transcription of aquaporin 2 in the inner medullary collecting duct cells resulting in the reduction of urine volume [23]. In the kidney FXR also plays a protective role against fibrotic events and may thus have potential as drug target for diabetic nephropathy and nephrosclerosis [24,25]. Mechanistically, FXR mediated inhibition of yes-associated protein (YAP) signaling appears to be a central anti-fibrotic mechanism in the kidney [26,27]. In the liver anti-fibrotic effects of FXR are under debate. Several studies suggested that FXR is functionally present in hepatic stellate cells (HSC), which are along with myofibroblasts the key cellular players in hepatic fibrogenesis. FXR activation may modulate HSC activity by restoring PPAR $\gamma$  and by FXR-SHP-dependent inhibition of AP-1 signaling on downstream

profibrogenic targets [28–31]. In contrast, other studies could neither detect FXR in mouse HSCs nor myofibroblasts at biologically significant levels and only to minimal amounts in human HSCs [31–34]. Newer research rather indicates that activated HSCs are unresponsive to FXR agonists due to enhanced SUMOylation of FXR. However, prophylactic FXR agonistic treatment in combination with SUMOylation inhibitors effectively reduced fibrotic markers [35]. FXR is not expressed in significant amounts in peripheral metabolically active tissue such as muscle or adipose tissue but can communicate with these tissues via FGF19 [36,37]. In adipose tissue very small amounts of FXR appear to regulate adipose differentiation since FXR $^{-/-}$  mice have small adipocytes [38] and moderate adipose specific overexpression of human FXR results in adipocyte hypertrophy and extracellular adipose matrix remodeling [39]. In adipocytes FXR also appears to assist lipogenesis through the enhanced expression of stearoyl-CoA desaturase (SCD) and fatty acid synthase in a PPAR $\gamma$ -dependent manner [40].

### 3. Mechanisms of transcriptional regulation by FXR (Fig. 1)

FXR acts by binding to cis-regulatory elements on DNA. In most scenarios a FXR-RXR heterodimer binds to the inverted DNA consensus sequence AGGTCA separated by one nucleotide (IR1). However, a recent study shows that the metabolically active isoforms FXR $\alpha$ 2/ $\alpha$ 4 are the dominating isoforms in response to FXR agonism and bind to almost 90% of binding sites in an isoform selective fashion via an everted repeat spaced by 2 nucleotides (ER-2), in addition to the canonical IR-1 FXR binding motif [19]. Noteworthy, FXR binding motifs apparently differ among tissues [41] and functional pathways. As such, FXR binds an uncommon DR-1 motif to transcriptionally repress autophagy related genes [42]. Interestingly, when comparing FXR-DNA binding between liver and intestine, only 11% of the FXR cistrome is shared among different tissues, which also translates into different transcriptional programs [41]. Besides the FXR-RXR heterodimer FXR can also bind as monomer or be tethered to the DNA via another transcription factor (e.g.



**Fig. 1.** Layers of FXR modulation. The activity of FXR can be modulated on several levels (see main text for details). 1. FXR can bind to different motifs in the FXR response element, which can have different transcriptional outcomes. 2. Different FXR isoforms activate different transcriptional programs. 3. FXR can bind to the DNA as monomer, heterodimer or even homodimer. 4. FXR can be bound by different potent agonists, antagonist or partial ant/agonists. 5. Posttranslational modifications (PTMs) may further activate or inhibit FXR action. 6. Co-activators, co-repressors and competence factors further impact on FXR activity. Depending on the combination of all of the contributing factors the transcriptional response will be modified ranging from transcriptional repression to transcriptional activation of subsets of FXR target genes.

NF $\kappa$ B [43]) [44,45]. FXR binding is also affected by the physicochemical properties of the agonist. There exist various structurally different synthetic and semi-synthetic FXR agonists and it appears that structurally different FXR agonists have also distinct transcriptional properties as has been shown in a comparison among the FXR agonists CDCA, fexaramine and GW4064 [46]. This has to be considered particularly for conditions when steroidal versus non-steroidal FXR agonists are compared. To date, there exists no isoform selective FXR agonist and the response to synthetic (pan)-FXR activation appears to be comparable between men and mice [47], although a few clinically important exceptions, in particular regarding apolipoprotein metabolism, exist. New research is directed to the development of selective bile acid receptor modulators, which may selectively activate certain pathways (e.g. metabolic or inflammatory) or even act gene-selectively (for a detailed excellent review see [45]).

Ligand binding by an agonist initiates conformational changes in the ligand binding domain, which allows dismissal of the co-repressor complex and recruitment of co-activator complexes, including PPAR $\gamma$  co-activator 1 $\alpha$  (PGC-1 $\alpha$ ) [48], steroid receptor coactivator 1 (SRC-1) [10,11] and the methyltransferases CARM1 [49] and PMRT1 [50,51]. The coactivator complex eventually prepares DNA for binding of the transcriptional machinery. Binding of certain co-factors or competence factors may also direct FXR binding. ChIP Seq analysis (in mice) revealed that FXR binding sites are co-enriched with monomeric binding sites for LRH-1 as competence factor, which recruits FXR to hepatic genes of lipid metabolism [52]. In addition, almost half of FXR binding sites in the liver overlap with HNF4 $\alpha$  binding sites and are enriched for complement, coagulation and drug metabolism cascades [53]. HNF4 $\alpha$  also physically interacts with FXR and increases its transcriptional activity [53]. A list and discussion of FXR co-activators and co-repressors can be found elsewhere [44,54].

Transrepression requires FXR binding to negative response elements on the DNA. There are a few examples where FXR directly transrepresses metabolic genes. As such, transrepression has been shown for ApoA1, where FXR binds the promotor as monomer. Repression of ApoA1 together with increased hepatic HDL uptake (see below) probably accounts for the HDL lowering effects of FXR ligands [55]. Similarly, FXR activation also transrepresses ApoC3, which may contribute to the TG lowering actions of bile acids and FXR agonists [56]. FXR also transrepresses expression of genes involved in glycolysis, in particular liver pyruvate kinase (LPK) by concomitant release of carbohydrate response element binding protein (ChREBP) from the LPK promotor and recruitment of co-repressors [57]. In addition, transrepression has been reported for autophagy genes via disruption of CREB/CRTC2 [58] or competition with PPAR $\alpha$  [42,59]. In line with a broader transrepressive role of FXR, an FXR ChIP-seq study which compared hepatic FXR binding in normal and obese mice found that several FXR associated genes in the obese condition were not inducible by FXR agonists indicating a higher number of functionally inactive FXR binding sites in obesity [60]. Unexpectedly, agonistic treatment often resulted in repression of gene expression [60].

Most of the negative effects of FXR are, however, not mediated by direct transrepressive effects but mediated by the transrepressor SHP. SHP is an unusual NR that lacks a DNA binding domain. Instead, SHP physically interacts with several other metabolically active NRs and inhibits gene transactivation by the NR with which it interacts, thus functioning as a negative regulator of metabolic NR-dependent pathways [61]. SHP inhibits transactivation by two mechanisms. On the one hand SHP interacts with the transactivation domain of other NRs thereby competing with co-activators. On the other hand SHP shows direct transcriptional repression [62]. From a metabolic point of view, the most important interacting partners for SHP are HNF4 and RXR [63], LRH-1 [62], LXR $\alpha$  and consequently a blunted SREBP-1c activation [64], PGC1 $\alpha$  [65], CAR [61] and PPAR $\alpha$  [66]. Therefore, FXR mediated SHP activation has profound physiological effects on bile acid, lipid and glucose homeostasis.

