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LXR IN CHOLESTEROL METABOLISM

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Abbreviations:

LXR: liver X receptor; **LXRE**: LXR response element; **FXR**: farnesoid X receptor; **PXR**: pregnane X receptor; **RXR**: retinoid X receptor (RXR); **RCT**: reverse cholesterol transport; **HDL:** high-density lipoprotein; **TG**: triglyceride; **LDLR**: low-density lipoprotein receptor

Abstract

The liver X receptors (LXRs) are nuclear receptors that are activated by endogenous oxysterols, oxidized derivatives of cholesterol. There are two isoforms of LXR, LXRα (NR1H3) and LXRβ (NR1H2). Both LXRα and LXRβ regulate gene expression by binding to DNA sequences associated with target genes as heterodimers with isoforms of the retinoid X receptor (RXR), RXRα (NR2B1), RXRβ (NR2B2) and RXRγ (NR2B3). LXRs act as cholesterol sensors: when cellular oxysterols accumulate as a result of increasing concentrations of cholesterol, LXR induces the transcription of genes that protect cells from cholesterol overload. In this review, we summarize the roles of LXRs in controlling cholesterol homoeostasis, including their roles in bile acid synthesis and metabolism/excretion, reverse cholesterol transport (RCT), cholesterol biosynthesis and uptake, and cholesterol absorption/excretion in the intestine. The overlapping and distinct roles of the LXRα and LXRβ isoforms, and the potential use of LXRs as attractive targets for treatment of cardiovascular disease are also discussed.

Liver X receptor (LXR)

The liver X receptors (LXRs), LXRα (NR1H3) and LXRβ (NR1H2), belong to the nuclear receptor superfamily of ligand-activated transcription factors (Janowski *et al.* 1996). LXRα was initially isolated from a rat liver cDNA library (Apfel *et al.* 1994) as a novel orphan nuclear receptor, i.e. receptors with no known physiological ligands, hence the name liver X receptor. Several groups identified the LXRβ isoform by screening of different cDNA libraries (Shinar *et al.* 1994, Song *et al.* 1994, Teboul *et al.* 1995). The human LXRα gene is located on chromosome 11p11.2, while the human LXRβ gene is located on chromosome 19q13.3. LXRα expression predominates in metabolically active tissues such as the liver, small intestine, kidney, macrophages and adipose tissue, whereas LXRβ is more ubiquitously expressed with particularly high levels in the developing brain (Fan *et al.* 2008), suggesting regulation of different physiological functions for the two receptors. Human LXRα and LXRβ share almost 80% amino acid identity in their DNA-binding domain and ligand-binding domain. The LXR paralogues are highly conserved between rodents and humans. Human LXRα and rat LXRα show close to 100 % homology in amino acid sequence in their DNA-binding domain and ligand-binding domain (Lee *et al.* 2008).

With the discovery of oxysterols (Janowski *et al.* 1999, Janowski *et al.* 1996) as endogenous ligands for LXRs, these receptors were included in the group of "adopted" nuclear receptors, i.e. receptors where a physiological ligand has been identified subsequent to the identification of the receptor.

Oxysterols, oxidized derivatives of cholesterol including 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S),25-epoxycholesterol, 20(S)-hydroxycholesterol and 27-hydroxycholesterol, are ligands for LXRs. Among them, 24(*S*),25-epoxycholesterol is the most potent agonist. It has been demonstrated that these oxysterols bind directly to the LXRs with K_d values ranging from 0.1 to 0.4 μM. LXRα and LXRβ show similar affinities for these compounds (Janowski *et al*. 1999). However, cholesterol itself is not a ligand for LXRs (Janowski *et al*. 1999). Recently, high concentrations of D-glucose and phytosterols, particularly β-sitosterol, were reported to be activators of LXRs (Mitro *et al.* 2007a, Plat *et al.* 2005). A subset of natural bile acids has been reported to selectively activate LXRα (Song *et al.* 2000), whereas N-acylthiadiazolines have selectivity for LXRβ, however with modest potency (Molteni *et al.* 2007). Recently, a phenethylphenyl phthalimide derivative has been shown to be a potent LXRα-selective antagonist (Motoshima *et al.* 2009). As regulators of metabolism, LXRs have been considered as potential drug targets by the pharmaceutical industry and synthetic LXR ligands have been developed that are widely used as tools in biomedical research. Synthetic LXR ligands include T0901317 (Schultz *et al.* 2000) and GW3965 (Collins *et al.* 2002). In general, these synthetic ligands show poor LXR subtype selectivity. The use of T0901317 as an LXR ligand is limited by its agonistic effect on farnesoid X receptor (FXR) (Houck *et al.* 2004) and pregnane X receptor (PXR) (Mitro *et al.* 2007b).

