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The function of xenobiotic receptors in metabolic diseases

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Running Title Page: Xenobiotic Receptors in metabolic diseases

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AHR; aldo-keto reductase 1B10, AKR1B10; activator protein 1, AP-1; cholic acid, CA; constitutive androstane receptor, CAR; chenodeoxycholic acid, CDCA; 6-(4-Chlorophenyl) imidazo [2,1-b] thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl) oxime, CICTO; carnitine palmitoyl-transferase 1A, Cpt1a; cAMP-response element-binding protein, CREB; deoxycholic acid, DCA; Dicyclohexyl phthalate, DCHP; dexamethasone, DEX; 3',4'-Dimethoxyflavone, DMF; de novo lipogenesis, DNL; (-)-epigallocatechin-3-gallate, EGCG; fatty acid, FA; fatty acid synthase, Fasn; fructose 1,6-bisphosphatase, FBPase; farnesyl-diphosphate farnesyltransferase 1, FDFT1; fibroblast growth factor-19, FGF-19; 5,11-dihydroindolo[3,2-b] carbazole-12-carbaldehyde, FICZ; forkhead box protein O1, Foxo1; farnesoid X receptor, FXR; glucose 6-phosphatase, G6Pase; glucokinase, GCK; glucose transporter 2, GLUT2; glutathione peroxidase, GPx; glutathione S-transferases, GSTs; 3-hydroxy-3-methylglytaryl-CoA synthetase, HMGCS; hepatocyte nuclear factor 4α, HNF4α; hormone-sensitive lipase, HSL; insulin-induced gene-1, Insig-1; 2-(10-H-indole-3-carbonyl) thiazole-4-carboxylic acid methyl ester, ITE; lithocholic acid, LCA; long non-coding RNAs, lncRNAs; lipopolysaccharide, LPS; Lanosterol synthase, LSS; multidrug resistance mutation 1, MDR1; microRNAs, miRNAs; 3'-methoxy-4'-nitroflavone, MNF; multidrug resistance proteins, MRPS; microsomal triglyceride transfer protein, MTP; nuclear factor kappa B, NF-κB; non-alcoholic fatty liver disease, NAFLD; NPC intracellular cholesterol transporter 1, NPC1; Niemann-Pick C1-like 1, NPC1L1; organic anion transporting polypeptides, OATPs; pregnenolone-16a-carbonitrile, PCN; plasma proprotein convertase subtilisin/kexin type 9, PCSK9;

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phosphoenolpyruvate carboxykinase, PEPCK; phosphor-gluconate dehydrogenase, PGD; patatin-like phospholipase domain-containing protein 3, Pnpla3; peroxisome proliferator-activated receptor γ, PPARγ; pregnant X receptor, PXR; stearyl CoA desaturase, SCD1; squalene epoxidase, SQLE; sterol regulatory element binding transcription factor 1, SREBP1; signal transducer and activator of transcription 3, STAT3; sulfotransferase 1E1, SULT1E1; type 2 diabetes, T2D; tributyl citrate, TBC; 2,3,7,8-tetrachlorodibenzo-p-dioxin,TCDD; 1,4-Bis-[2-(3,5-dichloropyridyloxy)] benzene, 3,3′,5,5′-Tetrachloro-1,4-bis (pyridyloxy) benzene, TCPOBOP; Toll-like receptor 4, TLR4; Takeda G protein-coupled receptor 5, TGR5; tumor necrosis factor α, TNF-α; retinoid X receptor, RXR; uncoupling protein 1, Ucp1; uridine diphosphate glucuronosyltransferase, UGT; wild-type, WT; xenobiotic receptor, XR;

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Abstract

Metabolic diseases are a series of metabolic disorders that include obesity, diabetes, insulin resistance, hypertension, and hyperlipidemia. The increased prevalence of metabolic diseases has resulted in higher mortality and mobility rates over the past decades, and this has led to extensive research focusing on the underlying mechanisms. Xenobiotic receptors (XRs) are a series of xenobiotic-sensing nuclear receptors that regulate their downstream target genes expression, thus defending the body from xenobiotic and endotoxin attacks. XR activation is associated with the development of a number of metabolic diseases such as obesity, nonalcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D), and cardiovascular diseases, thus suggesting an important role for XRs in modulating metabolic diseases. However, the regulatory mechanism of XRs in the context of metabolic disorders under different nutrient conditions is complex and remains controversial. This review summarizes the effects of XRs on different metabolic components (cholesterol, lipids, glucose, and bile acids) in different tissues during metabolic diseases. As chronic inflammation plays a critical role in the initiation and progression of metabolic diseases, we also discuss the impact of XRs on inflammation to comprehensively recognize the role of XRs in metabolic diseases. This will provide new ideas for treating metabolic diseases by targeting XRs.

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Key Words: Xenobiotic receptor, metabolic disease, metabolic components, inflammation, insulin resistance.

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Significance Statement

This review outlines the current understanding of xenobiotic receptors on nutrient metabolism and inflammation during metabolic diseases. This work also highlights the gaps in this field, which can be used to direct the future investigations on metabolic diseases treatment by targeting xenobiotic receptors.

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Introduction

Metabolic diseases represent a cluster of conditions that primarily result from excessive nutrient and metabolite (including but not limited to lipids, cholesterol, glucose, and bile acid) dysregulations(Rochlani et al., 2017). Over the past twenty years, more than a billion people have been suffering from metabolic diseases related to the global epidemic of obesity and T2D(Eckel et al., 2005, Jeffery and Richardson, 2021, Saklayen, 2018). Metabolic diseases seriously affect quality of life and increase mortality risk when they cannot be effectively controlled. Therefore, further investigations examining the underlying mechanisms responsible for metabolic disorders will provide evidence for novel treatments.

Exogenous substances such as xenobiotics, pathogens, and food can interfere with intestinal homeostasis. In obesity and other metabolic abnormalities, chronic inflammation is a key contributor to insulin resistance and causes malfunctions in insulin-target organs(Hotamisligil, 2006, Kawai et al., 2021, Yun Sok Lee, 2021). Evidence suggests that exogenous substances promote systemic inflammation and are related to metabolic diseases(Di Tommaso et al., 2021). To defend from these exogenous substances, host cells express Xenobiotic receptors (XRs) such as pregnant X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AHR) that were originally considered to be receptors that were expressed by host cells to sense and defend against xenobiotics(Nieves et al., 2022). Numerous metabolites have been demonstrated to promote the expression of XRs and to exert metabolic functions through XRs activation. PXR is a member of the nuclear receptor

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superfamily that affects energy homeostasis and inflammatory bowel disease(Koutsounas et al., 2013). CAR can regulate the metabolism of glucose, lipids, and bile acids involved in metabolic diseases(Daujat-Chavanieu and Gerbal-Chaloin, 2020). Moreover, AHR has also been demonstrated not only to play endogenous roles in natural physiology but also to be involved in the pathology of metabolic diseases and inflammation, thus suggesting a significant role for XRs in the context of metabolic diseases(Petriello et al., 2017). Here, we review the growing knowledge regarding the physiology of XRs in metabolism focusing on the role of XRs in different nutrient metabolism processes during different metabolic diseases. Understanding the complex roles of xenobiotic receptors in metabolic diseases may shed light on improved treatments for these diseases.

1. The overall view of the xenobiotic-sensing receptors

XRs consist of a group of receptors that are capable of binding to xenobiotics and sensing endogenous and exogenous toxic byproducts(Mackowiak and Wang, 2016). XRs are capable of regulating the enzyme activity and transporters involved in drug metabolism(Cai et al., 2021). Upon ligand binding, XRs translocate to the nucleus and couple with the nuclear receptor retinoid X receptor (RXR), thus initiating transcription in cells to execute their functions. In addition to metabolizing xenobiotics, XRs can support normal tissue function by regulating their downstream target genes expressiom in different organs(Oladimeji and Chen, 2018). PXR, CAR, and AHR are major XRs that have been well studied. Here, we review ligands and their biology.