Although FXR and SHP work in concert, the simultaneous deletion of

both nuclear receptors has more profound metabolic effects than would have been anticipated from the single knockout mice [67–69]. This suggests that FXR/SHP is interacting in a non-linear model and that FXR is not just simply epistatic to SHP [67,68]. Most prominent examples are Cyp17a1, which is overexpressed selectively in DKO mice and might contribute to the severe cholestatic phenotype of young DKOs [67] and Elovl3, which is reduced in FXR single knockouts but almost absent in the DKOs and contributes to the protection of age-related metabolic decay in old liver specific DKOs [68]. Interestingly however, deletion of SHP is sufficient to protect against steatosis and regulates triglyceride levels in the liver independent of FXR. This effect is also bile acid independent and may be attributed to lower expression of hepatic Srebp1c, PPAR $\gamma$  and Fsp27 $\beta$  [69]. Furthermore, an older report suggests that overall FXR plays a more important role for bile acid excretion and liver injury whereas SHP plays a more dominant role in bile acid synthesis of bile acids and regulation of bile acid composition [70].

#### 4. Factors affecting FXR activity (Fig. 1)

As outlined above, transcriptional activity of FXR is primarily regulated by permissive ligand binding with differences in FXR activation depending on the bile acid species. Bile acids are released from the gallbladder when food enters the duodenum and appear in the plasma 30 to 60 min postprandially [71,72]. In the postprandial period serum bile acids therefore increase approximately 2-fold along with increases of bile acid levels in the portal vein and liver [73,74]. In the fasting state bile acid secretion from the liver equals hepatic re-uptake of bile acids [73]. Of note, serum bile acids oscillate in a circadian rhythm [75] and are increased in the postprandial phase [76], while in metabolic diseases such as obesity and non-alcoholic fatty liver disease (NAFLD) bile acid levels and FXR expression are reduced [77,78]. Circadian expression of FXR itself revealed controversial findings, with either circadian cycling of FXR [79], or only cycling of FXR $\beta$  but not of FXR $\alpha$  [80]. Datasets in the Circadiomics database do not show circadian expression of FXR ([circadiomics.igb.uci.edu](http://circadiomics.igb.uci.edu)). Also, FXR isoforms affect FXR signaling and are dependent on the feeding and fasting state [20]. As such, fasting, exercise and a “healthy” liver increase the FXR $\alpha$ 2 isoform in the liver, which more effectively promotes fatty acid  $\beta$ -oxidation, reduces hepatic lipogenesis, enhances glycerate metabolism and improves ammonia clearance [19]. FXR $\alpha$ 1 is rapidly induced in the refeeding state to become the predominant isoform and starts a transcriptional program that primarily leads to reduced de-novo lipogenesis [20] (a review by S. Van Mil and colleagues in this special issue series discusses the metabolic effects of various FXR isoforms in more detail).

Another important level of modulating FXR activity are post-translational modifications (PTMs) (Table 1). Indeed, PTMs of hepatic FXR might be more relevant to feeding and fasting cycles than bile acid flux. In fed states, when hepatocellular glucose concentrations peak, part of the glucose is channeled into the hexosamine biosynthetic pathway and utilized for O-GlcNAcylation of FXR [81]. Increased O-GlcNAcylation of FXR enhances its transcriptional activity and protein stability in the fed state by inactivating co-repressor complexes. This suggests that FXR can (indirectly) sense glucose concentrations and translate the feeding signal of bile acids and glucose synergistically into enhanced transcriptional output. Interestingly, the O-GlcNAcylation sites appear to be conserved in the different FXR isoforms [81]. Since O-GlcNAcylation is altered in metabolic diseases, such as diabetes or cancer [82], it would be obvious that this PTM affects transcriptional outcomes under metabolic stress conditions. In fact, FXR ChIP and RNA Seq in normal and obese mice show a profoundly altered FXR-cistrome and transcriptome [60].

FXR is also dynamically acetylated and deacetylation via p300 and SIRT1, respectively, in response to the cellular energy state and the feeding/fasting cycle [83]. In response to feeding and bile acids FXR interacts with p300, which acetylates histones and generates an immediate favorable epigenetic condition for gene transcription. However,

**Table 1**  
Posttranslational modifications of FXR.

PTM	Position	Species	Condition	Effect	Signaling pathway	Comment	Ref.
O-GlcNAcylation	S62	mice, AML-12 and HepG2 cells	high carbohydrate diet	activating	Hexosamine pathway	high glucose enhances FXR transcriptional activity	[81]
Phosphorylation	Y67	mice and primary mouse hepatocytes	mice were fasted overnight	activating	FGF15 signaling	sterol transport and cholesterol efflux	[85]
	S135	HepG2 and HEK cells	normal	activating	PKC	enhance agonist binding	[87]
	S154	HepG2 and HEK cells	normal	activating	PKC	activation of FXR degradation	[87]
	S250	mice and multiple cell lines	6 h fasting	inhibiting	AMPK	starvation/Metformin activating AMPK; FXR isoform dependent nuclear localization	[86]
Acetylation	T442	multiple cell lines	fasting in charcoalstripped media	activating	PKC		[215]
	K157	mice	mice were fasted overnight	inhibiting/activating	P300, SIRT1	increased FXR stability but blocking dimerization with RXR	[83]
	K217	mice and monkey kidney cells (CV-1)	mice were fasted overnight	inhibiting/activating	P300, SIRT1	inhibiting in the fed state, activating in the starved state	[83,91]
Sumoylation	K122	mice and HepG2 cells and monkey kidney cells (CV-1)	normal	inhibiting	Sumo1	blocking dimerization with RXR	[90]
	K275	mice and HepG2 cells and monkey kidney cells (CV-1)	normal	inhibiting	Sumo1	blocking dimerization with RXR	[90]
	K277	mice and monkey kidney cells (COS-1)	mice were fasted overnight	inhibiting	Sumo2	tethering to NFkB resulting in repression of inflammatory genes, no influence on FXR target genes	[91]
Methylation	K206	HUH-7 cells and monkey kidney cells (COS-1)	normal	activating	Set7/9	enhances binding to FXRE	[216]

p300 also acetylates FXR which inhibits FXR heterodimerization with RXR and terminates DNA binding and transcription. In fasting conditions, low energy responsive NAD<sup>+</sup> sensitive SIRT1 is active and deacetylates FXR which then can again bind to DNA and is prepared for the next feeding cycle. In obese conditions SIRT1 is depleted and FXR constantly acetylated and transcriptional activity of FXR reduced [83]. Thus, both PTMs, O-GlcNAcylation and acetylation, may impair proper FXR signaling under metabolic stress conditions, such as NAFLD and/or obesity.