Transcriptional regulation by LXR

LXRs activate target genes by binding to DNA sequences associated with target genes. LXRs bind to consensus elements (LXREs) as heterodimers with isoforms of

the retinoid X receptor (RXR), RXRα (NR2B1), RXRβ (NR2B2) and RXRγ (NR2B3) (Makishima 2006). LXRE consists of two direct repeats (DR) of the consensus sequence AGGTCA separated by four nucleotides (DR-4) (Chawla *et al.* 2001). IR-0 (inverted repeat of the same consensus sequence with no spacer region) and IR-1 (inverted repeat of the same consensus sequence separated by 1 bp spacer) have also been shown to mediate LXR transactivation (Mak *et al.* 2002, Uppal *et al.* 2007). LXRs have been shown to regulate gene expression via LXREs in the promoter regions of LXR target genes such as UGT1A3 (UDP glucuronosyltransferase 1 family, polypeptide A3) (Verreault *et al.* 2006), fatty acid synthase (FAS) (Joseph *et al.* 2002a), carbohydrate response element binding protein (ChREBP) (Cha and Repa 2007), phospholipid transfer protein (PLTP) (Mak *et al.* 2002) and sterol regulatory element binding protein (SREBP) 1c (Repa *et al.* 2000a, Yoshikawa *et al.* 2001). LXREs have also been reported to be present in introns of target genes such as the ATP binding cassette transporter G1 (ABCG1) (Kennedy *et al.* 2001, Sabol *et al.* 2005). LXRs have been shown to activate gene expression via the IR-1 sequence for genes such as the human ileal bile acid-binding protein (I-BABP) and the organic solute transporter (Ost) (Landrier *et al.* 2003, Okuwaki *et al.* 2007). LXRs induce expression of the mouse Sult2a9 gene through binding to an IR-0 sequence in the promoter (Uppal *et al.* 2007). Recently, Wang *et al.* (Wang *et al.* 2008) have proposed a novel mode of regulation by LXR in which LXR represses gene expression via negative LXR DNA response elements (nLXREs) present in the gene promoters.

Cholesterol metabolism

Cholesterol is the essential precursor of steroid hormones (progesterone, estrogen, testosterone, glucocorticoids and mineralocorticoids), bile acids and vitamin D. It is

also a vital constituent of cell membranes that modulates the fluidity and permeability of the membrane. Cholesterol can be derived from the diet as well as from endogenous biosynthesis, the latter being the major source in humans. Homeostasis of cholesterol involves the movement of cholesterol between peripheral tissues and the liver. The liver regulates *de novo* biosynthesis of cholesterol, the excretion of cholesterol into bile (directly or after conversion to bile acids), the secretion of cholesterol into blood as very low-density lipoproteins (VLDL), the modulation of receptor-mediated cellular cholesterol uptake, the formation of cholesteryl esters, which are more hydrophobic than cholesterol itself, and the storage of cholesterol. The intestine regulates cholesterol absorption and excretion into feces.

LXR as cholesterol sensors

LXRs act as cholesterol sensors: when cellular oxysterols accumulate as a result of increasing concentrations of cholesterol, LXR induces the transcription of genes that protect cells from cholesterol overload. LXR activation regulates bile acid synthesis and metabolism/excretion, reverse cholesterol transport (RCT), cholesterol biosynthesis, and cholesterol absorption/excretion in the intestine (see Fig. 1).

