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Review

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Liver X receptors (LXRs). Part I: Structure, function, regulation of activity, and role in lipid metabolism

Receptory wątrobowe X (LXR). Część I: Budowa, funkcja, regulacja aktywności i znaczenie w metabolizmie lipidów

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Summary

Liver X receptors (LXRs) α and β belong to a family of nuclear receptors which form heterodimers with the retinoid X receptor and, upon ligand binding, stimulate the expression of target genes. LXRs were initially described as orphan receptors and oxidized cholesterol derivatives (oxysterols) were later identified as their natural ligands. In addition, several synthetic LXR agonists such as T0901317 and GW3965 were synthesized. Oxysterols are formed in amounts proportional to cholesterol content in the cell and therefore the LXRs operate as cholesterol sensors which protect from cholesterol overload by: 1) inhibiting intestinal cholesterol absorption, 2) stimulating cholesterol efflux from cells to high-density lipoproteins through the ATP-binding cassette transporters ABCA1 and ABCG1, 3) activating the conversion of cholesterol to bile acids in the liver, and (4) activating biliary cholesterol and bile acid excretion. In addition, LXR agonists activate *de novo* fatty acid synthesis by stimulating the expression of a lipogenic transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c), leading to the elevation of plasma triglycerides and liver steatosis. Here we describe the structure and function of the LXRs, their endo- and exogenous agonists and antagonists, the regulation of LXR expression and activity, and their role in the regulation of cholesterol and lipid metabolism. In the accompanying article we characterize other effects of LXRs, alterations in LXR expression, and changes in the level of their endogenous agonists in pathological conditions as well as therapeutic implications.

Key words: **liver X receptor • oxysterols • reverse cholesterol transport • lipogenesis • atherosclerosis**

Streszczenie

Receptory wątrobowe X (LXR) typu α i β należą do rodziny receptorów jądrowych, które tworzą heterodimery z receptorem retinoidowym X i po związaniu ligandu pobudzają transkrypcję genów. Początkowo zostały opisane jako "receptory sieroce", a następnie wykazano, że ich naturalnymi ligandami są utlenione pochodne cholesterolu (oksysterole). Otrzymano też kilka syntetycznych agonistów tych receptorów, takich jak T0901317 oraz GW3965. Oksysterole powstają w ilościach proporcjonalnych do zawartości cholesterolu w komórce, i dzięki temu receptory LXR działają jako "mierniki cholesterolu" chroniące przed jego nadmiarem poprzez: 1) hamowanie wchłaniania cholesterolu z przewodu pokarmowego, 2) stymulację usuwania cholesterolu z komórek do lipoprotein o wysokiej gęstości przez transportery wiążące ATP, ABCA1 i ABCG1, 3) pobudzanie przekształcania cholesterolu w kwasy żółciowe w wątrobie oraz 4) aktywację wydalania cholesterolu i kwasów żółciowych do żółci. Ponadto, agoniści LXR pobudzają syntezę kwasów tłuszczowych stymulując ekspresję lipogennego czynnika transkrypcyjnego, białka wią-

żącego element odpowiedzi na sterole typu 1c (SREBP-1c), co prowadzi do wzrostu stężenia trójglicerydów w osoczu oraz stłuszczenia wątroby. W pracy omówiono budowę i działanie receptorów LXR, ich endo- i egzogennych agonistów i antagonistów, regulację ekspresji i aktywności LXR oraz ich znaczenie w regulacji metabolizmu cholesterolu i lipidów. W towarzyszącym artykule opisano inne działania receptorów LXR, zmiany ekspresji tych receptorów oraz poziomu ich endogennych agonistów w warunkach patologicznych oraz implikacje terapeutyczne.

Słowa kluczowe: **receptory wątrobowe X • oksysterole • zwrotny transport cholesterolu • lipogeneza • miażdżyca**

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Abbreviations: **9cRA** – *9-cis* retinoic acid; **ACAT** – acyl-CoA: cholesterol acyltransferase; **ACC** – acetyl-CoA carboxylase; **CETP** – cholesterol ester transfer protein; **ChREBP** – carbohydrate response element binding protein; **CNRE** – cyclic AMP response element and a negative response element; **CTX** – cerebrotendinous xanthomatosis; **EL** – endothelial lipase; **FAS** – fatty acid synthase; **FF-MAS** – follicular fluid meiosis-activating sterol; **FXR** – farnesoid X receptor; **HDL** – high-density lipoproteins; **HPL** – hepatic lipase; **LCAT** – lecithin: cholesterol acyltransferase; **LDL** – low-density lipoproteins; **LPL** – lipoprotein lipase; **LXR** – liver X receptor; **LXRE** – liver X receptor response element; **PLTP** – phospholipid transfer protein; **PPAR** – peroxisome proliferator-response element; **ROS** – reactive oxygen species; **RXR** – retinoid X receptors; **SCD-1** – stearoyl-CoA desaturase-1; **SREBP** – sterol response element binding protein; **T-MAS** – testes meiosis-activating sterol; **TR** – thyroid hormone receptor; **VDR** – vitamin D receptor; **VLDL** – very-low-density lipoproteins.

1. Introduction

Liver X receptors (LXRs) belong to a large family of nuclear receptors which bind to the regulatory region of target genes and, upon ligand binding, stimulate their transcription. LXRs were initially isolated from a human liver cDNA library as an orphan receptor since its natural ligand was unknown [171]. Later, oxidized cholesterol derivatives or oxysterols were identified as specific ligands of LXRs, which are therefore also named "oxysterol receptors". Studies performed during the last decade suggest that LXRs are "cholesterol sensors" which, in response to excess cholesterol, stimulate its transport to the liver and biliary excretion. In this review we summarize the current knowledge about the structure and function of LXRs, their endo- and exogenous ligands, the regulation of LXR expression and activity, and their role in cholesterol and lipid metabolism. In a related article we characterize other roles of LXRs, alterations of endogenous LXR signaling in pathological conditions, the therapeutic potential of LXR modulators, and the effect of currently used drugs on the LXR signaling system.