Another important PTM is FXR phosphorylation induced via Fgf15/19 [84,85]. In the postprandial period FGF19 leads to hepatic phosphorylation of FXR via the non-receptor tyrosine kinase Src. This specific phosphorylation is important for FXR binding and transcriptional induction of bile acid metabolism genes (e.g. Shp, Mafg, Bsep, Mrp2) and the biliary cholesterol transport proteins Abcg5/8, thus for maintaining bile acid and cholesterol homeostasis [84,85]. FXR also interacts with the nutrient-sensitive and low energy kinase AMPK, which leads to FXR phosphorylation and reduced transcriptional FXR activity [86]. Also, PKC kinase can phosphorylate FXR at a different phosphorylation site, which promotes interaction of FXR with the co-activator PGC1 $\alpha$  and increases transcriptional activity [87]. Since bile acids can signal via PKC [88], even this PTM may be linked to the feeding/fasting cycle. Yet another PTM comprises the phosphorylation of the FXR isoforms FXR $\alpha$ 1 and  $\alpha$ 3 via PKA in the fasting condition to mediate hepatic glucose production via a glucagon/cAMP/PKA/FXR axis [89].

FXR may also be sumoylated which inhibits its transcriptional activity [90]. Sumoylation increases the interaction of FXR with NFkB but blocks that with RXR $\alpha$ , so that sumoylated FXR trans-represses inflammatory genes without affecting classical FXR/RXR $\alpha$  target genes [91]. Interestingly, acetylation at a certain position of FXR in excessive nutrient conditions prevents its sumoylation and promotes hepatic inflammation in obesity [91]. In addition, an atypical sumoylation event on FXR even coordinates an activation-degradation cycling pathway that regulates FXR responsive genes [92].

Overall, nutrient and energy sensitive PTMs of FXR enforce FXR signaling in the feeding state but may constitute a significant nutrient dependent factor potentially corrupting FXR activity in metabolic diseases. This may explain findings why the metabolic background is

significantly shifting FXR binding profiles and transcriptional outcome after ligand activation. These variations might be considered when FXR is targeted pharmaceutically in clinics. Of note, different FXR isoforms may harbor also different PTMs [86].

## 5. Physiological effects of FXR on metabolic homeostasis (Fig. 2, Table 2)

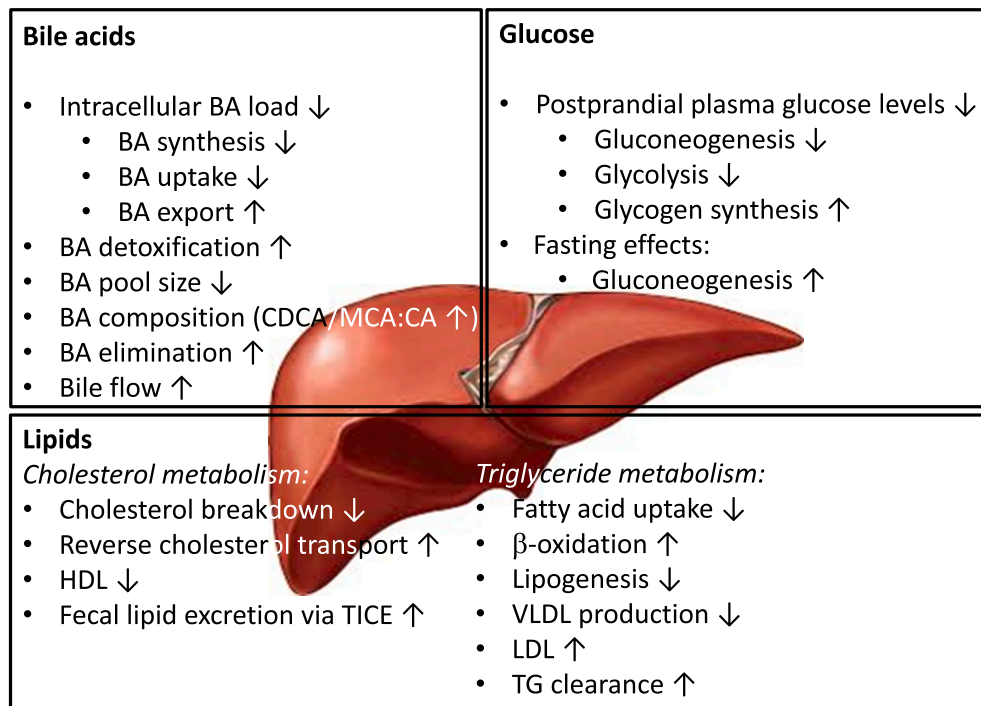
### Effects of FXR on bile acid metabolism:

Bile acids, which are generated from cholesterol in hepatocytes, undergo enterohepatic circulation between the liver and the intestine [51,93]. The bile acid concentration within the enterohepatic circulation is tightly controlled. Depending on the actual bile acid load further bile acids can be produced and more efficiently conserved within the enterohepatic circulation or their production can be repressed and bile acid excretion favored. The main sensor of bile acids in the enterohepatic circulation is FXR. Briefly, FXR activation reduces intracellular bile acid load in target tissues by repressing bile acid import transporters (i.e. *NTCP* and *ASBT*) and inducing bile acid export pumps (i.e. *BSEP*, *MRP2*, *OST $\alpha$ / $\beta$* ) along with suppression of bile acid synthesis (i.e. *CYP7A1*) [51,93].

FXR downregulates bile acid synthesis in hepatocytes via SHP-induced repression of CYP7A1 and from the intestine via induction of FGF19 and FGF19 mediated CYP7A1 repression [94]. However, for proper CYP7A1 repression by intestinal FGF19 sufficient hepatic SHP expression is required [94]. In addition, FXR primes the liver for intestinal FGF19 signaling by induction of the obligate co-receptor for FGF19,  $\beta$ -Klotho, thereby stabilizing the FGF receptor 4 [95]. Another level of FXR-depend regulation of Cyp7a1 consists of a rapid post-transcriptional degradation of Cyp7a1 mRNA via the FXR regulated RNA-binding protein Zfp3611 [96]. Hepatic FXR also regulates CYP8B1 [97] and thereby affects the ratio of the primary bile acids CDCA and CA. In contrast to Cyp7a1, the repressive effects on Cyp8b1 do not depend on intestinal FXR [98] and are less well explored. In addition to an inhibition of Cyp8b1 via FXR-Shp, FXR induces the transcriptional repressor Mafg, which represses Cyp8b1 (and other enzymes of the alternate bile acid synthesis pathway) and thus reduces cholic acid synthesis [99].

FXR activation also favors bile acid detoxification via induction of





**Fig. 2.** FXR is a metabolic homeostat for bile acid, glucose and lipid metabolism in the liver. **Bile acids:** FXR activation limits intracellular bile acid levels and overall results in BA elimination from the body. **Glucose:** FXR activation overall reduces postprandial glucose levels via limitation of hepatic glucose generation. **Lipids:** FXR activation overall reduces hepatic fatty acid generation, storage and release. However, long-term activation increases LDL and lowers HDL plasma levels (details see main text).