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1.1 PXR and its ligands

PXR is a ligand-regulated nuclear receptor which is over-expressed in the liver and intestines. It modulates key steps of xenobiotic and endobiotic metabolism in enterohepatic tissues(Stefano Fiorucci, 2012). Like other nuclear receptors, PXR includes an N-terminal domain, a DNA-binding domain, hinge region, and ligand-binding domain(Cai et al., 2021). Therefore, PXR can bind to large-scale ligands and regulate the expression of downstream target genes. Activated PXR directly regulates its target genes expresion, including Cytochrome P450 (CYPs), carboxylesterases, glutathione S-transferases (GSTs), glutathione peroxidase (GPx), ATP-binding cassette family proteins (ABCs), organic anion transporting polypeptides (OATPs), and estrogen sulfotransferase 1E1 (SULT1E1)(Xing et al., 2020).

PXR ligands can be divided into small-molecule chemicals, natural herbs, and endogenous biomolecules. The well-known agonist ligands of PXR contain rifampicin, dexamethasone, ritonavir, lovastatin, ritonavir, phenobarbital, clotrimazole, pregnenolone-16a-carbonitrile (PCN), natural herb St. John's Wort, 5β-pregnane-3,20-dione, estradiol, and lithocholic acid (LCA)(Brewer and Chen, 2016, Chai S. C. et al., 2016, Satoru Kakizaki, 2011, Stefano Fiorucci, 2012). A large number of agonist ligands activate PXR and exhibit species differences. For example, since the low homology between mouse and human ligand-binding domains, rifampicin exhibits a high affinity for human PXR, while PCN is a potent agonist of mouse PXR (Cai et al., 2021, S A Kliewer, 1998, W Xie, 2000). The calcium channel blocker felodipine was recently identified as a novel PXR agonist that can induce

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mouse PXR expression (Reddy and Nyunoya, 2021). Additionally, the activation of PXR through the process of ligand binding exhibits tissue specificity. A recent study suggested that tributyl citrate (TBC), an FDA-approved plasticizer for food and pharmaceutical applications, is a selective and latent PXR agonist in both mice and humans. Interestingly, TBC only activates intestinal PXR and does not influence PXR activity in liver(Sui et al., 2015). With the gradual development of knowledge concerning the ocean, many studies have also identified a series of natural molecules isolated from marine organisms that possess a potent ability to bind to PXR(Carazo et al., 2019).

In addition to ligand-dependent activation, the post-translational modulation of PXR can also regulate its activity. Poly (ADP-ribosyl) ation and SUMOylation of PXR stimulates its activity by influencing PXR protein stability and dimerization(Cui et al., 2015, Priyanka et al., 2016, Wang C. et al., 2018a). However, PXR phosphorylation, acetylation, and ubiquitination inhibit PXR activity by regulating its subcellular localization, co-regulatory interaction, and degradation(Biswas et al., 2011, Cui et al., 2020, Hu et al., 2020, Rana et al., 2013). There is mounting evidence that non-coding RNAs, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), modulate the function and expression of PXR post-transcriptionally(Smutny et al., 2021).

1.2 CAR and its ligands

CAR is also a member of the nuclear receptor superfamily (also known as

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NR1I3) that is mainly expressed in the liver (di Masi et al., 2009). CAR was discovered as a XR for both endogenous and exogenous ligands and participates in drug and energy metabolism and systematic inflammation. CAR is located in the cytoplasm and shuttles into the nucleus when activation(Modica et al., 2009). Once CAR is activated, it dephosphorylates and migrates to the nucleus, heterodimerizes with RXR, and recruits co-activator factors to allow for the transcription of its target genes(Oliviero et al., 2020). After activation, CAR increases the expression of the CYP phase I enzymes CYP2B, CYP3A and CYP2C to detoxify drugs and external compounds(Li H. et al., 2021a). CAR activation induces the expression of phase II enzymes, including uridine diphosphate glucuronosyltransferase (UGT) and sulphotransferases (SULT). Activation also increases the expression of uptake and efflux transporters such as multidrug resistance mutation 1 (MDR1), multidrug resistance proteins (MRPS), and organic anion-transporting polypeptides (OATP)(Li D. et al., 2015).

Currently, large amounts of compounds have been confirmed as CAR ligands that activate or suppress CAR activity, and these range from synthetic compounds to natural products. Ligands activating human CAR include 6-(4-Chlorophenyl) imidazo [2,1-b] thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl) oxime (CICTO), phenobarbital, androstanol, valproic acid, efavirenz, and flavonoids(Bae et al., 2021, Cerveny et al., 2007, Cherian et al., 2015). The compounds 1,4-Bis-[2-(3,5-dichloropyridyloxy)] benzene, 3,3′,5,5′-Tetrachloro-1,4-bis (pyridyloxy) benzene (TCPOBOP), phenobarbital, androstanol, paclitaxel, and diallyl sulfide are ligands

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that activate murine CAR(Bhushan et al., 2021, Fisher et al., 2007, Gao Y. et al., 2021, Goettel et al., 2020). Phenobarbital and androstanol can stimulate both murine and human CAR. Endogenous molecules, including steroids (androstanes, estrogens, and progestins), cholesterol metabolites, bilirubin, and bile acids, have also been confirmed as CAR ligands(Küblbeck et al., 2020).

CAR has been demonstrated to shares several common ligands with PXR like phenobarbital and 5β-pregnane-3,20-dione. Interestingly, clotrimazole that is a PXR activator behaves as a CAR antagonist and exhibits an inverse activity between the two receptors(Moore L. B. et al., 2000). Previous investigations have suggested that CAR is activated via two pathways that are ligand-dependent and ligand-independent. Cytoplasmic retention of CAR is modulated by upstream phosphorylation cascades and cytoplasmic chaperones that are necessary for CAR activation(Yan J. et al., 2015). The molecular target of phenobarbital induction has now been confirmed as phosphorylation at threonine 38 of CAR, and this dissociates CAR from the promoter and exports it back to the cytoplasm(Negishi, 2017).

Proteasomal inhibition interrupted CAR function, inhibits CAR nuclear trafficking, disrupts CAR interaction with co-activators in nuclear, and inhibits the induction of CAR target gene responses, and this leads to the accumulation of ubiquitinated human CAR(Chen T. et al., 2014, Daujat-Chavanieu and Gerbal-Chaloin, 2020). These findings suggest that CAR activity is not only influenced by ligand interactions but also by post-transcriptional modification.

1.3 AHR and its ligands

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The aryl hydrocarbon receptor (AHR) is a member of the basic helix-loop-helix (bHLH) superfamily of transcription factors and is activated by ligands(Murray et al., 2014). AHR binds to several factors (embracing hsp90, AIP/XAP2, and p23) within the cytoplasm. Binding of AHR ligands leads to the nuclear translocation of the bound receptor complex, and this further activates or suppresses downstream target gene expression(Safe et al., 2020). These genes are related to oxidative balance, endocrine homeostasis, and diverse metabolic and immunological progression(Goya-Jorge et al., 2020). The key downstream target genes of AHR are primarily ligand-metabolizing enzymes (CYP1A and CYP1B), immunoregulatory factors, and growth factors (IL-10, ARG1, IL-6, IL-22, and VEGF)(Shinde and McGaha, 2018). AHR participates in inflammatory responses, drug metabolism, and barrier homeostasis by regulating its downstream target genes.

AHR is a sensitive sensor for small molecules and endogenous and exogenous xenobiotics such as dioxins, microbial bioproducts, phytochemicals, and tryptophan-products(Furue et al., 2019). The canonical AHR agonist ligands include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 5,11-dihydroindolo[3,2-b] carbazole-12-carbaldehyde (FICZ), 2-(10-H-indole-3-carbonyl) thiazole-4-carboxylic acid methyl ester (ITE), tryptophan metabolites such as kynurenine and other gut microbial products, and leukotrienes(Safe et al., 2020, Shinde and McGaha, 2018). AHR antagonists include quercetin, resveratrol from the diet, and synthetic compounds such as CH223191, 3’,4’-dimethoxyflavone (DMF), 3’-methoxy-4’-nitroflavone (MNF), and others(Shinde and McGaha, 2018). Recently, the novel small molecule tapinarof

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was identified as a therapeutic ligand for AHR and has been used for psoriasis and atopic dermatitis treatment in clinical practice(Bissonnette et al., 2021).