2. Liver X Receptor: An Overview

There are two LXR isoforms in mammals, termed LXRα (NR1H3) and LXRβ (NR1H2). LXRα is abundantly expressed mainly in the liver, intestine, kidney, spleen, and adipose tissue, whereas LXRβ is ubiquitously expressed at a lower level [7,136]. Both isoforms share almost 80% identity of their amino-acid sequences. The LXR molecule consists of four domains: 1) an N-terminal ligand-independent activation function domain (AF-1) which may stimulate transcription in the absence of a ligand, 2) a DNA-binding domain (DBD) containing two zinc fingers, 3) a hydrophobic ligand-binding domain (LBD) required for ligand binding and receptor dimerization (see below), and 4) a C-terminal ligand-dependent transactivation sequence, also referred to as activation function-2 (AF-2), which stimulates transcription in response to ligand binding.

LXRα exists in three variants originating from alternative promoter usage and mRNA splicing: LXRα1, LXRα2, and LXRα3. [36]. LXRα1 is the major variant in most tissues except the testis, where the LXRα2 predominates, whereas LXRα3 is expressed at low levels in the lung, thyroid gland, and spleen. All three variants bind DNA, but LXRα2 is less active in stimulating transcription than LXRα1, whereas LXRα3 is unable to bind ligands and fails to stimulate transcription; LXRα3 may in fact antagonize the function of the other isoforms.

Both LXRα and LXRβ function as heterodimers with the retinoid X receptor (RXR), a common partner for several

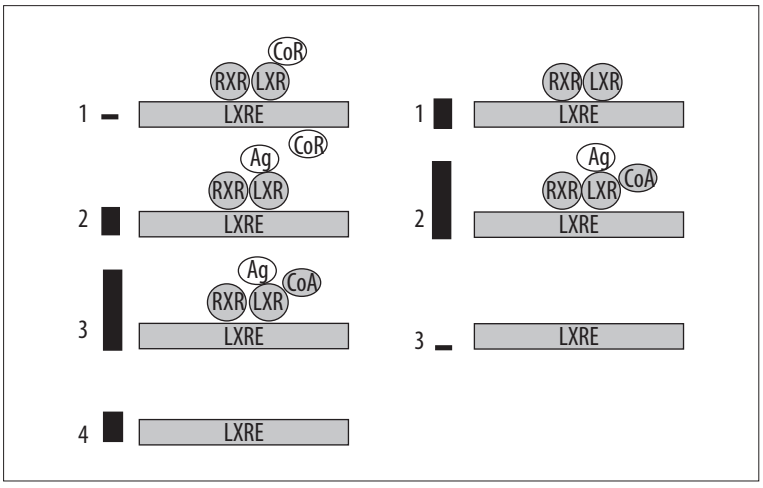

Figure 1. Two models of the regulation of gene expression by the LXR/RXR heterodimer. Left: unliganded LXR/RXR actively suppresses transcription (1) by recruiting co-repressors (CoR). Agonist (Ag) binding induces dissociation of the co-repressor, which results in moderate stimulation of transcription (2), and later recruits co-activators (CoA), leading to maximal stimulation of transcription (3). Removal of active suppression by LXR knockout (4) causes moderately higher expression of a target gene compared with the wild-type phenotype. Right: unliganded LXR/RXR heterodimer moderately stimulates transcription because co-repressors are not bound (1). Agonist binding induces a maximal stimulation of transcription by recruiting co-activators (2). In the absence of LXR, transcription is minimal (3) because the baseline stimulatory effect of unliganded LXR is removed. Vertical black bars qualitatively represent the level of gene expression. Various genes may be regulated according to the 1st or the 2nd model

nuclear receptors, including peroxisome proliferator-activated receptor (PPAR), vitamin D receptor (VDR), thyroid hormone receptor (TR), and farnesoid X receptor (FXR). LXR/RXR is a so-called "permissive heterodimer" which may be activated by either LXR agonist or 9-cis retinoic acid (9cRA), a specific RXR ligand. Simultaneous application of the agonists of both partners usually elicits a stronger response than either agonist alone. The LXR/RXR complex binds to a liver X receptor response element (LXRE) in the promoter region of target genes. The "ideal" LXRE sequence is a direct repeat-4 (DR-4) DNA fragment consisting of two AGGTCA hexameric half-sites separated by a 4-nucleotide spacer. However, analysis of functional LXRE in the known target genes revealed that the consensus sequences contain a number of invariant nucleotides in each half-site, but the nucleotides at other positions may vary considerably. Although LXRα and LXRβ have similar affinities to the idealized LXRE, they may have different affinities to specific nonideal LXRE sequences in certain genes [45,73].

Interestingly, similarly to PPAR and FXR, LXRs may stimulate transcription to some extent even in the absence of a ligand. In contrast, VDR/RXR and TR/RXR heterodimers do not exhibit any ligand-independent stimulatory activity. However, LXRs may also actively repress the expression of some genes in the unliganded state [45]. Therefore, a "three-step" model has been proposed to describe the activation of LXRs (Figure 1). According to this model, in the absence of agonist, the LXR/RXR heterodimer actively inhibits transcription by recruiting nuclear co-repressors. Ligand binding first induces dissociation of the co-repressors, leading to moderate stimulation of transcription, and then elicits recruitment of co-activator(s), thus causing a maximal stimulation of transcription. This model explains why the expression of some target genes is higher in LXR-deficient mice than in wild-type animals, although it is of course unresponsive to stimulation by LXR agonists (Figure 1). At least two studies [90,103] have demonstrated that the LXR/RXR heterodimer may also nonspecifically bind to the FXR response element (FXRE).