*CYP3A4*, *SULT2A1* and *UGT2B4* [100] and stimulates biliary phospholipid excretion via *MDR3* (*ABCB4*) [101]. Although stimulation of *MDR3* is predicted to counteract cholesterol gallstone formation, treatment with the FXR agonist obeticholic acid (OCA) in human volunteers decreased cholesterol solubility in bile by increasing gallbladder cholesterol saturation and bile acid hydrophobicity. This finding rather suggests that pharmacological activation of FXR increases the risk of gallstone formation [102].

In the ileum FXR activation controls shuttling of bile acids across enterocytes via regulation of bile acid uptake (via ASBT) and export (via OST $\alpha/\beta$ ) into the portal circulation [103]. High bile acid concentrations thus reduce bile acids returning back into enterohepatic circulation and favor their fecal elimination. Importantly, FXR stimulation in ileocytes induces FGF19 (FGF15 in mice) expression, which not only suppresses further bile acid synthesis in the liver [94] but also prepares the liver for the postprandial state by inhibiting gluconeogenesis and increasing glycogen and protein synthesis (for the pleiotropic effects of FGF19 on metabolic homeostasis the reader is referred to excellent recent reviews [104,105]).

Overall, intestinal and hepatic FXR activation profoundly feeds back in a quantitative as well as qualitative manner on bile acid pool size and composition. These changes not only reciprocally affect hepatic FXR signaling but equally importantly impact on extrahepatic bile acid signaling molecules such as TGR5 with profound effects on overall energy homeostasis [51,106].

#### Effects of FXR on glucose metabolism:

FXR is directly involved in glucose homeostasis in the liver and is sensitized for activation in the fed state. In the fed state FXR mRNA is increased [107] and glucose itself induces FXR mRNA levels in hepatocytes, while insulin reduces FXR expression, as shown in rodent diabetic models [108]. As discussed above, FXR can indirectly sense glucose levels also via O-GlcNAcylation, which renders FXR transcriptionally more active in the fed state. The effects of FXR on glucose homeostasis in part depend on the physiological context. In the feeding state FXR activation lowers plasma glucose levels via repression of gluconeogenesis and favors glucose disposition and glycogen storage. Thus, FXR<sup>-/-</sup> mice show glucose intolerance, insulin resistance and reduced hepatic glycogen stores [38,88,89,98]. The repressive effects on the

gluconeogenic genes G6pase and PEPCK depends on the FXR-SHP axis and is absent in either FXR<sup>-/-</sup> or SHP<sup>-/-</sup> mice [109,110]. Part of the negative effects of FXR on gluconeogenesis is also mediated via SHP inhibitory effects on HNF4 and FOXO1, the latter one being a pro-gluconeogenic transcription factor [111]. In addition, intestinal FGF19 also aids in reducing gluconeogenesis in the fed condition via inactivation of cAMP response element binding protein (CREB) and consequently reduction of PGC1 $\alpha$ -mediated PEPCK and G6Pase stimulation [112]. This may also explain why glucose sensitizing effects can also be achieved when selectively intestinal FXR is targeted with the non-absorbable FXR ligand fexaramine [113]. The effects rely on the release of intestinal FGF19 and mimic the effects of FGF19 over-expressing models [112]. In line with the glucose tolerant effects of FGF19, FGF15<sup>-/-</sup> mice are hyperglycemic.

In contrast to the fed state, FXR activation increases the expression of the gluconeogenic key enzymes PEPCK and G6Pase in the fasted state. One mechanism involves activation of the glucocorticoid receptor by FXR in the fasted state [114], another the phosphorylation of FXR via PKA, as mentioned in the PTM section [89]. A potential third mechanism includes a glucagon-FOXO2-mediated transcriptional repression of FXR-activated SHP [89]. In addition, in the fasting state the FXR effects are also counteracted by PPAR $\alpha$  and FGF21 release, which activate PGC1 $\alpha$  to stimulate gluconeogenesis [115]. Noteworthy, most authors report an inhibitory effect of FXR on gluconeogenesis (in the fed condition), however, the overall literature is controversial [116–118]. For example, FXR<sup>-/-</sup> mice have a decreased hepatic glucose production along with reduced PEPCK expression after short term feeding suggesting that FXR expression is necessary for maintaining basal hepatic PEPCK expression [118]. Along these lines Stayrook et al. report that FXR stimulates PEPCK via a mechanism including PPAR $\alpha$  activation and a signaling cascade resulting in FOXO1 activation and PEPCK transcription [116].

FXR activation also increases hepatic glycogen levels [110]. This effect appears to depend on two FXR dependent mechanisms. First, FXR represses glycolysis via binding to and releasing of ChREBP and HNF4 from the LPK promotor [57]. The main purpose of ChREBP is to induce expression of glycolytic and lipogenic genes, which is interfered by FXR. The repression of glycolysis by FXR disposes glucose for glycogen synthesis via insulin action. Second, FXR-induced release of intestinal

**Table 2**  
Effects of FXR activation on liver metabolism.

	Function	Factors involved	Gene/protein/ pathway
Bile acid metabolism	BA synthesis ↓	SHP, FGF19	↓ CYP7A1
	BA uptake ↓	SHP	↓ NTCP, OATP1B1
	BA export and elimination ↑		↑ BSEP, MRP2, MDR3, OSTα/β ↓ ASBT
	BA detoxification ↑		↑ CYP3A4, SULT2A1, UGT2B4
Glucose metabolism	BA composition	SHP, Mfag	↓ CYP8B1
	Bile flow ↑		↑ BSEP, MRP2, MDR3
	Gluconeogenesis ↓	SHP, inhibition of HNF4 and FOXO1 FGF19, CREB and PGC1α inhibition	↓ PEPCK, G6pase
	Glycolysis ↓	ChREBP and HNF4 inhibition	↓ glycolytic genes
Lipid metabolism	Glycogen synthesis ↑	FGF19 and GSK3 inhibition, SHP	↓ LPK
	cholesterol breakdown ↓	SHP, FGF19	↓ CYP7A1
	Reverse cholesterol transport ↑		↑ SR-B1, PLTP, ApoE, ApoC1, ApoC4 ↓ ApoA1; ↑ CETP, Pon1
	HDL ↓		↓ ApoA1; ↑ CETP, Pon1
	Fecal lipid excretion via TICE	FGF19	indirect: intestinal ↑ ABCG5/8 and ↓ NPC1L1
	Fatty acid uptake ↓ b-oxidation ↑	SHP, HNF4 PPARα	↓ CD36 ↑ MCAD, ACOX, PDK4
		FGF19	inhibition of ACC2, induction of CPT1
	Lipogenesis ↓	SHP, inhibition of LXR and SREBP1c inhibition of ChREBP	↓ FAS, ACC, SCD1 ↓ LPK
		FGF19, inhibition of SREBP1c	↓ FAS, ACC, SCD1
	VLDL secretion ↓	SHP, inhibition of HNF4	↓ MTP, ApoB
Protein metabolism autophagy	LDL ↑		↓ LDLR, PCSK9
	TG clearance ↑		increase in LPL activity by ↑ ApoC2 and ↓ ApoC3 ↑ VLDLR, SCD1 ↑ eIF4B, eIF4E, P- S6
	Protein synthesis ↑	FGF19, ERK, mTOR	↑ eIF4B, eIF4E, P- S6
	Amino acid breakdown ↑		
	Ammonium detoxification ↑		
Autophagic degradation ↓		Inhibition of PPARα, disruption of CRT2-CREB	↓ autophagic genes, ↑ Rubicon