LXR and bile acid synthesis, metabolism and excretion

Bile acid synthesis and secretion constitute the major route for elimination of cholesterol from the body. Oxysterols, natural ligands for LXRs, are generated when cholesterol levels are high. The classical pathway of bile acid synthesis is initiated by 7α-hydroxylation of cholesterol catalyzed by the cytochrome P450 cholesterol 7α-hydroxylase (CYP7A1), which encodes the rate-limiting enzyme of this pathway (Russell and Setchell 1992). In rodents, LXRα stimulates the expression of CYP7A1

via binding to an LXRE present in the CYP7A1 promoter. Thus rats and mice have the capacity to convert dietary cholesterol to bile acids (Peet *et al.* 1998). As a consequence, these species quickly adapt to a diet rich in cholesterol by increasing its conversion to bile acids. The importance of LXRα activated CYP7A1 in regulating cholesterol balance in the rodent liver became evident from studies of LXR knockout mice (Peet *et al.* 1998). LXRα, but not LXRβ (Alberti *et al.* 2001), knockout mice accumulate large amounts of cholesterol esters in the liver after being fed a high-fat cholesterol diet due to failure of inducing expression of the CYP7A1 gene.

In contrast to observations in rats and mice, LXRα agonist treatment suppresses expression of CYP7A1 in primary human hepatocytes (Goodwin *et al.* 2003). This repression is, at least in part, due to the direct induction of small heterodimer partner (SHP), a gene that has a repressive effect on CYP7A1 via liver receptor homologue 1(LRH1; also called FIF in rat and CPF in humans) (Goodwin *et al.* 2000). These results suggest that different species may employ distinct molecular strategies to regulate cholesterol homeostasis, emphasizing the importance of valid experimental models for the development of pharmaceuticals for human use.

In addition to its role in controlling bile acid anabolism, LXR also plays a role in regulating bile acid catabolism. Recent reports indicate that ligand-activated LXRα up-regulates human UGT1A3 gene expression through binding to an LXRE-like sequence in the promoter (Barbier *et al.* 2009). UGT1A3 is one of the most active enzymes for glucuronide conjugation of bile acid. Bile acid glucuronidation allows their conversion into urinary excretable metabolites. Based on these observations, it

159 was proposed that LXRα activation may facilitate definitive cholesterol elimination in
160 the form of urinary bile acid glucuronides.
161
162 Most bile acids are *N*-acyl amidates with glycine or taurine to decrease toxicity and
163 increase solubility for secretion into bile (Hofmann 1999). Taurine occurs naturally in
164 many foods and is known to lower cholesterol profiles (Chen *et al.* 2004, Zhang *et al.*
165 2004). Additionally, taurine has been shown to induce CYP7A1 activity thereby
166 increasing bile acid synthesis (Yokogoshi *et al.* 1999). Interestingly, it has been
167 shown that taurohyodeoxycholic acid can activates the LXRE in the CYP7A1
168 promoter via LXRα, suggesting that activation of LXR signaling is one mechanism
169 by which taurine activates CYP7A1 activity (Song *et al.* 2000).
170
171 Excretion of free cholesterol into the bile is another major route for eliminating excess
172 cholesterol from the liver. In the liver, ABCG5 and ABCG8 have been proposed to
173 transport cholesterol from hepatocytes to the bile canaliculi. ABCG5 and ABCG8 are
174 half transporters that form obligate heterodimers, and are both regulated by LXR
175 activation (Berge *et al.* 2000, Repa *et al.* 2002). ABCG5 and ABCG8 are expressed in
176 the apical membrane of enterocytes and at the canalicular membrane of hepatocytes.
177 These transport proteins promote secretion of hepatic cholesterol into bile. Mice
178 lacking ABCG5 or ABCG8 exhibit profound reduction in biliary cholesterol levels
179 and an accumulation of cholesterol in the liver after cholesterol feeding (Yu *et al.*
180 2002). Mutations in the genes encoding either ABCG5 or ABCG8 result in β-
181 sitosterolemia, an autosomal recessive disorder characterized by an increased risk of
182 atherosclerosis and elevated plasma levels of phytosterols (Lee *et al.* 2001, Lu *et al.*
183 2001). The human ABCG5 and ABCG8 genes are oriented in a head-to-head

configuration separated by a 374-bp intergenic region. No LXREs have been identified in the promoters of ABCG5 or ABCG8, but the intergenic region was found to act as a bidirectional promoter and be partially responsive to treatment with LXR agonists (Remaley *et al.* 2002).