In addition to regulating its activity, several ligands have also been confirmed as regulators of AHR expression, containing endogenous metabolites such as FICZ and indoxyl sulfate and also extensively used drugs such as omeprazole and leflunomide and dietary compounds such as quercetin(Goya-Jorge et al., 2020, Kim M. et al., 2021a, Rey-Bedon et al., 2022, Stejskalova et al., 2011). In the AHR signaling pathway, the aryl hydrocarbon receptor repressor (AHRR) competes with AHR to prevent AHR from binding to the xenobiotic-response element(Oshima et al., 2009). Investigations have confirmed that AHRR post-translational modifications, containing SMUOylation, demethylation, and DNA methylation, can regulate the function of AHRR, and this further influences the activation of AHR signaling(Oshima et al., 2009, Philibert et al., 2012, Tian et al., 2017). Thus, AHR signaling activation can be divided into three isoforms that include stimulation by ligand binding, regulation of AHR expression, and AHR post-translational modification.

Previous studies have revealed that XRs play a vital role in maintaining tissue homeostasis and defending against toxicants by binding to ligands to further induce their downstream target gene expression. Their ligands include a series of endogenous substances and xenobiotics derived from internal synthetic compounds, natural products, and toxic substances. As PXR and CAR belong to the same superfamily, they share common ligands that can activate the two receptors simultaneously. Ligand stimulation also exhibits differences in specific species and tissues, thus suggesting a

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complex function. In the future, the complex functions and specific conditions should be considered before considering ligands of XRs as treatment drugs.

2. PXR in the context of metabolic disease

As a xenobiotic-sensing nuclear receptor that modulates the expression of drug metabolism-related enzymes and transporters, PXR can be regulated by various factors, containing pharmaceuticals, nutrients, and dietary and environmental factors. Most of these factors are closely associated with metabolic diseases(Ma X. et al., 2008, Sultana et al., 2021). Numerous studies have confirmed that PXR plays a vital role in regulating obesity, insulin resistance, non-alcoholic fatty liver disease (NAFLD), and cardiovascular diseases(Jinhan He, 2013, Sayaf et al., 2021, Zhou C., 2016). Moreover, PXR activation has been observed to regulate glucose, lipid, bile acid metabolism, and other important components of metabolic diseases. PXR can regulate inflammatory responses that are an important component of metabolic diseases. Here, we summarize the functional role of PXR in the context of nutrient metabolism and inflammation during metabolic diseases.

2.1 Functional roles of PXR in lipid homeostasis

Increased lipid levels largely contribute to the development of atherosclerotic cardiovascular disease, fatty liver, and insulin resistance(Chen L. et al., 2019b, Li Y. H. et al., 2018b). Lipid metabolism is often accompanied by complex physiological processes. The liver is the core organ participated in lipid metabolism through *de novo* lipogenesis (DNL), catabolism of lipids (β-oxidation), lipid uptake, and secretion(Alves-Bezerra and Cohen, 2017, Geng et al., 2021). In addition to the liver,

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adipose tissue is not only responsible for sufficient lipid storage by lipogenesis but also for fatty acid (FA) availability by lipolysis that provides the substrate for energy metabolism via β-oxidation(Bódis and Roden, 2018). The regulatory circuits for fuel storage and oxidation in white adipose tissue and brown adipose tissue play a key role in systemic energy homeostasis. Circuit dysregulation is an important cause of metabolic diseases, including obesity, insulin resistance, chronic inflammation, and cardiovascular diseases(Chouchani and Kajimura, 2019). As PXR plays a complex role in lipid metabolism, we reviewed previous studies examining the effects of PXR on lipid metabolism in the liver and adipose tissue.

2.1.1 The effects of PXR on liver lipid metabolism

The liver is the major organ participated in metabolic degradation of xenobiotics(Jetter and Kullak-Ublick, 2020). In liver samples from NAFLD patients, sterol regulatory element binding transcription factor 1(SREBP1) and SREBP1 target genes have been demonstrated to be upregulated during steatohepatitis, whereas PXR protein levels were reduced, thus suggesting the role of PXR in lipogenesis during NAFLD(Bitter et al., 2015). An *in vivo* study suggested that pharmacological activation of PXR significantly increased the mRNA levels of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) and two lipogenic enzymes that included stearyl CoA desaturase (SCD1) and fatty acid elongase, with increased levels of hepatic triglycerides(Amélie Moreau, 2008, Zhang et al., 2013). In human hepatic cells, ligand-dependent activation and knockout of PXR promotes *de novo* lipogenesis and steatosis by inducing the SREBP1 pathway and aldo-keto reductase 1B10

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(AKR1B10)-mediated increase in acetyl-CoA carboxylase (ACC) activity(Bitter et al., 2015). S14 is a thyroid-responsive gene that transduces nutrient-related and hormone-related signals to genes involved in liver lipogenesis(Kinlaw et al., 1995). Amélie *et al*. confirmed that PXR activation facilitates aberrant *de novo* lipogenesis via the S14 pathway activation in liver(Moreau et al., 2009). Interestingly, four-week-old male AKR/J mice were fed a high-fat diet for 7 weeks with twice-weekly injections of PCN (50 mg/kg, IP). PCN treatment reduced hepatic Srebp1 expression and fatty acid synthase (Fasn) genes, and this further alleviated lipid deposition in the liver(Ma Y. and Liu, 2012). (-)-epigallocatechin-3-gallate (EGCG), a major component of green tea, was determined to activate PXR and inhibit lipogenesis (Srebp1, Fasn, and ACC1) in an HFD and STZ-induced type 2 diabetes model (Li X. et al., 2018a). However, further study is required to determine if the roles of EGCG on lipogenesis are dependent upon PXR activation.

For lipid β-oxidation, PCN treatment decreased carnitine palmitoyl-transferase 1A (Cpt1a) expression and serum 3-hydroxybutyrate levels(Nakamura et al., 2007). In PXR-/- mice, after HFD feeding Cpt1a expression was markedly increased, and this was accompanied by enhanced liver mitochondrial β-oxidation(He et al., 2013). Investigation of this mechanism confirmed that PXR directly binds to FoxA2 and represses its activation of Cpt1a and Hmgcs2 promoters in the fasting liver, thus further inhibiting lipid β-oxidation in the liver(Nakamura et al., 2007). A free fatty acid uptake transporter, CD36, is positively regulated by PPARγ. Zhou *et al*. reported that PXR could directly regulate CD36 or through its activation of PPARγ to thereby

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further accelerate liver lipid deposition and induce liver-specific hepatic steatosis and insulin resistance in a liver specific way(Zhou J. et al., 2008). Numerous substrates regulate lipid metabolism that is dependent upon the activation of PXR such as tricresyl phosphate, naturally occurring lignan sesamin, and the pyrethroid pesticide cis-bifenthrin(Tai et al., 2019, Xiang et al., 2018, Xiang and Wang, 2021). These findings suggest that both whole-body and liver PXR activation enhanced hepatic lipid accumulation and decreased serum 3-hydroxybutyrate by influencing lipid DNL, β-oxidation, and uptake. Interestingly, two studies have demonstrated different effects of PXR on lipid metabolism compared to others, and this may be due to the complex effectes of PXR in nutrient metabolism during different metabolic diseases. Investigating the effects of PXR in specific states during metabolic diseases may help to provide a deeper understanding of the effect of PXR.

2.1.2 The effects of PXR on adipose lipid metabolism

The primary lipid metabolism pathways in adipose tissue are lipolysis and lipogenesis. Although adipose tissue plays a notable role in metabolic diseases, few studies have considered the role of PXR on lipid metabolism in adipose tissue, and this may be owing to the low expression of PXR in adipose tissue. Only one study revealed that PCN treatment of ARK/J mice on a HFD increased the expression of hormone-sensitive lipase (HSL), an enzyme known to hydrolyze triglycerides in mammalian adipose tissue, in brown adipose tissue(Ma Y. and Liu, 2012, Zimmermann et al., 2004). In turn, adipose tissue elevated lipolysis could increase the fatty acid level in the circulation, and this would translocate into the liver and

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dysregulate PXR, ultimately further developing insulin resistance and hepatic steatosis(Renu et al., 2019). Few reports have examined the regulatory role of PXR in adipose tissue, thus suggesting that PXR may exert few effects on lipid metabolism in adipose tissue considering its expression level. Fatty acids from adipocytes may conversely regulate PXR activation.