FGF19 stimulates hepatic glycogen synthesis independently of insulin [119]. Intestinal FGF19 also aids in reducing gluconeogenesis in the fed condition via reduction of PGC1α-mediated PEPCK and G6Pase stimulation. This explains why glucose sensitizing effects can also be achieved when selectively intestinal FXR is targeted with the non-absorbable FXR ligand fexaramine [113]. The effects rely on the release of intestinal FGF19 and mimic the effects of FGF19 overexpressing models [112]. In line with the glucose tolerant effects of FGF19, FGF19<sup>-/-</sup> mice are hyperglycemic. FGF19 mimetics are therefore an interesting drug target for the treatment of diseases associated with insulin resistance [120]. In contrast to FXR stimulation, FXR<sup>-/-</sup> mice show glucose intolerance, insulin resistance and reduced hepatic glycogen stores [109,110,118]. The reduced glycogen content in FXR<sup>-/-</sup> mice may, however, be responsible for the fact that these mice show transient hypoglycemia

upon short term fasting [38].

In contrast to the physiological effects of FXR on glucose homeostasis during feeding and fasting cycles, the contribution of FXR on insulin resistance in the pathophysiological setting of obesity is less clear. In direct contradiction to the antidiabetic reports of FXR activation appear findings that long-term feeding of obese mice with various FXR agonists accelerates glucose intolerance [121]. One plausible explanation for this finding may be related to the long-term reduction of the bile acid pool size and reduced energy expenditure. These negative effects of FXR on glucose homeostasis in obesity are supported by other reports in obese FXR<sup>-/-</sup> mice, which show that in the absence of FXR glucose homeostasis and insulin sensitivity is improved in ob/ob and high-fat diet (HFD) fed mice [122,123]. Interestingly, these effects were only seen in whole body and not in liver-specific FXR<sup>-/-</sup> mice, indicating that extrahepatic, likely intestinal, FXR effects on glucose metabolism are critical. The importance of intestinal FXR for glucose homeostasis in obesity is supported by several observations. Bile acid sequestrants such as cholestyramine or cholestevam, which bind luminal bile acids and therefore reduce small-intestinal FXR signaling improve glucose homeostasis in type 2 diabetes [124–127]. The beneficial effects of bile acid sequestrants on glucose homeostasis may be attributed to (FXR-independent) colonic TGR5 stimulation of resin bound bile acids and subsequently GLP-1 secretion [124–127], which promotes insulin release and lowers glucagon secretion from the pancreas. In addition, sequestrants treatment reduces bile acid absorption and intestinal FGF19 release, which derepresses hepatic bile acid synthesis and shifts bile acid composition towards CA-enriched bile acid pools. CA is converted to DCA by gut bacteria to activate both FXR and TGR5 in colonic L cells and stimulates GLP-1 secretion [128,129]. The most compelling evidence for the beneficial metabolic effects of intestinal FXR antagonism, however, comes from a series of elegant studies, which show that alterations in the gut microbiome may produce directly acting intestinal FXR antagonists such as tauro-β-muricholic acid in mice [130,131] or glyco-ursodeoxycholic acid in human [132]. These intestinal FXR antagonists reduce FXR-dependent production of intestinal ceramides, which could otherwise give rise to hepatic ER stress and lipotoxicity as well as a shift in the ratio of white to brown adipocytes [133]. Similar effects have also been seen with antibiotic treatment and in intestinal FXR<sup>-/-</sup> mice [130,134]. Parts of the antidiabetic effects of metformin also appear to work via modulation of the gut microbiome and the induction of an intestinal FXR antagonist [132]. The intestine may also contribute to lower glucose plasma levels by delayed glucose uptake into enterocytes in FXR<sup>-/-</sup> conditions [135]. However, as already mentioned above, also activation of intestinal FXR has substantial metabolic benefits in models of obesity. Selective activation of intestinal FXR with the poorly absorbable FXR agonist fexaramine significantly reduced weight and improved insulin sensitivity in HFD-fed mice [113]. These effects were mainly attributed to a FGF15-induced switch in the bile acid composition with high relative amounts of serum lithocholic acid (LCA). LCA is the most potent endogenous activator of TGR5, which results in browning of white adipose tissue in these animals with subsequent increased thermogenesis. Likewise, effects of fexaramine are reduced in TGR5<sup>-/-</sup> mice [113]. An interesting follow up study however, found that effects of fexaramine can be prevented by antibiotic treatment [136]. Fexaramine and intestinal FXR activation shape the gut microbiota to produce LCA, activate intestinal TGR5 and subsequently release GLP-1. Antibiotic treatment reduces LCA producing bacterial strains and completely reversed fexaramine-induced metabolic effects on LCA synthesis, adipose tissue browning and insulin sensitivity [136]. Effects of the gut microbiome on bile acid metabolism and FXR signaling are well-appreciated, though not yet fully understood [137]. An elegant comprehensive study demonstrated that the gut microbiota promoted weight gain, insulin sensitivity and hepatic steatosis in a strictly FXR-dependent manner and reciprocally, FXR contributes to increased adiposity by altering the microbiota composition [138]. Microbiota transfer from obese wild-type and FXR<sup>-/-</sup> mice to germ-free recipients

showed that the altered microbiota partly contributed to the improved metabolic profile of FXR<sup>-/-</sup> mice [138]. A full recapitulation of several other elegant studies is, however, beyond the scope of this review.

Recent studies also show that abrogation of hepatic FXR signaling contributes to improved metabolic phenotypes. In aged mice and HFD-fed obese the combined deletion of the hepatic FXR/SHP axis reverses the aging and metabolic phenotype of body weight gain, increased adiposity, and glucose/insulin tolerance [68,69]. Interestingly, this phenotype is not observed in single FXR<sup>-/-</sup> or SHP<sup>-/-</sup> mice [68]. Disruption of the hepatic FXR/SHP axis facilitates efficient fat usage in WAT and BAT gene expression via activation of TGR5 in BAT by high serum bile acid levels FXR<sup>-/-</sup>/SHP<sup>-/-</sup> mice (68). Adding to overall complexity and controversial findings, it also appears that different genetic FXR<sup>-/-</sup> models show divergent effects on glucose homeostasis [139].

Taken together, FXR activation is a cornerstone in the hepatic disposal and distribution of glucose in the physiological transitions between feeding and fasting cycles. To effectively control glucose homeostasis hepatic FXR acts in immediate concert with intestinal FXR and FGF19 signaling. When the rhythmic transition between fed-fast states is interrupted, e.g. when FXR signaling is overridden by long term pharmacological or genetic manipulations or alterations of the gut microbiome, long-term adaptations such as changes in bile acid pool size and composition may alter expected physiological FXR signaling responses.