LXR and reverse cholesterol transport (RCT)

RCT is a pathway by which accumulated cholesterol is transported from peripheral tissues to the liver followed by biliary secretion and subsequent disposal via the feces. High-density lipoprotein (HDL) cholesterol is believed to play a key role in the process of RCT, as it promotes the efflux of excess cholesterol from peripheral tissues and returns it to the liver for biliary excretion. Accumulation of cholesterol in macrophages in the vessel wall is considered a primary event in the development of atherosclerosis and, therefore, removal of excess of cholesterol from these cells is important for prevention and /or treatment of atherosclerotic cardiovascular diseases.

LXR, by regulating expression of several genes, including ABCA1, ABCG1, ApoE and PLTP plays a critical role in RCT. LXR activation increases cholesterol efflux important for RCT from peripheral tissues and has antiatherogenic potential by inhibiting the progression of and even promoting the regression of atherosclerosis in mice (Joseph *et al.* 2002b, Levin *et al.* 2005, Naik *et al.* 2006). Consequently, the development of pathway-selective LXR agonists represents an attractive therapeutic approach for atherosclerosis.

ABCA1 was initially found to be induced by pharmacological activation of LXR with T0901317 (Repa *et al.* 2000b), and later an LXRE was identified in this gene (Costet

209 *et al.* 2000). ABCA1 is expressed at the basolateral membrane of the enterocyte, in
210 hepatocytes and in macrophages. ABCA1 mediates transport of phospholipids and
211 cholesterol to lipid-poor apolipoproteins such as apo-A1, which stabilizes the HDL
212 particle and is thus responsible for the initial step of RCT. Accordingly,
213 overexpression of hepatic ABCA1 raises HDL cholesterol levels (Basso *et al.* 2003,
214 Wellington *et al.* 2003). Studies in mice with tissue-specific knockout of ABCA1
215 revealed that hepatic and intestinal ABCA1 contribute ~80% and ~20%, respectively,
216 to HDL biogenesis in mice (Brunham *et al.* 2006, Timmins *et al.* 2005). ABCA1 is
217 important for macrophages to regulate sterol homeostasis. In support of this, ABCA1
218 knockout mice show evidence of cholesterol accumulation in a variety of
219 macrophage-rich tissues including lung, spleen, lymph nodes, thymus, and skin
220 (Christiansen-Weber *et al.* 2000, McNeish *et al.* 2000). Recently, macrophage-
221 specific knockout of ABCA1 in mice was shown to lead to an increase in free and
222 esterified cholesterol in macrophages, and enhanced inflammatory responses (Zhu *et*
223 *al.* 2008). Overexpression of ABCA1 in macrophages in low-density lipoprotein
224 receptor knockout ($LDLR^{-/-}$) mice inhibits atherosclerotic lesion progression and
225 exerts a protective role against atherosclerosis with minimal effects on plasma HDL
226 (Van Eck *et al.* 2006).
227
228 ABCG1 expression is also induced by LXR activation and LXREs have been
229 identified in the promoter region of this gene (Kennedy *et al.* 2001, Sabol *et al.* 2005).
230 Studies in ABCG1 knockout mice revealed that ABCG1 is primarily expressed in
231 macrophages, endothelial cells and lymphocytes. However, it is also found in Kupffer
232 cells and hepatocytes (Kennedy *et al.* 2005). Based on the observation that ABCG1
233 knockout mice fed a high-fat and high-cholesterol diet accumulate considerable

amounts of cholesterol and neutral lipids in macrophages and liver, it was proposed that ABCG1 plays an important role in cholesterol efflux (Kennedy *et al.* 2005). In contrast to ABCA1 that transports cholesterol to lipid-poor apolipoproteins, ABCG1 transports cholesterol to phospholipid-containing acceptors such as HDL. A synergistic relationship between ABCA1 and ABCG1 has been proposed. ABCA1 promotes lipidation of lipid-poor particles and generates acceptors for ABCG1 mediated cholesterol efflux (Gelissen *et al.* 2006).