LXR ligands may also inhibit the transcription of certain genes through several mechanisms, including: 1) stimulation of an intermediate regulatory protein which inhibits transcription or destabilizes mRNA, 2) binding of LXRs to other transcription factors and inhibiting their activity, 3) competition between LXRs and other transcription factors for a common pool of RXR, 4) competition between LXR/RXR heterodimers and other transcription factors for the same coactivators, and 5) binding of the LXR/RXR heterodimer to the LXRE sequence overlapping with the response element for other nuclear receptors.

Recently, an interesting novel mechanism through which LXRs may regulate gene expression was described [157,158]. Namely, in renal juxtaglomerular apparatus, cyclic AMP increases renin gene expression through an LXRα-dependent mechanism by stimulating the binding of LXRα to a specific DNA sequence referred to as "cyclic AMP response element and a negative response element" (CNRE) within a promoter region of the renin gene. This effect is not mimicked by classic LXR or RXR agonists and, vice versa, cAMP does not induce LXR binding to LXRE. Subsequently, Anderson et al. [4] have shown that apart from renin, many other genes may also be regulated in this manner.

3. Oxysterols as the Natural LXR Ligands

3.1. Definition, classification, and origin of oxysterols

It is widely accepted that the major physiological LXR ligands are some monooxygenated derivatives of cholesterol referred to as oxysterols [70,92]. LXRs thus belong to a family of nonhormone nuclear receptors which, similarly to PPAR or FXR, are activated by metabolic intermediates normally found in the biological milieu at relatively high concentrations. Indeed, the EC_{50} value of LXRs for most oxysterols is within the low micromolar range, which is close to the normal levels of these compounds in plasma and tissues. The metabolic fate of oxysterols is faster

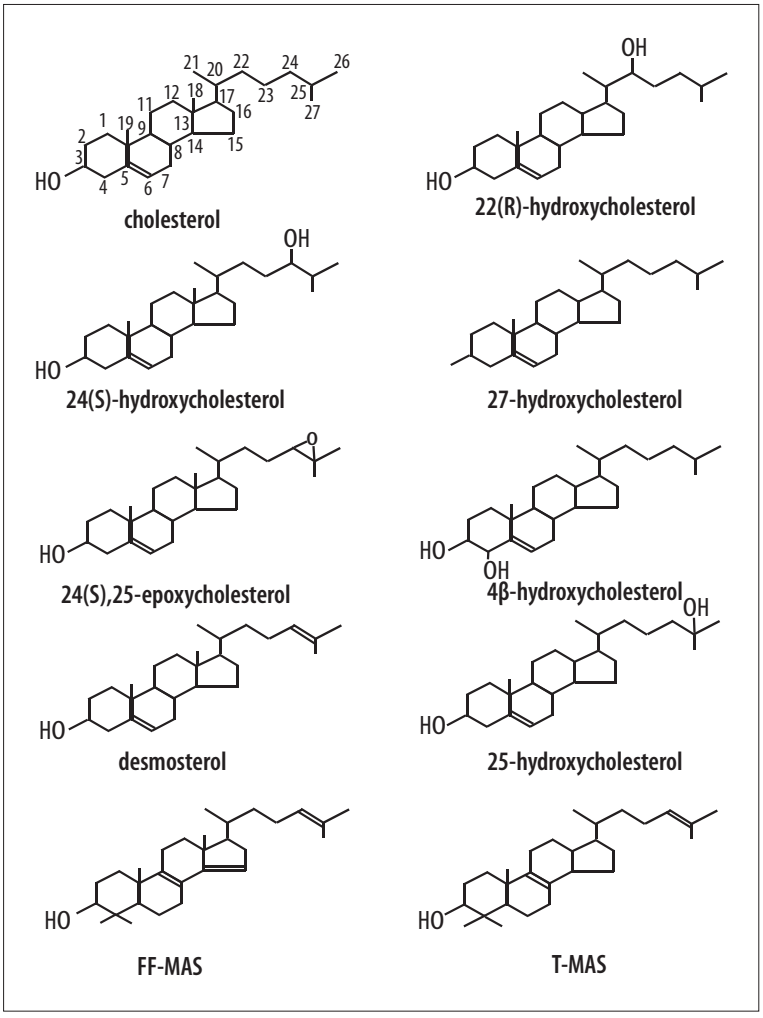

Figure 2. Endogenous sterol LXR agonists. FF-MAS – follicular fluid meiosis-activating sterol, T-MAS – testes meiosis-activating sterol

than that of cholesterol and, therefore, their concentration better reflects short-term changes in cholesterol balance, which makes oxysterols good candidates for regulatory molecules. Although cholesterol has no measurable affinity to LXRs, some authors question the regulatory role of oxysterols, arguing that they are always present together with a 10^5–10^6 excess of cholesterol [13]. It should be noted that oxysterols also have other effects unrelated to LXR stimulation. For example, certain oxysterols inhibit the processing of sterol regulatory element-binding proteins (SREBPs) and thus inhibit cholesterol biosynthesis more efficiently than cholesterol itself.

There are three sources of oxysterols in the animal body:
- Endogenous production in enzymatic reactions.
- Endogenous production through the non-enzymatic reactive oxygen species (ROS)-dependent oxidation of cholesterol.
- Delivery from alimentary sources.