Effects of FXR on lipoprotein and lipid metabolism:

FXR is a key regulator of cholesterol and lipid homeostasis and – from a clinical perspective – can determine the atherosclerotic risk and fat content of the liver. The impact on cholesterol metabolism is established by regulating cholesterol breakdown and cholesterol distribution. FXR directly regulates CYP7A1, the rate limiting enzyme in the conversion of cholesterol into bile acids. This is probably the most crucial function of FXR because thereby it also controls – in a feedback loop – its own physiological ligand availability. For sufficient reduction of hepatic CYP7A1 crosstalk between hepatic SHP and intestinal FGF19 release is necessary (see above and [94]). In mice, but not in humans, the FXR-dependent regulation of Cyp7a1 is directly opposed by LXR, which stimulates cholesterol breakdown [140]. However, FXR<sup>-/-</sup> mice, which have increased Cyp7a1 levels, do not show reduced cholesterol levels but instead are markedly hypercholesterolemic [97,141]. This phenotype has been attributed to the ability of FXR to control gene expression of cholesterol transporting apolipoproteins as well as cholesterol transporting membrane proteins. Cholesterol and cholesterol esters are transported in the systemic circulation packed as lipoproteins. VLDL and LDL transport cholesterol esters from the liver to the periphery whereas HDL returns cholesterol to the liver (reverse cholesterol transport) for further metabolism into bile acids and/or biliary secretion. Thus, LDL is regarded as “bad” cholesterol since it promotes vascular plaque formation and HDL is termed “good” since it protects from cholesterol depositions in the periphery [6].

The effects of FXR on lipoprotein metabolism are in part controversial and there exist ligand, sex specific and species differences, besides differences in the methodological design of the experiments [6,142,143]. Different FXR ligands possess different potential to induce transhepatic cholesterol efflux from plasma into feces, which was related to different capacities to induce genes mediating clearance of cholesterol efflux (e.g. SR-B1, and others) [143]. Sex-specific differences in lipid metabolism are to a large extent modulated via the gut microbiota and bile acid composition [144]. Sex is one of the variables affecting the gut microbiota [145] which synthesizes a number of FXR agonists and antagonists that compete for the activation or suppression of intestinal FXR [133] and therefore lead to sex specific differences.

A newly developed mouse model with a humanized chimeric liver may however help to overcome species differences and may allow for the urgently awaited detailed studies of FXR agonism on lipoprotein metabolism [146]. Overall, in “regular” mice FXR ligand activation is anti-atherogenic by enhancing reverse cholesterol transport and limiting

intestinal cholesterol absorption, which leads to reduced atherosclerotic plaque formation [147,148]. These effects are largely independent of SHP and for unknown reasons are less effective in female mice [148]. In line, FXR antagonists exacerbate the dyslipidemic profile in susceptible mice [149]. FXR<sup>-/-</sup> mice display a pro-atherogenic profile with increased serum cholesterol levels composed of increased HDL and LDL levels. When FXR<sup>-/-</sup> mice are challenged with cholesterol enriched diet and/or crossbred into the pro-atherogenic ApoE<sup>-/-</sup> background atherosclerotic plaque formation exacerbates [150]. The increase in serum HDL levels in FXR<sup>-/-</sup> mice is caused by a reduced reverse cholesterol transport and HDL clearance rate because scavenger receptor B1 (SR-B1), the hepatic transporter responsible for re-uptake of HDL back into liver, is under direct positive control of FXR [141]. Of note, reverse cholesterol transport is regulated by LXR and FXR in a coordinated manner. LXR stimulates the first step in reverse cholesterol transport, which is the transfer of cholesterol to HDL via ABCA1 and ABCG1 [151,152], while FXR regulates the uptake of HDL into the liver via SR-B1 [141].

There exist significant differences in HDL metabolism between mice and humans and from the plasma lipoprotein levels it appears that FXR activation in humans rather shows a pro-atherogenic profile. In human hepatocytes ApoA1, which is the main lipoprotein of HDL is under direct negative regulation of FXR, and FXR stimulation reduces HDL levels [55]. The role of FXR in bile-acid mediated repression of human ApoA1 has recently been questioned [153], but murine ApoA1 is not repressed by bile acids and/or FXR [141,153]. The human finding is further supported by additional evidence, that bile acid sequestrants which reduce bile acid pools increase ApoA1 and HDL [154], whereas patients with prolonged cholestasis and increased serum bile acids have lowered ApoA1 and HDL cholesterol levels [55,155]. Patients treated with the FXR ligand obeticholic acid (OCA) also display reduced HDL [156]. The pro-atherogenic impact of FXR on human HDL metabolism is extended by the stimulating effects on cholesterol ester transfer protein (CETP), which promotes the exchange of cholesteryl esters and TGs between HDL and ApoB-containing lipoproteins, resulting in a pro-atherogenic plasma lipid profile with increased VLDL and LDL cholesterol levels and decreased HDL cholesterol [157]. FXR also regulates phospholipid transfer protein, which is important for the maintenance of HDL metabolism [158] as well as hepatic lipase [159], but the relevance for the atherogenic risk profile of these enzymes is not entirely clear. Potential anti-atherogenic properties of FXR may consist in a reduction of the potent atherogenic lipoprotein(a) [160]. Eventually, clinical studies in humans with prolonged FXR activation have to determine whether activation of FXR reduces atherogenic plaque formation as a convincing read-out marker.

The increase in LDL cholesterol in FXR<sup>-/-</sup> mice can be attributed to increased hepatic synthesis of ApoB containing particles, which are the main component of VLDL and LDL, and enforced VLDL loading via microsomal triglyceride transfer protein, MTP, which is under direct control of HNF4 and can be blocked via FXR-SHP-HNF4 inhibition [161]. VLDL particles are transformed by the action of intravascular lipoprotein lipases, which release TGs and enable hydrolysis of TGs into fatty acids. FXR regulates the expression of the secreted cofactors required for LPL activity. Activation of FXR induces ApoC2 [162], which activates LPL and reduces the expression of ApoC3, which inhibits LPL [56]. Thus, FXR activation paradoxically also results in increased LDL cholesterol [156,163] by the transformation of TG-rich VLDL into TG-poor but cholesterol-rich LDL particles. In addition, hepatic LDL-receptor mRNA and its modulator PCSK9 are decreased upon bile acid treatment and thus also contribute to an LDL increasing profile [164–166].

FXR activation promotes cholesterol excretion on hepatic and intestinal levels. FXR activation represses CYP7A1 and the accumulating hepatocellular cholesterol is rerouted via ABCG5/G8, the canalicular cholesterol heterodimer transporters, into bile. CA feeding increased Abcg5/8 expression [167] and FXR<sup>-/-</sup> mice have decreased expression

of Abcg5/8 [141], but the regulation of this cholesterol transporter likely is via LXR [168]. Another mechanism for FXR dependent cholesterol export is a route directly from blood into the intestinal lumen without using the biliary route. This pathway is known as transintestinal cholesterol excretion (TICE), which can account for up to 30% of fecal neutral sterol output in mice. FXR activation in the intestine along with FGF19 and a shift in the bile acid composition can significantly increase sterol elimination via enhanced ABCG5/8 activity in the intestine [169]. In addition, postprandially raised intestinal FGF19 results in a phosphorylation PTM of SHP which subsequently inhibits intestinal cholesterol absorption via NPC1L1 [170].