2.2 Functional roles of PXR in glucose homeostasis

Glucose is a major source of energy for the human body to that is required to maintain homeostasis that involves a balance between glucose production and utilization. Glucose metabolism disorders lead to severe metabolic diseases, containing diabetes, fatty liver, and cardiovascular disease (Chen L. et al., 2019b). The liver is the major organ responsible for approximately 90% of the endogenously synthesized glucose production through gluconeogenesis and glycogenolysis(Moore M. C. et al., 2012). The sources of glucose synthesis include diverse factors including pyruvate, lactate, amino acids, and glycerol during fasting(Schutz, 2011). There are three key rate-limiting enzymes during gluconeogenesis: phosphoenolpyruvate carboxykinase (PEPCK) that catalyzes the decarboxylation and phosphorylation of oxaloacetate to generate phosphoenolpyruvate; fructose 1,6-bisphosphatase (FBPase) that converts fructose 1,6-bisphosphate to fructose 6-phosphate; glucose 6-phosphatase (G6Pase) that produces glucose from glucose 6-phosphate(Kirchner et al., 2008).

The role of PXR on glucose metabolism primarily depends upon its target genes expression such as PEPCK, G6Pase, glucose transporter 2 (GLUT2), and MDRs.

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Studies have demonstrated that prototypical PXR agonists PCN and rifampin administration results in increased glucose levels during OGTT in humans and rats (Rysä et al., 2013). In contrast, upon HFD feeding PXR ablation ob/ob mice exhibited significantly ameliorative glucose tolerance and decreased serum glucose levels during fasting and feeding, and this was accompanied by decreased hepatic PEPCK and G6Pase levels(He et al., 2013, Ling et al., 2016). In regard to the underlying mechanism of PXR in regulating gluconeogenesis, Susumu *et al*. reported that PXR suppressed G6Pase by binding to cAMP-response element-binding protein (CREB) directly (Kodama et al., 2007). FoxO1 and PXR reciprocally co-regulate their target genes, thus modulating gluconeogenesis(Kodama et al., 2004). Additionally, ligand-activated PXR interferes with HNF-4 signaling through targeting the common coactivator PGC-1 during glucose metabolism(Bhalla et al., 2004). A recent research confirmed that low glucose stimulates PXR phosphorylation at Ser350 and further increases gluconeogenesis in human HepG2 cells, thus suggesting positive feedback between PXR and glucose metabolism(Gotoh et al., 2017). Interestingly, the expression of G6Pase and PEPCK was decreased in the livers of high-fat diet-fed AKR/J mice treated with PCN, a PXR agonist, and this was accompanied by improved insulin sensitivity(Ma Y. and Liu, 2012). Another study also reported that a major green tea component, EGCG, ameliorated glucose homeostasis and restrained gluconeogenesis (PEPCK and G6Pase) through direct or indirect activation of PXR in HFD-induced type 2 diabetes models(Li X. et al., 2018a).

PXR not only decreases glucose production but also influences glucose uptake

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and utilization in liver cells. Pre-treatment with rifampicin and PCN markedly inhibited glucose uptake caused by decreased levels of GLUT2, the primary glucose transporter in mammalian liver and wild-type mouse hepatocytes(Hassani-Nezhad-Gashti et al., 2018). The PXR activators rifampin and PCN could also decrease glucose consumption and glucokinase (GCK) expression that is a key regulator of glucose metabolism in hepatocytes(Ling et al., 2016).

Conversely, nutrient conditions can influence the degree of PXR activity. PCN can amplify the expression of multiple well-known PXR target genes in glucose-fed mice compared to levels at the basal status(Hassani-Nezhad-Gashti et al., 2019). Hyperglycemia is a dangerous factor that induces diabetes, fatty liver disease, and cardiovascular disease. Therefore, it is an effective approach for the treatment of metabolic diseases by targeting hyperglycemia(Chen L. et al., 2019b). Based on this, there is a need to develop novel therapeutic drugs that contrapuntally inhibit hepatic glucose production. The effect of PXR in the context of glucose metabolism is complex and may be influenced by the nutrient status, species, and other environmental factors. Key aims for future research targeting PXR include the development of drugs possessing tissue selectivity and clinical safety with a specific nutrient statement.

2.3 Roles of PXR in cholesterol metabolism

Cholesterol homeostasis is a vital substrate of the cell membrane and is vital for cell membrane maintenance and systemic function. Intracellular cholesterol metabolism is maintained by a complex network that regulates cholesterol

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biosynthesis, uptake, export, esterification, and trafficking(Xu H. et al., 2020) as presented in Figure1. Dysregulated cholesterol metabolism can not only cause cardiovascular diseases but can also induce a large number of other metabolic diseases.

As a xenobiotic receptor, PXR has been demonstrated to possess the effects to induce hypercholesterolemia and modulate the content of cholesterol. Treating wild-type (WT) mice with PXR agonists increased serum HDL-C and apoA1 levels(Bachmann et al., 2004). PXR activation diminishes plasma LDL-cholesterol levels and leads to hepatic steatosis in LDL-knockout mice(Hoekstra et al., 2009). A recent study revealed that dicyclohexyl phthalate (DCHP) is an underlying intestinal PXR-selective agonist that elevates plasma cholesterol levels in wild-type mice. Interestingly, DCHP exposure also led to higher circulating ceramide levels and increased the expression of intestinal genes mediating lipogenesis in a PXR-dependent manner, thus suggesting that intestinal PXR plays a vital role in cholesterol and lipid metabolism(Sui et al., 2021).

For the underlying mechanism, the cholesterol biosynthesis enzyme squalene epoxidase (SQLE) was confirmed as an immediate transcriptional target of PXR and caused the elevated cholesterol biosynthesis(Gwag et al., 2019). Tuire *et al*. determined that an agonist of the liver X receptor (LXR), circulating 4β-hydroxycholesterol (4βHC), is elevated by PXR activation. PXR activation induces cholesterol efflux and its transporters ABCA1 and ABCG1 while suppressing the influx of cholesterol and its transporter lectin-like oxidized LDL receptor-1. This is

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dependent upon elevated 4βHC levels and further LXR activation(Salonurmi et al., 2020). Rifampicin induces CYP27A1 gene transcription, increases intracellular 27-hydroxycholesterol (27-HOC) levels and induces ABCA1 and ABCG1 mRNA expression only in intestinal cells, and this stimulates cholesterol efflux from intestinal cells to apolipoprotein A-I and HDL in the circulation(Li T. et al., 2007, Lin Y. N. et al., 2018). Quetiapine, one of the antipsychotic medicines, has recently been determined to specifically activate intestinal PXR that further stimulates intestinal expression of the cholesterol transporter Niemann-Pick C1-Like 1 (NPC1L1) and microsomal triglyceride transfer protein (MTP) to thereby increase lipid absorption in intestinal (Meng et al., 2019). PXR activation elevates the atherogenic lipoproteins VLDL and LDL levels due to the decreased expression of CD36, ApoA-IV, and CYP39A1, all of which are participated in cholesterol metabolism and lipoprotein transportation(Zhou C. et al., 2009). Interestingly, in apoE-/- mice deficiency in PXR did not change cholesterol levels in plasma but instead attenuated atherosclerosis development, and this may result from reduced CD36 expression and lowered CD36-mediated oxidized LDL uptake in peritoneal macrophages(Sui et al., 2011, Zhou C. et al., 2009). In human volunteers, PXR activation by rifampicin increased intermediate-density lipoprotein (IDL), LDL, total cholesterol, and the lathosterol-cholesterol ratio (a marker of cholesterol synthesis), thus suggesting elevated cholesterol synthesis. The elevated cholesterol levels were caused by widespread induction of cholesterol synthesis genes, including the rate-limiting Hmgcr, and by upregulation of the intermediates in the Kandutsch-Russell cholesterol synthesis pathway in the

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liver(Karpale et al., 2021). Additionally, the activation of PXR induces plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) that is an inhibitor of hepatic LDL uptake, and these roles are mediated by elevated proteolytic activation of SREBP2 in response to PXR activation(Karpale et al., 2021).