In general, oxysterols produced in enzymatic reactions (Figure 2) are potent LXR agonists, whereas nonenzymatically generated ones have weak or no agonistic activity. Interestingly, natural oxysterol enantiomers have much higher affinity to LXRs than stereoisomers which are not endogenously generated [70].

Enzymatically generated oxysterols which are recognized by LXR ligands may be divided into three groups:
- Intermediates of the cholesterol biosynthetic pathway; until now 24(S),25-epoxycholesterol is the only representative of this group (Figures 2 and 3).
- Intermediary compounds in the synthesis of steroid hormones from cholesterol, i.e. 22(R)-hydroxycholesterol and, to a lesser extent, 20(S)-hydroxycholesterol (Figures 2 and 4).

- Several hydroxysterol compounds formed from cholesterol by "sterol hydroxylases", many of them being various isoforms of cytochrome P450 (CYP).

3.2. 24(S),25-epoxycholesterol (24(S),25-EC)

24(S),25-EC is a potent LXR agonist with an EC_{50} of about 300 nM [70]. It is synthesized from dioxidosqualene in the shunt pathway of the mevalonate cascade (Figure 3) and is detected in high amounts in the liver and, to a lesser extent, in many other tissues [15]. In contrast to many oxysterols mentioned below, 24(S),25-EC is unlikely to be increased, but is rather reduced by cholesterol overload, since cholesterol reduces the expression and/or activity of several enzymes localized proximally to 24(S),25-EC in the mevalonate cascade.

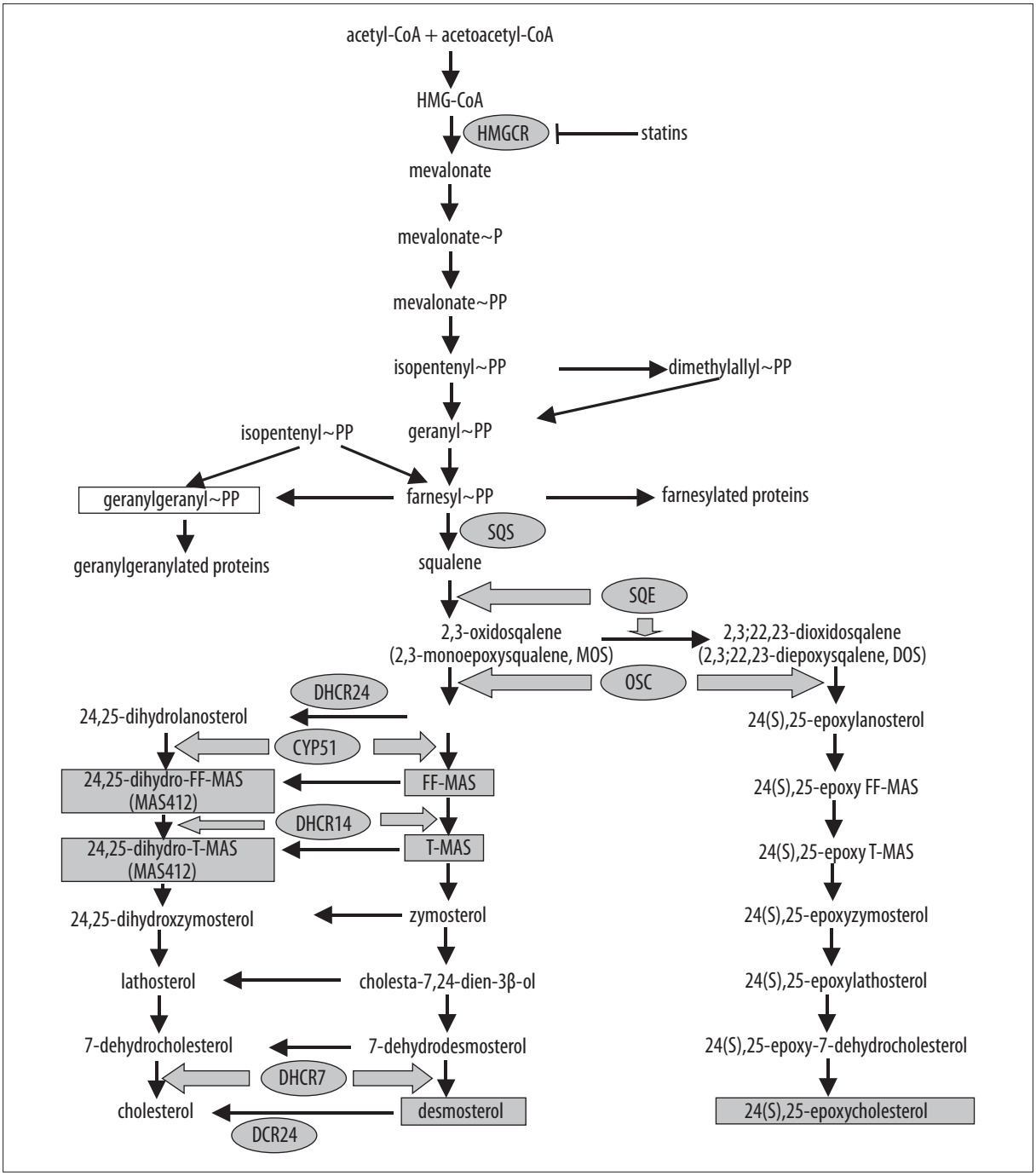

Figure 3. Cholesterol biosynthetic pathway. Recognized LXR agonists are presented on the gray background. Geranylgeranyl-pyrophosphate, an LXR antagonist, is presented in the white frame. It should be noted that many intermediates in the synthesis of 24(S),25-epoxycholesterol from 24(S),25-epoxylanosterol have not been tested in the context of LXR agonism so far. Only enzymes relevant to this review are presented. HMGCR – 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34), SQS – squalene synthase (EC 2.5.1.21), SQE – squalene epoxidase (EC 1.14.99.7), OSC – oxidosqualene: lanosterol cyclase (EC 5.4.99.7), DHCR24-3β-hydroxysterol Δ^{24}-reductase (desmosterol reductase, EC 1.3.1.72), CYP51 – lanosterol 14α-demethylase (sterol 14-demethylase, EC 1.14.13.70), DHCR14 – sterol 14α-reductase (EC 1.3.1.70), DHCR7–7-dehydrocholesterol reductase (EC 1.3.1.21)