FXR also regulates lipogenesis and TG metabolism and thus contributes to the overall “fat” content of the liver. Of note, the effects on lipogenesis are inverse to the pro-lipogenic effects of LXR [6]. Overall, FXR activation reduces fatty liver in mice and humans and FXR<sup>-/-</sup> mice are prone to fatty liver disease (for review [171]). A separate review in this special issue is focusing on the role of nuclear receptors for non-alcoholic fatty liver disease (NAFLD), therefore the effects of FXR modulation on NAFLD are not further expanded here. The TG-lowering effect in the liver is not only achieved by TG clearance via stimulating VLDL export and endothelial LPL activity but also engage a more direct mechanism. FXR reduces the major transcription factor for lipogenic pathways, SREBP-1c via induction of SHP and thereby reduces fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and sterol CoA desaturase (SCD1) [64], although SHP independent repression appears to exist [107]. In addition, FGF19 supports the repressive effects on SREBP-1c and lipogenic enzymes [172], but eventually hepatic lipid accumulation under dietary challenge depends on the existence of hepatic FXR [173]. For regulation of lipogenesis FXR directly opposes the effects of hepatic LXR, which is a positive regulator of SREBP-1c and de-novo lipogenesis [174,175].

The role of FXR for fatty acid oxidation is less clear and species specific differences may exist. FXR activation induces human but not murine PPAR $\alpha$  expression and its target genes [176], suggesting cross-talk of FXR with PPAR $\alpha$ -mediated  $\beta$ -oxidation. Moreover, FXR is a direct activator of carboxylesterase-1 (CES1), which also hydrolyzes TGs and results in increased fatty acid oxidation, potentially via stimulation of PPAR $\alpha$  [177]. Also, the ability of FXR activation to increase pyruvate dehydrogenase kinase PDK4 should suppress glycolysis and favor  $\beta$ -oxidation [178]. In contrast, CA feeding in mice inhibited PPAR $\alpha$  target genes [179] and enzymes of  $\beta$ -oxidation are increased in FXR<sup>-/-</sup> mice [109].

Taken together, FXR and FGF19 reduce hepatic lipogenesis in the postprandial state and therefore counteract and terminate the lipogenic effects of insulin, ChREBP and LXR. Simultaneously, FXR increases HDL return and reduces VLDL secretion. Cholesterol is shifted towards biliary and transintestinal elimination and lipids may further be broken down and oxidized.

#### *Effects of FXR on protein metabolism:*

FXR is not only orchestrating hepatic glucose and lipid disposal but also regulating amino acid metabolism. In the fed state amino acids in the liver are either used for protein synthesis or can be transformed into glucose and fatty acids or used for energy generation. The action of FXR on amino metabolism appears to be a dual one as it is involved in anabolic (i.e. stimulation of protein synthesis) [119] but also catabolic (i.e. amino acid break-down and ammonium detoxification) [180] processes. FXR activation is probably able to stimulate protein synthesis via FGF19 signaling in hepatocytes. It has been shown that FGF19 treatment increases phosphorylation of components of the eukaryotic initiation factor 4 complex (i.e. eIF4B and eIF4E), which mediates mRNA binding to the ribosome. In addition, phosphorylation of the ribosomal subunit S6 enhances total protein synthesis including albumin synthesis in mouse liver [119]. The mechanisms of stimulating protein synthesis by the hormone FGF19 are through the Ras/ERK pathway and thus different to that of insulin, which signals via the PI3K/Akt/mTOR signaling pathway [119]. mTOR signaling is the central kinase signaling

hub to integrate nutritional inputs with anabolic responses and cell-cycle progression in the fed state [181]. A recent report, however, shows that FGF19 can very well activate mTOR signaling in the presence of amino-acids. Interestingly, not only the proliferative effects of FGF19 are mediated by this FGF19-mTOR axis but also some of the metabolic effects, such as fatty acid oxidation, glucose metabolism and bile acid metabolism [182]. This suggests that mTOR is an indispensable mediator of mitogenic and importantly also metabolic FGF19 effects. However, it has not been studied whether FXR activation utilizes this signaling pathway in the postprandial phase or whether FXR can directly interact with the mTOR pathway.

The effects of FXR-FGF19 on protein synthesis may have broader energetic implications as it not only affects the transcription of a single gene but more broadly regulates transcription of secretory proteins in the postprandial phase independently of the diverse functions of the single proteins [59]. The consequence for overall hepatic energy homeostasis becomes apparent when considering that the liver is among the most active secretory organs (next to the pancreas and the salivary glands) [183] and approximately half of the proteins produced by hepatocytes are secreted [59,183]. Interestingly, genome wide profiles indicate that PPAR $\alpha$  is engaged in the opposite reaction and spares energy resources in the fasted state [59]. The FXR-PPAR $\alpha$  dualism is further underpinned by the recent finding that FXR represses the steroidogenic enzyme Cyp17a1 in the fed state. In the fasted state Cyp17a1 is de-repressed and produces a hormone-ligand (i.e. dehydroepiandrosterone) for PPAR $\alpha$  [184]. Overall, a concept is emerging where FXR and FGF19 fuel protein secretion in the nutrient-rich fed state and therefore coordinate energy fluxes in the postprandial phase [59].

Surprisingly, FXR is also directly involved in amino acid catabolism and the urea cycle, which transforms ammonia from amino acid catabolism into urea for renal elimination [180]. Functionally, FXR activation promotes protein degradation and ammonium clearance in mice through direct binding to genes of amino acid degradation, ureagenesis and glutamine synthesis. FXR<sup>-/-</sup> mice have reduced plasma urea concentration after a protein rich diet, but accumulated precursors of ureagenesis, which is due to reduced hepatic expression of enzymes that regulate ammonium detoxification. This mechanism is important, since hyperammonia is a typical problem of patients with acute or chronic liver diseases and FXR activation might promote ammonium clearance in these patients [180].

#### *Effects of FXR on autophagy:*

Autophagy is a catabolic lysosomal degradation pathway that recycles cellular components to provide nutrients upon starvation conditions [185]. Autophagy is regulated by feeding and fasting signals, most prominently by the mTOR pathway. In the feeding state autophagy is suppressed and in the fasting state it is activated. Recently, the spectra of nutrient dependent signals that regulate autophagy was extended to the NRs FXR and PPAR $\alpha$ , which inversely regulate autophagy to maintain cellular energy homeostasis [42](for review [186]). FXR agonists such as GW4064 suppress autophagy even in the fasted state and the repressive effects of feeding were blunted in FXR<sup>-/-</sup> mice. Genome wide FXR binding studies confirmed that FXR was targeted to autophagy related genes including LC3a and LC3b. Surprisingly, motif analysis detected an FXR-untypical DR-1 binding motif, which however is a typical positive response element for the PPAR $\alpha$ /RXR heterodimer. In fact, PPAR $\alpha$  shows exactly opposite effects on autophagy. The PPAR $\alpha$  agonist GW7467 induces autophagy genes even in the fed condition and the induction of autophagy upon fasting was blunted in PPAR $\alpha$ <sup>-/-</sup> mice. Detailed studies show that FXR recruits the co-repressor NCOR to its DR-1 binding site but also appears to directly compete in an alternating manner with PPAR $\alpha$  for the binding sites in promoters and enhancers of autophagy-related genes. A different mechanism for FXR mediated repression of autophagy depends on the FXR mediated disruption of the CRTC2-CREB complex, which upregulates autophagy-related genes in the fasting condition [58]. Of note, in none of these studies effects of FXR on the mTOR pathway have been noted and it remains open if the



reported suppressive effect of FGF19 on autophagy [187] involves mTOR as previously shown for protein synthesis and some of the FXR related metabolic effects (see above and [182]).