Through complex coordinated regulation, PXR regulates key aspects of cholesterol metabolism and participates in metabolic diseases, including cholesterol synthesis, remodeling, absorption, cholesterol uptake by peripheral tissues, and reverse cholesterol transport. However, in different tissues and specific pathological environments, the effects of PXR may vary through different mechanisms. Certain clinical drugs exhibit the potential to activate PXR, and their clinical use should be considered due to their role in regulating cholesterol metabolism that may be a risk factor for hypercholesterolemia.

2.4 Roles of PXR in bile acid metabolism

Bile acids are key agents that are responsible for intestinal nutrient absorption and the biliary secretion of toxic metabolites, lipids, and xenobiotics(Chiang J. Y., 2013). Bile acids are primarily synthesized from cholesterol in the liver via classical and alternative pathways and comprise a major part of cholesterol metabolism(Wang Y. et al., 2021, Yang and Zhang, 2020). Cholesterol is converted to cholic acid (CA) and chenodeoxycholic acid (CDCA) that are two major BAs in the liver. In rodents, CDCA is further metabolized to α/β-muricholic acid (α/β-MCA). BAs are primarily conjugated with taurine and glycine, catalyzed by CYPs, secreted into the bile, and stored in the gallbladder. After a meal, BAs are secreted into the intestine and

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converted into secondary BAs by bacterial enzymes, including deoxycholic acid (DCA) and lithocholic acid (LCA)(Russell, 2003, Wang X. et al., 2018b).

In enterohepatic circulation, bile acids are secreted from the liver to the intestine and then back to the liver dependent on their micelle-forming properties. These physiological functions play a key role in nutrient absorption and distribution, metabolic regulation, and homeostasis (Ahmad and Haeusler, 2019). Bile acids are being increasingly investigated as complex metabolic integrators and signaling factors and not just as lipid solubilizers but also as simple modulators of bile acid homeostasis(Thomas et al., 2008). Disruption of bile acid metabolism can cause cholestatic liver disease, diabetes, dyslipidemia, and cardiovascular diseases (Li T. and Chiang, 2014). Previous studies have suggested that bile acids primarily activate the farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) to modulate lipid and glucose metabolic homeostasis and energy metabolism in the liver, gut, and peripheral tissues. However, certain investigations have confirmed that PXR also participates in bile acid metabolism and influences metabolic diseases(Castellanos-Jankiewicz et al., 2021, Chávez-Talavera et al., 2017, Chiang J. Y. L. and Ferrell, 2018).

Four-day treatment with a high dose of DEX (75 mg/kg) has been used in assorted investigations to activate PXR signaling in mice(Klaassen and Aleksunes, 2010). A recent study revealed that 75 mg/kg of DEX did not alter the total BA concentrations in mice serum but significantly decreased the total unconjugated BAs and total secondary BAs in the liver, thus suggesting the beneficial effects of PXR

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activation on cholestasis by reducing BA concentrations in the liver(Wang X. et al., 2018b). The role of PXR activation was mediated by increased levels of fibroblast growth factor-19 (FGF-19), a key modulator in inhibiting hepatic bile acid synthesis, as confirmed by another study(Guthrie et al., 2020). Analysis of specific components of bile acids in serum revealed that PXR activation by 75 mg/kg DEX treatment elevated TMCA and TCA content and decreased DCA and TDCA in the serum, thus suggesting that PXR activation may exert an inhibitive effect on intestinal bacteria that synthesis secondary BAs(Wang X. et al., 2018b). Further studies have confirmed that PXR activation induced by PCN downregulates BA-metabolizing bacteria in the intestine and regulates BA homeostasis in a gut microbiota-dependent manner(Dempsey et al., 2019). Besides regulating intestinal bacteria and secreted factors, PXR can regulate bile acids by modulating the expression of its synthetic enzymes, including the key enzymes cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1), in the classical BA synthesis pathway(Zhao L. Y. et al., 2017). Besides as BAs synthetic enzymes, PXR regulates BAs oxidation and conjugation by controlling the expression of glutathione-S-transferase (GST), UGT, and SULT. Moreover, PXR modulates the expression of genes responsible for BAs secretion including ABC and OATP membrane transporters(Modica et al., 2009).

As one of the key ligands of PXR, bile acids can regulate PXR activity. The severity of bile acid malabsorption is closely related to PXR deactivation. Enterohepatic circulation of bile acids is a key factor for preserving baseline hepato-intestinal PXR activity (Iwamoto et al., 2013). Bile acids are also determined to be a

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major factor in the cross-talk mediated by PXR and activated CYPs(Hafner et al., 2011). PXR responds to secondary bile acids and induces their catabolism(Makishima, 2005). These findings illustrate a feed-forward regulatory pathway between BAs and PXR, where BAs can regulate PXR activity and induce their own metabolism.

2.5 Roles of PXR in innate immunity

Metabolic diseases are often accompanied by systemic inflammations. It is also widely recognized that chronic inflammation plays a critical role in the initiation and progression of metabolic diseases(Baker et al., 2011). It has long been confirmed that exposure to xenobiotics and PXR activation can impair the immune system. Recently, a large number of investigations have demonstrated the modulatory effects and underlying mechanisms of PXR on inflammation.

PXR mitigates inflammatory injury through the negative regulation of inflammatory pathways and cytokines such as nuclear factor kappa B (NF-κB), Toll-like receptor 4 (TLR4), signal transducer and activator of transcription 3(STAT3), IL-6, tumor necrosis factor α (TNF-α), and other pathways.

St. John's wort, a PXR agonist, can activate PXR and further inhibit tumor necrosis factor (TNF) α-induced NF-κB translocation and activation(Yan T. et al., 2021). Intestinal PXR activation exerts a protective effect in the context of inflammatory bowel disease (IBD), and this is partially due to the attenuation of NF-κB signaling that leads to lower expression of cytokines(Cheng J. et al., 2012). Furthermore, PXR directly interacts pro-inflammatory with NF-κB and activator

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protein 1 (AP-1) to decrease inflammation-induced expression of chemokine CXCL2 and suppress neutrophil infiltration in the liver(Okamura et al., 2020). Further studies suggested that the anti-inflammatory effect of PXR was mediated by the inhibition of NF-κB activity by enhancing IκBα (a suppressor of NF-κB) expression in tetrachloromethane (CCl_4)-induced mouse liver inflammation. PXR/RXRα binds to two sites in the upstream regulation of IκBα and enhances IκBα expression in a dose-dependent way(Ye et al., 2016).

PXR has also been confirmed to negatively regulate TLR4 signaling(Huang et al., 2018). PXR activation reduces TLR4 expression by decreasing TLR4 mRNA stability(Huang et al., 2018). PXR regulates pathogen-induced inflammation and host defense against viral infection by regulating the TLR4 signaling(Qiu et al., 2016). The effects of PXR activation on TLR4 signaling have been reported mostly in intestinal diseases. The intestinal microbial metabolite indole 3-propionic acid (IPA), a ligand for PXR *in vivo*, modulates mucosal integrity and inflammation via a pathway that involves luminal sensing and signaling by TLR4(Venkatesh et al., 2014). Rifaximin improved *Clostridium difficile* toxin-induced intestinal epithelial cell apoptosis and inflammation via the PXR-dependent TLR4/MyD88/NF-κB pathway and may be effective in *Clostridium difficile* infections treatment (Esposito et al., 2016). Following intrarectal exposure to TcdA/B, PXR-deficient mice (Nr1i2-/-0076) exhibited reduced survival, and this effect was related to elevated levels of innate immune cell influx. This exacerbated response was abolished by TLR4 signaling blocking(Erickson et al., 2020). Regarding the underlying mechanism, a study

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reported that patchouli alcohol increased the expression of the nuclear receptor PXR and promoted the PXR/Toll-like receptor 4 (TLR4) axis to suppress the nuclear import of NF-κB (p50 and p65) during osteoporosis(Lu et al., 2021). IL-35-producing Bregs and Treg cells critically modulate chronic illnesses worldwide through mechanisms related to the disruption of gut microbiota composition. Treatment with 3-idoleacetic acid together with lipopolysaccharide (LPS) induces IL-35 + B cell generation through PXR and TLR4 (Su et al., 2022). These findings reveal a negative regulatory effect of PXR on TLR4 signaling during intestinal disease. However, the underlying mechanisms by which PXR regulates TLR4 in the context of metabolic diseases must be clarified.