3.3. Intermediates of steroidogenesis (Figure 4)

The conversion of cholesterol to pregnenolone is a first and rate-limiting step in steroidogenesis, i.e. the formation of steroid hormones from cholesterol. This reaction is catalyzed by CYP11A1, also referred to as "cytochrome P450 side-chain cleavage enzyme (P450scc)" or "cholesterol desmolase", an enzyme contained in the inner mitochondrial membrane of steroidogenic tissues, including the adrenal cortex, ovaries, testes, and placenta. CYP11A1 is also detected at low levels in the nervous system, heart, pancreas, and skin [127]. This enzyme catalyzes three consecu-

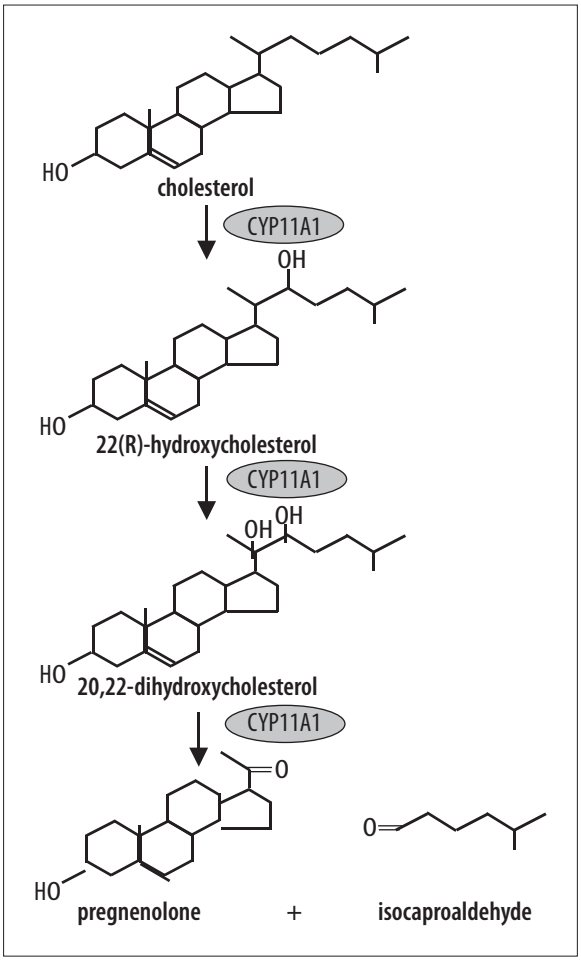

Figure 4. Initial steps of steroidogenesis

tive reactions leading to the conversion of cholesterol to pregnenolone with 22(R)-hydroxycholesterol (22(R)-HC) and 20,22-dihydroxycholesterol as intermediates (Figure 4), both of them being potent LXR activators. The monohydroxylated 20(S)-hydroxycholesterol is also an LXR agonist, but this compound is not endogenously generated. In contrast, pregnenolone as well as unnatural enantiomers, i.e. 22(S)-HC and 20(R)-HC, are inactive; in fact, 22(S)-HC has been reported to be an LXR antagonist [70].

3.4. Cholesterol hydroxylases and hydroxylated cholesterol derivatives

3.4.1. 24(S)-hydroxycholesterol

The family of hydroxylated cholesterol derivatives known to activate LXRs includes 24(S)-hydroxycholesterol (24(S)-HC), 25-hydroxycholesterol (25-HC), and 27-hydroxycholesterol (27-HC) (Figures 2 and 5). 24(S)-hydroxycholesterol, also referred to as "cerebrosterol", is the most abundant hydroxysterol in the brain [96]. It is synthesized in neurons, but not in glial cells, by a cytochrome P450 CYP46 isoform (cholesterol 24-hydroxylase) [102]. 24(S)-hydroxycholesterol plays an important role in brain cholesterol homeostasis. Because cholesterol does not cross the blood-brain barrier, it cannot be removed from this organ through the classical reverse-transport mechanism involving high-density lipoproteins (HDLs). In contrast, 24(S)-HC crosses the blood-brain barrier and thus the conversion of cholesterol to 24(S)-HC is a main route of cholesterol efflux from the brain [17]. 24(S)-HC is detected in the blood at a concentration proportional to the brain weight/body weight ratio and is then taken up by the liver and further metabolized. In the liver, 24(S)-HC is excreted into the bile as sulfated or glucuronidated conjugates or is first converted to 7α,24(S)-dihydroxycholesterol by microsomal 24-hydroxycholesterol 7α-hydroxylase (CYP39A1) or by cholesterol 7α-hydroxylase (CYP7A1) and then further oxidized to bile acids [95,120]. The plasma concentration of 24(S)-HC reflects the balance between cerebral production and hepatic metabolism [21]. Due to the high relative brain weight, plasma 24(S)-HC is highest during the first year of life and then declines by a factor of four during the first two decades [21]. 24(S)-HC may be a relevant LXR activator in the brain since its concentration in this organ is about 100 μM, in contrast to only about 0.15 μM in plasma. Interestingly, the distribution of CYP46A1 closely resembles that of LXR in the brain [17]. In addition, CYP46A1 is expressed only in neurons, but not in glial cells. It was proposed that 24(S)-HC derived from neurons stimulates the efflux of cholesterol from glial cells to extracellular lipid-free apolipoprotein E by stimulating ABCA1 in an LXR-dependent manner (see below). This extracellular cholesterol may then be taken up by neurons and converted to 24(S)-HC [12]. Thus, feed-forward cross-talk between neurons and glial cells may link these two pathways of cholesterol removal from brain cells. Knockout of the *CYP46A1* gene in mice results in a feedback suppression of cholesterol biosynthesis in the brain [83].