Apolipoprotein E (ApoE) has been shown to be up-regulated by LXR activation through its direct interaction with LXREs present in the enhancers of this gene (Laffitte *et al.* 2001). Secretion of ApoE promotes incorporation of cholesterol into the lipid-poor HDL particles. In agreement with this, a massive accumulation of lipoproteins and lipoprotein remnants have been observed in the plasma of both humans and mice lacking functional ApoE (Plump *et al.* 1992, Zhang *et al.* 1992). ApoE is also an important modulator of atherogenesis. This is supported by findings that *ApoE*−/− mice develop atherosclerosis on a normal chow diet (Reddick *et al.* 1994), and that selective re-expression of ApoE in macrophages of *ApoE*−/− mice through bone marrow transplantation or transgenic expression decreases atherosclerosis (Zhu *et al.* 1998).

PLTP is a target for LXR activation in the liver and in macrophages (Laffitte *et al.* 2003). It has been proposed that plasma PLTP facilitates the transfer of phospholipids and cholesterol from triglyceride-rich lipoproteins (TRL) into HDL. PLTP is capable of generating preβ-HDL through HDL conversion. The generation of preβ-HDL particles, a very efficient acceptor of peripheral cell cholesterol, enhances cholesterol

efflux from peripheral cells (Lee *et al.* 2003). These results suggest that PLTP is important for the prevention of atherosclerosis. Consistent with the proposed role for PLTP in lipoprotein metabolism, the plasma of PLTP knockout mice showed a complete inability to transfer phospholipids from TRL into HDL both *in vitro* and *in vivo* (Jiang *et al.* 1999). In a transgenic mouse model engineered to overexpress human PLTP, there is a 30%-40% decrease in plasma levels of HDL cholesterol compared to wild-type mice. In addition, these mice showed an increased capacity to produce pre β-HDL (van Haperen *et al.* 2000). Moreover, plasma from these animals prevents accumulation of intracellular cholesterol in macrophages more efficiently than plasma from wild-type mice. These results suggest that PLTP is mediating an increase in cholesterol efflux.

LXR and cholesterol biosynthesis

Recently, Wang *et al*. (Wang *et al.* 2008) demonstrated that LXRα negatively regulated two genes, squalene synthase (*FDFT1*) and lanosterol 14α-demethylase (*CYP51A1*), that encode key enzymes in the cholesterol biosynthesis pathway. LXREs that confer LXR mediated repression were identified in these two genes. Based on these observations, it was proposed that LXRα plays an important role in suppression of cholesterol biosynthesis.

LXR and cholesterol uptake

The major part of cholesterol in human blood is transported within low-density lipoproteins (LDL-C). The LDLR mediates the removal of LDL and remnant lipoproteins from circulation by binding to apolipoprotein B-100 (ApoB-100) and ApoE. It also plays a major role in regulation of plasma cholesterol levels in humans

(Brown and Goldstein 1986). Recently, Zelcer *et al.* demonstrated that LXR decreases LDLR-dependent cholesterol uptake through a LXR-Idol (Inducible Degrader of the LDLR) pathway. LXR induces the expression of Idol, which in turn catalyzes the ubiquitination of the LDLR, thereby targeting it for degradation (Zelcer *et al.* 2009). On the contrary, induction of LDLR expression via an LXRE by LXR agonist has been reported by Ishimoto *et al.* (Ishimoto *et al.* 2006). The use of different cell lines and different LXR agonists in the two studies may account for the contradictory results. Clearly, the exact role of LXR in regulation of LDLR expression and subsequent cholesterol uptake needs to be further exploited.