PXR can modulate liver inflammation by regulating the inflammation-prone gut microbiome signature that differs between males and females. PXR knockout mice exhibit downregulation of hepatic genes involved in microbial responses and inflammation. This was due to the decrease in pro-inflammatory *Lactobacillus* and the elevation in anti-inflammatory *Bifidobacterium* in a PXR-dependent manner in the intestine(Kim S. et al., 2021b).

Conversely, the inflammatory response can modulate the expression of PXR and its downstream target genes. During lipopolysaccharide (LPS)-induced liver inflammation, LPS largely inhibited PXR expression in a dose-dependent way, and this was followed by the inhibition of CYP3A11 in the mouse liver(Xu D. X. et al., 2004). A further mechanistic study suggested that Kupffer cells and reactive oxygen species that are possibly produced by NADPH oxidase and xanthine oxidase are

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involved in LPS-induced downregulation of nuclear receptor PXR and its target gene CYP3A in the mouse liver(Xu D. X. et al., 2004). NF-κB-mediated inflammation can influence PXR phosphorylation and activation. NF-κB directly regulates PXR signaling via interacting with its dimerization partner RXRα(Bautista-Olivier and Elizondo, 2022). The inflammatory cytokine IL-6 can suppress PXR expression, and this is mediated by the NF-κB pathway(Li M. et al., 2021b). The regulatory effect of PXR during inflammation suggests that PXR is an anti-inflammatory modulator of inflammatory bowel disease and toxin-induced liver injury. Inflammation plays a complex role in metabolic diseases. In the initial stages of NAFLD, inflammation is responsible for the repair and defense of external substances. However, an inflammatory response promotes NAFLD progression after persistent external stimulation. The anti-inflammatory effects of PXR in the treatment of metabolic diseases such as NAFLD should consider the specific stage of these metabolic diseases.

PXR also exerts non-genomic and antithrombotic effects during atherosclerosis. PXR is expressed in human platelets. Treatment with PXR ligands inhibited platelet functions stimulated by a range of agonists with platelet aggregation, granule secretion, adhesion, and spreading on fibrinogen, all of which were attenuated along with a reduction in thrombus formation (both *in vitro* and *in vivo*). The PXR ligand-mediated inhibition of platelet function was determined to be related to the depression of Src family kinases (SFKs)(Flora et al., 2019).

PXR may participate in the metabolism of multiple nutrients, and its role in

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metabolic diseases is complex. Nutrient metabolism is a sophisticated metabolic network that often interwinds and regulates itself(Ahmad and Haeusler, 2019). PXR knockout alleviates HFD-induced obesity by inducing FGF15 expression, thus leading to the inhibition of bile acid synthesis and the reduction of lipid absorption, hepatic lipid accumulation, and liver triglyceride levels(Zhao L. Y. et al., 2017). Statins are therapeutic drugs that modulate serum cholesterol levels and decrease the risk of heart disease. They are confirmed to change the microbial composition, alter bile acid components, and increase fasting blood glucose levels and body weight through a PXR-dependent mechanism(Caparrós-Martín et al., 2017). The wide distribution of PXR in the body causes difficulties in specific metabolic diseases treatment. Concurrently, PXR in different tissues and specific pathological environments may exert different effects on disease, thus indicating its complex functions. Future investigations should focus on investigating the role and mechanism of PXR in the context of metabolic diseases in various pathological environments.

3. CAR in metabolic disease

In addition to acting as a xenobiotic receptor, The activation of CAR alleviates insulin resistance, represses lipogenesis and gluconeogenesis, and upregulates brown adipose tissue energy expenditure during metabolic diseases(Yan J. et al., 2015). CAR shares some common downstream target genes with PXR but can also modulate lipid, glucose, cholesterol, and BA metabolism in specific pathways.

3.1 CAR in lipid metabolism

Previous studies have suggested that activation of CAR results in decreased

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hepatic triglyceride levels(Yan J. et al., 2015). CAR activation downregulates liver lipid deposition and lipogenic gene (Srebp1c, Fasn, and Scd-1) expression in ob/ob mice, and this is dependent upon sulfotransferase 2B1b (SULT2B1) induction(Dong et al., 2009). The activation of CAR by TCPOBOP also induced lipid β-oxidation in the liver(Dong et al., 2009). A mouse-specific CAR ligand, TCPOBOP, can reduce liver and plasma triglyceride levels. However, TCPOBOP regulated lipid homeostasis by increasing serum and liver triglyceride levels and promoting hepatocyte hypertrophy in humanized CAR mice without human CAR activation. This suggests that the roles of TCPOBOP on lipid metabolism are independent of human CAR activation(Skoda et al., 2022). Liver X receptors (LXR, NR1H2/3) function as cholesterol sensors, thus protecting mammals from cholesterol overload. Specifically, CAR loss elevated lipogenic LXR target genes expression, ultimately resulting in elevated hepatic triglyceride accumulation(Xiao et al., 2010). CAR activation in the mouse liver leads to insulin-induced gene-1 (Insig-1) activation (an anti-lipogenic protein) and with a reduction in the active form of Srebp-1 levels (Roth et al., 2008). In the liver, CAR competitively binds to the DR1 motif and to GRIP-1 and PGC-1α that are common coactivators to inhibit HNF-4 activity. This may be a general underlying mechanism by which CAR downregulates key genes involved in hepatic lipid and glucose metabolism(Miao et al., 2006). Treatment of mice with phenobarbital, another CAR activator, suppresses the interaction between PPARα and PGC1α in the liver, thus attenuating PPARα-dependent lipid metabolism(Shizu et al., 2020). However, phenobarbital significantly elevated plasma triglyceride levels in

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contrast to the action of TCPOBOP(Skoda et al., 2022). Interestingly, Alice *et al*. reported that CAR activation induced hepatic lipogenesis and regulated patatin-like phospholipase domain-containing protein 3 (Pnpla3) expression in an LXR-independent pathway to promote nonalcoholic fatty liver disease (NAFLD)(Marmugi et al., 2016). CAR downregulated liver lipid metabolism through the LXR-Srebp1 pathway and its downstream target genes, such as SULT2B1, Insig-1, and HNF-4α. Interestingly, there is a series of reports regarding the effects of CAR on lipid metabolism. CAR activation by TCPOBOP induces hepatic lipogenesis and promotes NAFLD progression. In humanized CAR mice, TCOBOP increased triglyceride levels serum and liver. In hyperlipidemic mice, TCPOBOP treatment significantly reduced plasma triglyceride and intermediate-density lipoprotein/low-density lipoprotein cholesterol levels and very-low-density lipoprotein production in the liver and was accompanied by a decrease in hepatic triglyceride content and the repression of several genes involved in lipogenesis(Sberna et al., 2011b). TCPOBOP and phenobarbital exhibited the opposite effects in regard to the regulation of plasma triglyceride levels. These investigations revealed that the modulation of lipid metabolism by CAR is more complex than we initially assumed and depends upon the metabolic context. Different types of CAR ligand treatments may regulate lipid metabolism not only through CAR activation, and further investigation of the underlying mechanism is required in regard to their application in metabolic diseases.

3.2 CAR in glucose metabolism

CAR activation not only tightly modulates lipid metabolism but also influences

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hepatic glucose metabolism. In diabetic mice, CAR activation significantly decreases serum glucose levels and improves glucose tolerance by inhibiting glucose production and stimulating glucose uptake. The decreased glucose production by CAR activation was caused by interfering with forkhead box protein O1 (Foxo1) and influencing hepatocyte nuclear factor 4α (HNF4α) activity to downregulate PEPCK and G6pase expression (Kachaylo et al., 2012, Kodama et al., 2004, Miao et al., 2006). CAR stimulates glucose uptake via increasing hexokinase (HK) and phosphogluconate dehydrogenase (PGD) activities that are enzymes responsible for the first irreversible step in glycolysis and a rate-limiting enzyme in the pentose phosphate pathway, respectively(Chen F. et al., 2019a, Dong et al., 2009). Caitlin *et al.* reported an important species difference between human CAR and mouse CAR in terms of hepatic energy metabolism. Activation of mouse CAR inhibits genes related to gluconeogenesis, fatty acid synthesis, and lipogenesis. However, human CAR activation specicially depresses gluconeogenesis by decreasing G6pase and PEPCK expression without interfering with fatty acid synthesis(Lynch et al., 2014). Liver tissue metabolome analysis also demonstrated that CAR activation significantly decreased the components involved in key gluconeogenic pathways and increased the content contributing to glucose utilization pathways. These changes were due to the downregulation of the hepatic glucose sensor and the bidirectional transporter Glut2(Chen F. et al., 2019a).