3.4.2. 27-hydroxycholesterol and cholestenoic acid

27-hydroxycholesterol (27-HC) is generated by the mitochondrial cholesterol 27-hydroxylase (CYP27) expressed in the liver and other cell types, including macrophages, fibroblasts, and endothelial cells. CYP27 is unique among other cytochrome P450 isoforms in that it catalyzes three consecutive oxidation reactions of a C-27 methyl group to alcohol (27-hydroxycholesterol), aldehyde, and carboxylic acid (3βâ-hydroxy-5-cholestenoic acid) [14] (Figure 6). However, the product dissociates from the enzyme after each step and has to compete with cholesterol for its active center to be further oxidized. Therefore, when the cholesterol concentration is high, little 27-HC is further oxidized to cholestenoic acid.

In the liver, CYP27 is a first enzyme of a so-called alternative or acidic pathway of bile-acid synthesis, which accounts for the formation of about 4% of the bile-acid pool synthesized in humans [98]. In other cells, 27-HC is not only the LXR ligand, but may also be involved in cholesterol efflux, since both 27-HC and cholestenoic acid are removed from the cells in an HDL-independent manner. Up to 50% of cholesterol may be removed as 27-HC or cholestenoic acid from cultured cells [14]. This mechanism may be especially important in cells which have no direct contact with plasma and are thus relatively isolated from HDLs. Thus the conversion of cholesterol to 27-HC in peripheral tissues plays a role analogous to its conversion to 24(S)-HC in the brain. The amount of 27-HC formed in the cell is proportional to the cholesterol content

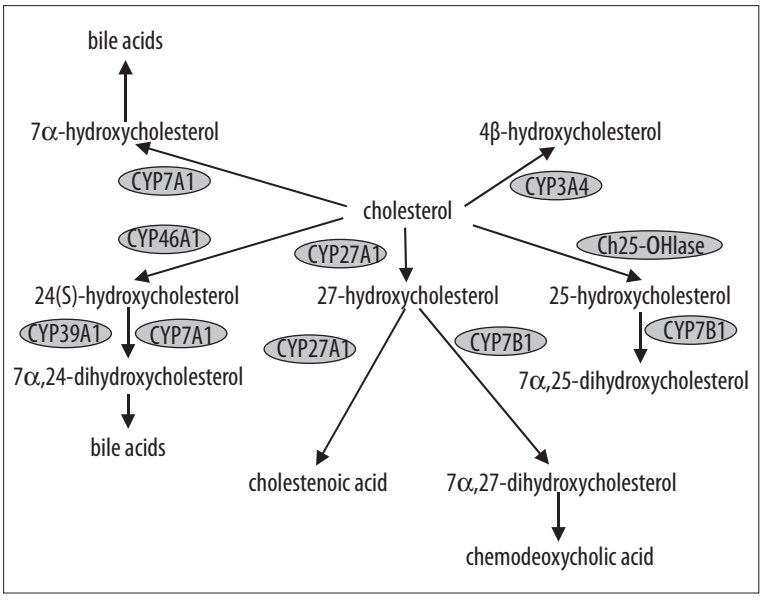

Figure 5. Synthesis and major metabolic pathways of cholesterol-derived oxysterols. CYP – cytochrome P450, Ch25-OHlase – cholesterol 25-hydroxylase not belonging to the CYP family

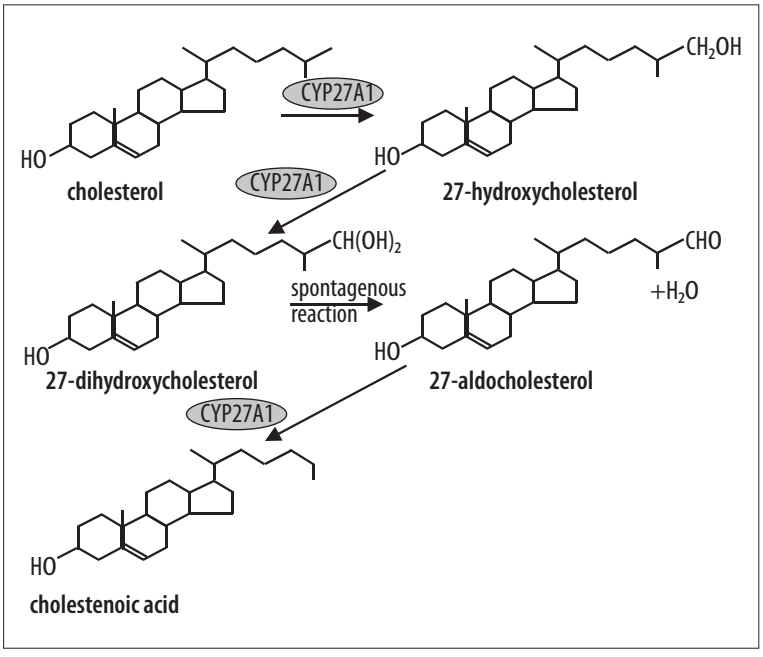

Figure 6. Synthesis of 27-hydroxycholesterol and cholestenoic acid by CYP27A1

and is markedly increased in fibroblasts and macrophages loaded with low-density lipoproteins [8,51]. Inhibition of CYP27 activity in cultured human macrophages induces a marked cholesterol accumulation [98]. These data confirm an important role of CYP27 in cholesterol efflux. Deficiency of CYP27 causes cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive disease characterized by dementia, ataxia, cataract, and multiple xanthomas in the brain and tendinous system. CTX is associated with premature atherosclerosis and increased cholesterol content in the vascular wall despite apparently normal plasma lipid profile [164].