LXR and intestinal cholesterol absorption

Intestinal cholesterol absorption has been shown to be a major determinant of plasma cholesterol levels. LXR activation results in a reduced absorption of intestinal cholesterol by regulating expression of several genes such as heterodimeric ABCG5/ABCG8 and Niemann-Pick C1-Like 1 (NPC1L1) involved in this process. LXR activation increases the expression of both ABCG5 and ABCG8, which transport absorbed cholesterol back to the lumen of the intestine. Consistent with this finding, administration of LXR agonists substantially decrease intestinal net cholesterol absorption in mice.

NPC1L1 is expressed in the small intestine, most likely in the brush border membrane of enterocytes, and it is required for intestinal cholesterol absorption (Altmann *et al.* 2004). It was recently reported that LXR activation downregulates NPC1L1 expression both in mice and in a human enterocyte cell line (Duval *et al.* 2006).

LXR and fecal neutral sterol excretion via intestine

Activation of LXR in mice leads to enhanced fecal neutral sterol loss (Plosch *et al.* 2002). Recent studies have revealed a major contribution of the intestine in excretion of cholesterol. In a study by Kruit *et al.* (Kruit *et al.* 2005), increased fecal neutral sterol excretion by LXR activation was observed in both wild-type mice and in *Mdr2*$^{-/-}$ mice, which are unable to secrete cholesterol into bile. These results suggest that an important part of excess cholesterol is excreted directly via the intestine. In addition, recent studies by van der Veen *et al.* (van der Veen *et al.* 2009) have revealed that trans-intestinal cholesterol excretion is a major route for removal of blood-derived free cholesterol in mice and this process is stimulated by activation of LXR upon treatment with T0901317. Moreover, ABCG5 knockout mice show evidence of impaired trans-intestinal cholesterol excretion, suggesting that ABCG5/ABCG8 heterodimers are involved in this pathway.

LXRs as therapeutic targets

As described above, LXRs function as cholesterol sensors with important roles in regulating cholesterol homeostasis, and thus there is a widespread interest in the development of synthetic LXR ligands as therapeutic agents. Indeed, the abundant expression of the LXRα protein in macrophages present in human atherosclerotic lesions supports the hypothesis that LXRα agonists could have a beneficial effect against development of atherosclerosis (Watanabe *et al.* 2005).

Recently, synthetic LXR ligands have been characterized in several animal models for the treatment of atherosclerosis. In a study by Joseph and co-workers (Joseph *et al.*

2002b), the influence of a nonsteroidal LXR agonist GW3965 on the development of atherosclerosis was investigated in both *LDLR*$^{-/-}$ and *ApoE*$^{-/-}$ mice. The results showed that GW3965 inhibits the development of atherosclerotic lesions in both murine models, providing direct evidence for an atheroprotective effect of LXR agonists. In the study by Terasaka *et al*. (Terasaka *et al.* 2003), T0901317, a synthetic LXR ligand, was administered to *LDLR*$^{-/-}$ mice. T0901317 significantly reduced the atherosclerotic lesions in *LDLR*$^{-/-}$ mice without affecting total plasma cholesterol levels. Moreover, an agonist for RXR, the obligate heterodimeric partner of LXRs, has been shown to be effective in reducing atherosclerosis (Claudel *et al.* 2001). These results suggest that LXR ligands may be useful therapeutic agents for the treatment of atherosclerosis. However, this therapeutic strategy needs to address that LXR activation is associated with stimulation of lipogenesis resulting in increased plasma triglyceride (TG) levels and hepatic steatosis. Several *in vivo* studies have shown that rodents treated with T0901317 have massive TG accumulation in the liver and increased plasma TG levels (Grefhorst *et al.* 2002, Repa *et al.* 2000a, Schultz *et al.* 2000). The LXR agonist, GW3965, also increases hepatic TG levels in mice (Grefhorst *et al.* 2005). Interestingly, a potent synthetic steroidal LXR activator, DMHCA (N,N-dimethyl-3b-hydroxy-cholenamide), has recently been demonstrated to reduce atherosclerosis in ApoE-deficient mice, without inducing hepatic and plasma TG levels. Based on these observations, DMHCA could be a candidate for further development as a therapeutic agent for treatment of atherosclerosis (Kratzer *et al.* 2009).