3.3 CAR in cholesterol and bile acid metabolism

CAR also plays a vital role in cholesterol and bile acid metabolism. CAR can

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elevate BA content and decrease hepatic cholesterol levels by regulating downstream target gene expression. Anne *et al*. determined that long-term CAR activation is related to decreased whole-body cholesterol content and atherosclerosis susceptibility(Sberna et al., 2011a). For the underlying mechanism, CAR activation suppressed Abcg5 and Abcg8 expression and consequently lowered the biliary cholesterol level. In the same study, CAR activation promoted the conversion of cholesterol to bile acids via elevating the expression of CYP7a1, a rate-limiting enzyme in BA synthesis. CAR activation enhances BA re-absorption by enhancing the expression of the bile acid transporters Abst and Ostβ, and this further alleviates cholesterol gallstone disease(Cheng S. et al., 2017). In addition to regulating the enzymes responsible for BA metabolism and transport, CAR can cross-talk with PXR by either competing for common coactivators or by disrupting the coactivation of other transcription factors(Pavek, 2016).

Bilirubin, LCA, and steroids can indirectly enhance CAR transcriptional activity in bile acid metabolism. Downstream target gene encoding for enzymes of CAR are participated in the oxidative metabolism (CYPs), conjugation (GSTs, UGTs, and SULTs), and transport (ABCs and OATPs) of bile acids(Modica et al., 2009). In summary, CAR acts in concert to establish a metabolic safety network against BA by inhibiting its synthesis and inducing the genes responsible for phase I (hydroxylation), phase II (conjugation), and phase III (excretion) metabolism. Indeed, by binding and activating CAR, BAs stimulate CAR in a feed-forward manner, thus regulating the expression of BA enzymes and membrane transporters that are responsible for BA

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clearance.

4. AHR in metabolic disease

The AHR is an evolutionarily conserved sensor that integrates environmental, microbial, metabolic, and endogenous signals into specific cellular responses. It is associated with autoimmune, neoplastic, metabolic, and degenerative diseases(Rothhammer and Quintana, 2019). Therefore, the study of AHR regulation and function may direct the development of novel therapeutic interventions for these diseases.

4.1 The functional role of AHR in inflammation during metabolic disease

As a cytoplasmic receptor and transcription factor, AHR has been previously speculated to play a key role in immunity and tissue homeostasis(Shinde and McGaha, 2018). AHR regulates adaptive immunity by inducing Th17 cells, stabilizing Treg and Tr1 cells, and priming anti-inflammatory dendritic cells(Rothhammer and Quintana, 2019).

During metabolic diseases, intestinal AHR plays a dominant role in regulating inflammation, and this further alleviates disease progression. In obese and type 2 diabetes patients, elevated plasma IL-22 and IL-17 levels were accompanied by increased AHR transcripts in peripheral blood mononuclear cells, thus suggesting that AHR may play a plausible role in the interaction between pro-inflammatory status and metabolism in T2D and obesity(Zhao R. X. et al., 2020). In an HFD mouse model, AHR activation elicited IL-22 production in the gut that improved intestinal barrier permeability and decreased endotoxemia(Lin Y. H. et al., 2019). AHR activation

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defended chemically induced damage to intestinal barrier integrity and decreased inflammatory cytokine expression in intestinal cells through the p38-MAPK pathway, thus further alleviating obesity(Postal et al., 2020). Myeloid cell AHR nuclear translocator knockout promoted the conversion from NAFL to steatohepatitis with increased hepatic macrophage infiltration and M1 macrophage marker expression (Scott et al., 2019). The studies suggest the protective role of AHR in inflammation during metabolic diseases that is different from its role in adaptive immunity.

4.2 The functional role of AHR in metabolic disease

In obese patients, serum AHR levels were determined to be significantly higher, and this is correlated with food consumption, thus suggesting a vital important role of AHR in obesity(Andac-Ozturk et al., 2021). In AHR whole-body knockout mice, adiposity was increased and was accompanied by decreased glucose tolerance. Liver steatosis was also accelerated with increased hepatic triglyceride accumulation and decreased blood lipids(Jin et al., 2021). Treatment with an AHR agonist alleviates insulin resistance and serum and liver triglyceride content in diabetic mice(Natividad et al., 2018). A recent study reported that saccharin/sucralose remarkably inhibited microbiota-derived AHR ligands and AHR expression in colon, and this further elevated serum and hepatic fatty acid levels, ultimately causing NAFLD in mice(Shi et al., 2021). Tissue-specific AHR deficiency in mature adipocytes through adiponectin-Cre promotes obesity, whereas hepatic AHR deficiency exacerbates steatosis without exerting significant effects on obesity, thus suggesting a different role for AHR in specific tissues(Gourronc et al., 2020). For the underlying

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mechanism, decreased microbiota production of AHR ligands leads to AHR inactivation, and this causes defective mucosal barrier integrity, reduced GLP-1 secretion, and the development of more severe metabolic syndrome(Natividad et al., 2018). AHR can also influence metabolic diseases by regulating inflammation. Lin *et al*. identified an AHR ligand agonist, indigo, as a promoter of IL-10 and IL-22 that defends from HFD-induced insulin resistance and fatty liver disease in a diet-induced obesity model. These effects are mechanistically related to downregulated inflammatory immune cell tone in tissues(Lin Y. H. et al., 2019). Interestingly, Xu *et al*. reported different results. AHR knockout protected against HFD-induced hepatic steatosis, insulin resistance, obesity, and inflammation resulting from higher energy expenditure accompanied by elevated mitochondrial β-oxidation gene expression and thermogenic gene uncoupling protein 1 (Ucp1)(Xu C. X. et al., 2015). In NAFLD, AHR activation can promote the expression of the estrogen metabolic enzyme CYP1A1 that is critical in the estrogen pathway, thus resulting in the inhibition of fatty acid oxidation, suppression of hepatic export of triglycerides, and an elevation in peripheral fat mobilization(Zhu et al., 2020). Further studies have revealed that an activated AHR is necessary but not sufficient to attain obesity, a status that mostly obtains fat from the diet, thus suggesting that the modulatory role of AHR is dependent upon the nutrient environment(Rojas et al., 2021).

Overall, AHR was confirmed to regulate metabolic diseases, and this was primarily due to its modulation of intestinal integrity, anti-inflammatory effects, and downstream target gene expression. However, the underlying mechanisms of AHR

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regulation of lipids, glucose, cholesterol, and bile acids remain poorly understood, and the effects of AHR on metabolic diseases in different physiological states remain controversial. More studies are required to investigate the regulatory roles of AHR on specific metabolic components in different physiological states, as this may expand the knowledge of AHR in the context of metabolic diseases.

Discussion

In this review, we highlight the regulatory effect of three xenobiotic receptors (PXR, CAR, and AHR) in lipid, glucose, cholesterol, and BA metabolism during metabolic diseases. Considering the increased prevalence of metabolic diseases and the growing concerns regarding human exposure to xenobiotics in the environment, knowledge of xenobiotic receptors and the regulation of metabolic diseases is important(Gulliver, 2017).

XRs are vital regulators of metabolic diseases. However, a number of ligands of XRs exert diverse effects in humans and rodents. Therefore, the confirmation of endogenous ligands, particularly those with species specificity, will expand our knowledge about the biology of XRs in different species. Concurrently, XRs share some common ligands and downstream target genes, thus further regulating metabolic diseases. XRs in different tissues and specific pathological environments may exert different effects on diseases, thus indicating their complex functions. Although PXR activation may promote hepatic fatty acid deposition and lipogenesis and lead to steatosis, its immunosuppressive effects on the intestine and immune cells may be beneficial for metabolic disorders by alleviating chronic inflammation(Gao J. and Xie,

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2012). Investigations examining selective XRs regulators may provide new therapeutic tools for metabolic disorder management. Furthermore, identifying novel downstream target genes during the regulation of XRs in metabolic diseases and exploring the treatment for metabolic diseases may be more effective.