Apart from being oxidized to cholestenoic acid, 27-hydroxycholesterol may be hydroxylated to 7α,27-dihydroxycholesterol by hepatic microsomal oxysterol 7α-hydroxylase (CYP7B1), distinct from cholesterol 7α-hydroxylase (CYP7A1). 7α,27-dihydroxycholesterol is further metabolized to chemodeoxycholic acid. Thus, 27-HC of peripheral origin may be a substrate for hepatic bile-acid production [10].

Cholestenoic acid is an even more potent LXR activator than 27-HC, with an EC_{50} of 200 nM, whereas its physiological concentration in plasma is 3–5 μM [151]. Most of the cholestenoic acid detected in plasma originates from the lung. Its concentration is reduced by 50% in patients after pulmonectomy and it also decreases in patients with obstructive or parenchymal lung diseases [9]. Most cells contain much more 27-HC than cholestenoic acid, whereas the amount of cholestenoic acid exceeds that of 27-HC in the culture medium because cholestenoic acid is more

efficiently removed from the cell due to its greater water solubility [10].

3.4.3. 25-hydroxycholesterol

25-hydroxycholesterol (25-HC) is a minor oxysterol formed by a specific microsomal enzyme not belonging to the CYP family, cholesterol 25-hydroxylase, which contains two non-heme iron atoms [99]. 25-HC is then taken up by the liver, hydroxylated to 7α,25-dihydroxycholesterol by CYP7B1, and further metabolized to bile acids (Figure 5).

3.4.4. 4β-hydroxycholesterol

4β-hydroxycholesterol (4β-HC) is formed in the liver and possibly in the small intestine by CYP3A4 and is one of the major circulating oxysterols [19]. Interestingly, both the formation and the metabolism of this compound are extremely slow and its half-life is close to 50 hours. Indeed, although 4β-HC is converted in the liver to bile acids, this occurs at a very slow rate since all CYP isoforms studied to date have very weak activity toward this compound [18]. The plasma concentration of 4β-HC is about 70 nM, i.e. almost fivefold more than the concentration of 4α-HC, which is a product of nonenzymatic cholesterol autooxidation [19].

3.4.5. 7α-hydroxycholesterol

Cholesterol 7α-hydroxylase (CYP7A1) is a rate-limiting enzyme in the classical pathway of bile-acid synthesis in the liver. Indeed, most plasma 7α-cholesterol originates from a hepatic CYP7A1-catalyzed reaction. However, 7α-hydroxycholesterol is a weak LXR agonist, whereas its derivatives, cholic and chemodeoxycholic acids, are completely inactive [69].

3.5. Nonenzymatic sources of oxysterols

Various oxysterols are generated in ROS-mediated reactions; however, most of them have only weak, if any, LXR agonistic activity. These oxysterols are normally detected in plasma and tissues at concentrations much lower than those of enzymatic products. However, their concentration increases in pathological conditions associated with oxidative stress, and they may exert many unfavorable effects, such as inhibition of endothelium-dependent vasorelaxation [81], protein ubiquitination [108], endoplasmic reticulum stress [125], and apoptosis [91]. It is suggested that oxysterols may contribute to the pathogenesis of oxidative stress-related diseases such as atherosclerosis, together with other lipid peroxidation products [16,22,148]. For example, certain oxysterols such as 7-keto-, 7α-hydroxy-, 7β-hydroxy-, 5,6-epoxycholesterol, and 3β,5α,6β-trihydroxycholestane are detected in oxidized low-density lipoproteins [32] and in human atherosclerotic lesions [28]. The plasma concentration of 7β-hydroxycholesterol correlates with the carotid artery intima-media thickness, a marker of subclinical atherosclerosis [140], and is reduced by antioxidant treatment [131].

Finally, oxysterols are present in certain foods, usually constituting up to 1% of dietary cholesterol. The major diet-derived oxysterols include 7-ketocholesterol, 7α- and 7β-hydroxycholesterol, 5α,6α-epoxycholesterol (α-EPOX), 5β,6β-epoxycholesterol (β-EPOX), and cholestan 3\β,5,6β-triol (αTRIOL). They originate most likely from the ROS-driven oxidation of cholesterol during food preparation and storage. Indeed, oxysterols are contained mainly in cholesterol-rich food, in particularly high amounts in food stored for prolonged periods of time or processed at a high temperature in the presence of oxygen, such as dried milk powder, cheese, etc. Alimentary oxysterols are absorbed in the small intestine and incorporated into chylomicrons. However, oxysterols of alimentary origin do not bind to LXRs with high affinity [69].