Specific roles of LXR isoforms in cholesterol metabolism

Isoform specific knockouts have yielded valuable information on individual physiological roles of the LXRα and LXRβ isoforms. LXRα$^{-/-}$ mice challenged with high-cholesterol diets fail to induce CYP7A1 expression, and as a result, accumulate large amounts of cholesterol esters in the liver (Alberti *et al.* 2001, Peet *et al.* 1998). Moreover, a recent report demonstrates that on a high fat diet, more cholesterol was accumulated in the liver of LXRα$^{-/-}$ and LXRαβ$^{-/-}$ mice than in wild-type and LXRβ$^{-/-}$ mice (Korach-Andre *et al.* 2009). These studies suggest that in the liver conversion of cholesterol to bile acids is controlled by LXRα. Although LXRβ is also expressed in the liver, its presence does not rescue the loss of LXRα in these mice. This is in line with literature showing that hepatic CYP7A1 and several genes involved in cholesterol metabolism were not induced in the liver of LXRα$^{-/-}$ mice treated with LXR ligands (Quinet *et al.* 2006).

Several studies have addressed specific roles of the LXRα and LXRβ isoforms in atherosclerosis. The work from Schuster *et al.* (Schuster *et al.* 2002) demonstrates that either receptor can play an atheroprotective role in macrophages and that the combined deficiency of both LXRα and LXRβ is required for foam cell-lipid accumulation in aortic lesions. Lund *et al*. (Lund *et al.* 2006) found that a synthetic compound, which actives both LXRα and LXRβ, induced ABCA1 expression and stimulated cholesterol efflux in macrophages from both LXRα$^{-/-}$ and LXRβ$^{-/-}$ mice. Moreover, treatment with an LXR agonist reduced atherosclerosis in ApoE$^{-/-}$/LXRα$^{-/-}$ mice suggesting that LXRβ alone is sufficient to mediate the anti-atherogenic functions of LXR activation (Bradley *et al.* 2007). One potential problem with LXRα/β agonists for treatment of atherosclerosis is their detrimental lipogenic effects dominated by LXRα. The overlapping and differential roles of LXRα and LXRβ

imply that LXRβ-selective targeting may separate the antiatherogenic and hypertriglyceridemic effects of the current dual agonists.

Conclusions

Studies in recent years have significantly enhanced our understanding of the molecular mechanisms of LXR signaling as an important global regulator of cholesterol homeostasis. The recent progress in the development of novel LXR ligands that reduce atherosclerosis, without displaying induction of non-desired effects observed by previous generations of LXR agonists, such as liver lipogenesis, show therapeutic promise for treatment of cardiovascular diseases. The future development of LXR subtype-specific ligands would provide critical tools for defining the mechanisms of distinct roles of LXRα and LXRβ, and might provide drug candidates with improved therapeutic profiles. Additionally, the development of novel ligands that possess tissue-specific agonist/antagonist properties provides another promising avenue for drug discovery.