XRs can be activated by a series of xenobiotics and can stimulate downstream target genes expression, including phase I and II enzymes and drug transporters. Most drugs and xenobiotics are metabolized in the liver. Among others, different cytochrome P450 (CYP) enzymes catalyze the metabolic conversion of foreign compounds, and various transport proteins are involved in the excretion of metabolites from hepatocytes(Hammer et al., 2021). The expression and activity of drug-metabolizing enzymes and transporters plays a decisive role in determining the pharmacokinetic properties of a compound in a given test system. Therefore, XRs are implicated in multiple drug-drug interactions that can lead to alterations in drug pharmacokinetics and cause fluctuating therapeutic efficacies. Among all of the downstream target genes, CYP3A4 is the primary isoform of cytochrome P450 oxidases participated in the metabolism of approximately 60% drugs, and its expression level is highly variable in human subjects(Du et al., 2013). CYP3A4 induction can result in serious toxicological consequences as a result of elevated drug metabolism that contributes to drug-drug interactions, the bioactivation of xenobiotics to carcinogenic or toxic metabolites, and possibly endocrine disruption(Chai X. et al., 2013). Besides CYPs, several other phase I enzymes also play notable roles in the clearance of drugs by hydrolysis, reduction, and oxidation. PCN could significantly

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influence the pharmacokinetics of ursolic acid in rats, and it exhibited discrepant effects on the messenger RNA expression of CYP and transporters in tissues(Jinhua et al., 2020). Moreover, PCN significantly elevated Oatp2 expression and influenced the pharmacokinetics of sorafenib. Microvascular density and vascular endothelial growth factor levels in tumor-adjacent tissues declined significantly, thus suggesting that elevated Oatp2 expression enhances the therapeutic effect of sorafenib in a rat model of liver cancer(Wen and Zhao, 2021). Other factors also affect drug pharmacokinetics through PXR. Interferon-alpha has been suggested to cause pharmacokinetic drug interactions by decreasing the expression of drug disposition genes by influencing NF-κB and PXR activities(Theile et al., 2021). Besides PXR, CAR can influence drug pharmacokinetics through the regulation of CYP1A, UGTs, and SULTs(Fu et al., 2020). A series of approved drugs such as imiquimod (IMQ) were confirmed to be metabolized by AHR-regulated CYP enzymes *in vivo* and *in vitro*(Mescher et al., 2019, Shimizu et al., 2021).

Considering the properties of XRs, it is recommended that researchers and clinicians consider their effects on drug metabolism when designing XRs as therapeutic targets for metabolic diseases. Concurrently, owing to the functions of XRs, agonists or antagonists of these receptors may possess therapeutic potential in regard to the correct management of certain diseases.

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Authorship contributions

Participated in research design:

Conducted experiments:

Contributed new reagents or analytic tools:

Performed data analysis:

Wrote or contributed to the writing of the manuscript: Jinhang Zhang, Qingyi Jia, Yanping Li and Jinhan He.

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Footnotes

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Figure Legends

Figure 1. Overview of whole-body cholesterol metabolism.

Cholesterol metabolism is maintained by an array of regulatory processes controlling absorption, de novo synthesis, hepatic lipoprotein production, lipoprotein uptake, and efflux. The enterocytes are responsible for absorbing diet cholesterol and converting it into chylomicron, which is further transported to the blood. LDL uptake through LDLR and hydrolysis to cholesterol via lysosome. In liver cells, the biosynthesis pathway converts acetyl-CoA into cholesterol through nearly 30 enzymatic reactions, during which HMGCR and SQLE are the two key speed-limiting enzymes. The cholesterol can be converted to bile acid, oxysterols, or esterified to lipid droplets. Liver cells synthesize HDL and efflux via ABCA1 and ABCG1. NPC1L1, Niemann-Pick C1-like 1; ABCG5/G8, ATP-binding cassette (ABC) transporters G5 and G8; ABCG1, ABC transporters G1; ABCA1, ATP-binding cassette transporter A1; acetyl-CoA, acetyl coenzyme A; ACAT2, acetyl CoA acetyltransferase2; HMGCS, 3-hydroxy-3-methylglytaryl-CoA synthetase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGCR, HMG-CoA reductase; Farnesyl-PP, Farnesyl pyrophosphate; FDFT1, farnesyl-diphosphate farnesyltransferase 1; SQLE, squalene epoxidase; LSS, Lanosterol synthase; NPC1, NPC intracellular cholesterol transporter 1.

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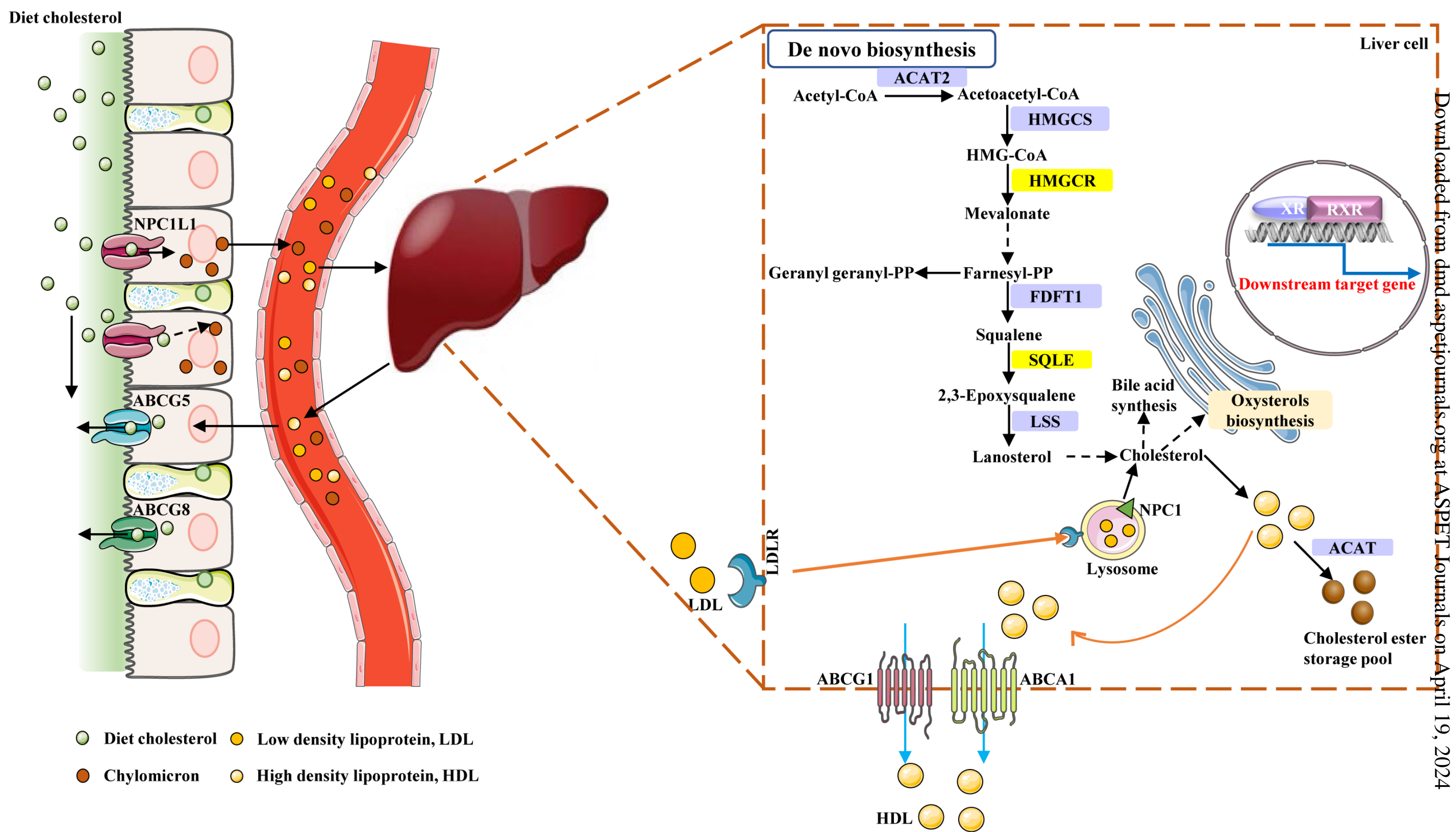

Figure 1

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