Our own study showed impaired autophagy in several cholestatic liver diseases, including PBC, PSC/SSC and genetic cholestasis [188]. Mechanistically, genome-wide FXR ChIP seq analyses showed that FXR bound sites in close proximity to genes of pathways for vesicle transport including the autophagosome-lysosome fusion pathway. These genes were selectively bound by FXR only in the cholestatic condition. Rubicon, which is an inhibitor of the autophagosome-lysosome fusion process is a bona-fide target of FXR in ChIP seq analyses and is robustly increased on transcriptional levels in liver tissues of cholestatic patients as well as in primary human hepatocytes treated with CDCA or the FXR agonist obeticholic acid (OCA) [188]. Interestingly, ursodeoxycholic acid (UDCA), which is antagonistic to FXR and the first line treatment for various cholestatic liver diseases, showed opposing effects. UDCA stimulates autophagy and reduces Rubicon in human liver tissue [188]. Whether FXR-induced Rubicon plays a role in the feeding and fasting regulation of autophagy in humans is not known.

## 6. FXR crosstalk with inflammatory pathways

Besides its role for liver metabolism, FXR activation also shows anti-inflammatory properties. FXR<sup>-/-</sup> mice show increased levels of hepatic inflammation, which is a driver for inflammation induced carcinogenesis in these mice [189,190]. In the intestine FXR<sup>-/-</sup> mice have a compromised epithelial barrier and reduced antibacterial factors such as angiogenin, inducible nitric oxide synthase (iNOS) and interleukin-18, which favors bacterial invasion and transition from gut derived toxins [191]. Likewise, FXR activation maintains the intestinal barrier function and inhibits inflammation in models of inflammatory bowel disease [192]. An anti-inflammatory key event is the capability of FXR to interact with nuclear factor- $\kappa$ B (NF $\kappa$ B) [43,193,194]. NF $\kappa$ B is a core transcriptional regulator of the inflammatory response and of cell proliferation which is rapidly activated in response to pro-inflammatory stimuli [195]. FXR activation transrepresses hepatocellular NF $\kappa$ B activation and reduces classical pro-inflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and iNOS [193]. Reciprocally, NF $\kappa$ B activation antagonizes FXR activity and target gene expression [193]. Interestingly, NF $\kappa$ B regulated anti-apoptotic genes are not repressed by FXR suggesting that FXR selectively inhibits only the effects of NF $\kappa$ B on inflammation [43,193]. From a clinical perspective it would be favorable to identify or develop FXR modulators which selectively repress inflammatory pathways without interfering with the metabolic effects of FXR. In such an effort mometasone furoate was identified as a compound that selectively reduced NF $\kappa$ B reporter activity in an FXR-dependent manner [43]. As already pointed out above sumoylation increases the interaction of FXR with NF $\kappa$ B but blocks that with RXR $\alpha$ , so that sumoylated FXR trans-represses inflammatory genes without affecting classical FXR/RXR $\alpha$  target genes [91].

Another level of FXR-dependent control of inflammation represents the inflammasome. Inflammasomes are cytoplasmic protein complexes, which sense inflammatory stimuli and induce an inflammatory response by mediating cleavage of pro-inflammatory cytokines [196]. While bile acids have been identified as stimuli (i.e. danger associated molecular patterns/DAMPs) which can activate the inflammasome in macrophages, FXR was found to be an important negative regulator of the NLRP3 inflammasome by direct physical interaction with NLRP3 and caspase 1 [197]. This suggests that FXR could play a role in the modulation of sepsis induced cholestasis [197]. In addition, FXR also has immunomodulatory effects by facilitating homing and function of myeloid-derived suppressor cells which function as a critical negative feedback loop in immune-mediated liver injury [193,198,199].

## 7. Perspective: FXR as a metabolic drug target

FXR has emerged as an intriguing pharmacological target for various metabolic diseases and its agonist OCA has already been approved as second line treatment for primary biliary cholangitis [200]. Although, disease determining or modifying genetic mutations of FXR are reported only for a minority of conditions (e.g. cholestasis [201] and gallstone disease [202]), FXR is a bona-fide target to correct metabolic imbalances. Currently there are more than 50 human clinical trials listed for various steroidal and non-steroidal FXR agonists and for FGF19 ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). Most of these clinical trials encompass cholestatic liver disease, NAFLD, effects on lipoprotein metabolism, but also bile acid malabsorption, diarrhea and lipodystrophy. The rationale for treating cholestatic liver disease is straight forward and supported by an already extensive publication record in rodent and human models of cholestatic liver diseases [203,204]. The beneficial effects of FXR agonism for Type 2 diabetes and NAFLD are less clear to dissect and the existing body of evidence in the literature is confounding particularly because of existing species differences. OCA treatment of patients with Type 2 diabetes resulted in improved insulin sensitivity in hyperinsulinemic-euglycemic insulin clamp experiments [205], which is likely a consequence of reduced gluconeogenesis. These studies also showed improvements of surrogate markers of liver fibrosis and liver injury and also some loss of total bodyweight [156,205,206]. The beneficial effects on markers of liver fibrosis have been shown to directly translate into improved histology in OCA-treated NAFLD patients [156]. Reduction of fat content, inflammation and anti-fibrotic effects may be direct FXR effects [207]. However, LDL cholesterol was increased and HDL cholesterol significantly decreased, which represents the cardiovascular hallmark of an atherosclerotic risk profile [208]. These pro-atherogenic lipid profiles resemble a significant obstacle for the treatment of patients with type 2 diabetes, obesity and NAFLD which often have increased cardiovascular risks. Another very common and disturbing side effect of FXR agonists is *de-novo* appearance or aggravation of existing pruritus in up to 23% to 70% of NAFLD or PBC patients, respectively [156,200]. The pruritogenic effects, however, appear to be more prevalent with the steroidal FXR agonist OCA than with the non-steroidal FXR agonists [209]. Mechanistically, activation of TGR5 may account for a proportion of OCA related pruritus since OCA might activate TGR5 in submicromolar concentrations [210,211].

Mouse experiments suggest that the even the beneficial effects of bariatric surgery in morbidly obese patients, which reroutes the natural flow of nutrients along the gastrointestinal tract and results in elevated bile acid levels [212], also depends at least in part on FXR [213]. However, it is not entirely clear if these effects are a consequence of intestinal or rather hepatic FXR and effects are robustly influenced by the gut microbiome [214]. Together, FXR agonists are promising for the treatment of metabolic diseases but may on the other hand also worsen distinct metabolic parameters. This may clinically not be apparent in short term clinical trials but may become a significant risk for long-term treatment.

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## Declaration of competing interest

**Katrin Panzitt:** has nothing to disclose of relevance for this manuscript

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