Declaration of interest

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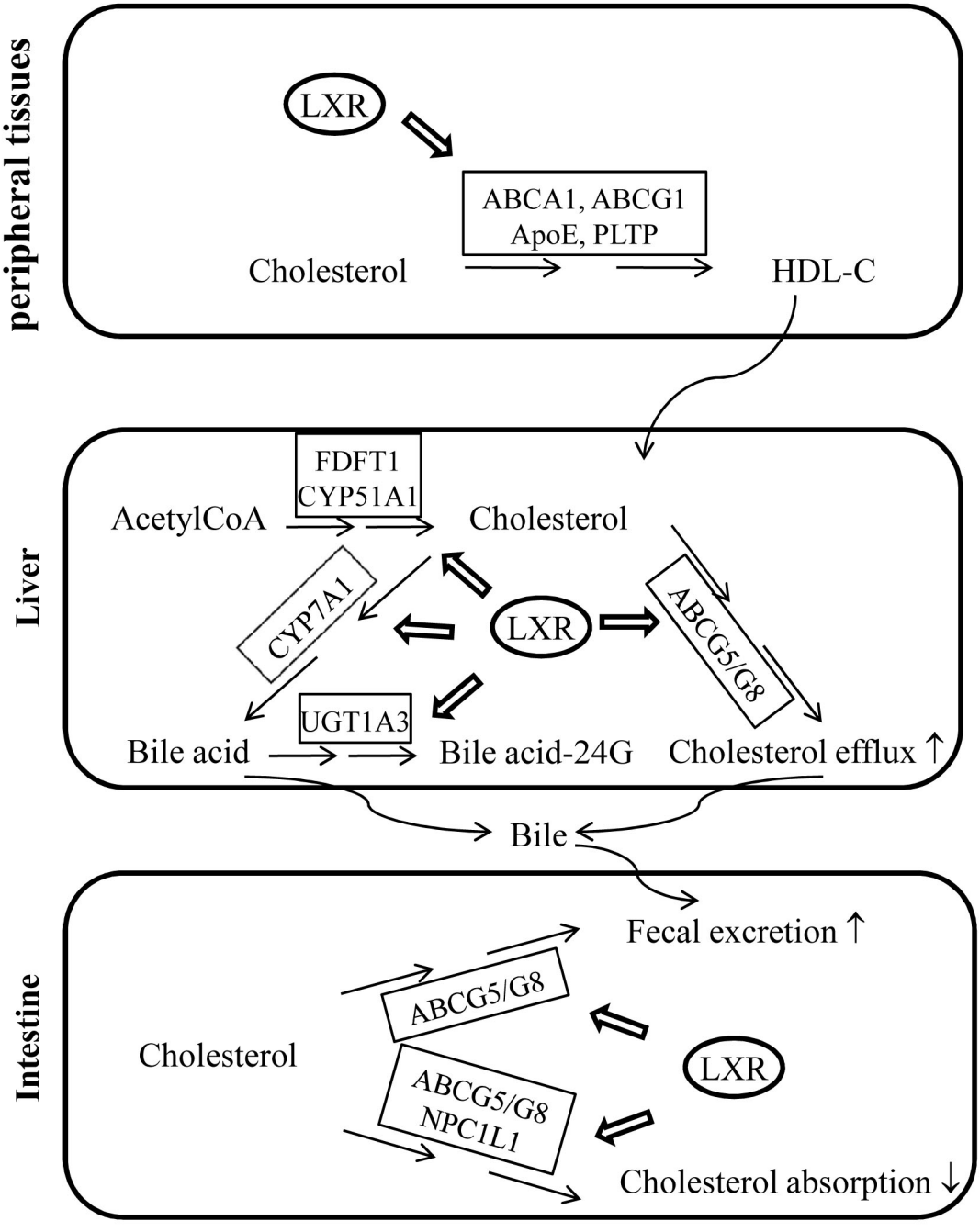
Legends to Figures

Figure 1. Role of LXR in cholesterol metabolism. In the liver, cholesterol biosynthesis/efflux and bile acid metabolism/excretion are all regulated by LXR. LXR increases efflux in the peripheral tissues, and in the intestine, LXR decreases absorption and increases fecal excretion. See text for details. *Yellow boxes* represent LXR target genes. HDL-C – high density lipoprotein cholesterol, ABC – ATP-binding cassette transporters, ApoE - apolipoprotein E, PLTP - phospholipid transfer protein, UGT1A3 - UDP glucuronosyltransferase 1 family, polypeptide A3, CYP7A1 - cholesterol 7α-hydroxylase, FDFT1 - farnesyl-diphosphate farnesyltransferase 1, CYP51A1 - cytochrome P450, family 51, subfamily A, polypeptide 1, NPC1L1 - Niemann-Pick C1-Like 1.

Figure 1


peripheral tissues
LXR
ABCA1, ABCG1
ApoE, PLTP
Cholesterol
HDL-C
Liver
FDFT1
CYP51A1
AcetylCoA
Cholesterol
CYP7A1
LXR
ABCG5/G8
UGT1A3
Bile acid
Bile acid-24G
Cholesterol efflux ↑
Bile
Intestine
Fecal excretion ↑
ABCG5/G8
Cholesterol
LXR
ABCG5/G8
NPC1L1
Cholesterol absorption ↓