3.6. Oxysterols in plasma

The total concentration of oxysterols in the plasma of healthy humans is about 1 μM, which is about 0.02% of cholesterol. This level may increase to up to 20–30 μM (0.5–0.75% of total cholesterol) in various disease states. The most available oxysterol in the blood is 27-HC (0.4 μM), followed by 24(S)-HC (0.15 μM), 7α-HC (0.1 μM), and 4βâ-HC (70 nM) [22]. Less available are βEPOX, α-TRIOL, and 7-KC (about 50 nM), whereas the concentrations of α-EPOX, 7β-HC, and 25-HC are only about 5 nM. Thus, enzymatically generated oxysterols (with the exception of 25-HC) circulate at higher amounts than nonenzymatically formed ones. The concentrations of nonenzymatically formed oxysterols may even be overestimated due to the rapid *ex vivo* oxidation of cholesterol during sample processing. Although oxysterols are slightly more hydrophilic than cholesterol itself, they circulate in plasma lipoproteins, and their distribution between lipoprotein fractions closely resembles that of cholesterol. The exception is cholestenoic acid, which is mostly detected in the water phase.

3.7. Oxysterols as LXR agonists

The precise order of potency of various oxysterols as LXR activators differs in various studies. However, it seems that the hydroxyl group in the side chain of the cholesterol molecule as well as cholesterol's 3βâ-hydroxyl group are essential for LXR activation. Sterols with a 3α hydroxyl group are inactive. In most studies, 22(R)-HC, 20(S)-HC, and 24(S)-HC are the most potent agonists, whereas 25-HC and 27-HC have relatively weak activity [69,70]. Triple-knockout mice lacking CYP46, CYP27, and cholesterol 25-hydroxylase exhibit an impaired response of hepatic LXR target genes to dietary cholesterol load, which confirms an essential role of endogenous oxysterols in LXR signaling [37].

Most oxysterols have similar affinity toward LXRα and LXRβ. In contrast, oxidation of the B ring of the cholesterol moiety to form, for example, 5,6-24(S),25-diepoxycholesterol leads to the generation of more specific LXRα agonists [69]. In addition, 6α-hydroxy bile acids have higher affinity to LXRα than to LXRβ [150]. Two 6α-hydroxy bile acids, hyodeoxycholic acid (HDCA) and hyocholic acid (HCA), are synthesized by CYP3A4 from litocholic and chemodeoxycholic acids, respectively, and are detected in the liver at a concentration of about 30 μM. HDCA and HCA bind to LXRα with EC_{50} of 55 μM and 17 μM, respectively, which are close to or within the physiological range. Thus, HDCA and HCA are physiological LXRα-selective agonists, at least in the liver.

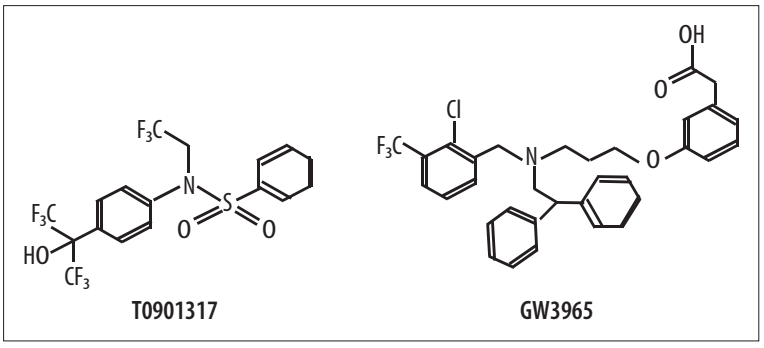

Figure 7. Most commonly used synthetic LXR agonists

4. Non-Oxysterol LXR Ligands

4.1. Intermediates of cholesterol biosynthesis

Recently, Yang et al. [176] demonstrated that desmosterol (24-dehydrocholesterol), one of the intermediates in cholesterol biosynthesis, is a potent activator of LXRs (EC_{50}=1 μM). In contrast, several other intermediates, such as lathosterol, lanosterol, and 7-dehydrocholesterol, are inactive (Figure 3). Inhibition of desmosterol reductase by triparamol leads to the up-regulation of LXR target genes due to the accumulation of endogenous desmosterol [176].

Janowski et al. [70] demonstrated that LXRα is activated by 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol, also referred to as follicular fluid meiosis-activating sterol (FF-MAS). FF-MAS was first isolated from preovulatory follicular fluid and stimulates meiosis of mammalian oocytes [25]. FF-MAS is an intermediate in cholesterol biosynthesis, which is produced from lanosterol by lanosterol 14α-demethylase (CYP51), and in most cells is rapidly converted to 4,4-dimethyl-5α-cholesta-8,24-dien-3β-ol (T-MAS) by sterol 14α-reductase (Figure 3). T-MAS (testis meiosis-activating sterol) was isolated from bull testis. In most tissues, including hepatocytes, where the cholesterol synthesis pathway is highly active, the levels of FF-MAS and T-MAS are below the detection limit since the latter is immediately converted to zymosterol. Due to low expression of the respective downstream enzymes, FF-MAS and T-MAS accumulate at high concentrations in the ovaries and testes, respectively (Figure 3). The affinity of FF-MAS and T-MAS to LXR is relatively low and these compounds most likely also possess LXR-independent activities. In addition, LXR-activating oxysterols have no effect on meiosis. Nevertheless, abnormalities of gonadal function in mice lacking LXRs suggest that FF-MAS/T-MAS-LXR signaling may be important for gonadal function.

4.2. Glucose

Mitro et al. [112] recently demonstrated that glucose and glucose-6-phosphate bind to and activate LXRα and LXRβ with EC50 of 300 μM and 3 μM, respectively. In contrast, other simple carbohydrates have no effect. In addition, feeding mice with glucose results in the up-regulation of LXR target genes in the liver and intestine. These data suggest that glucose is a physiological ligand of LXR, which thus may function not only as cholesterol, but also as a glucose sensor.

4.3. Synthetic and natural exogenous LXR ligands

Two nonsteroid synthetic LXR agonists, T0901317 and GW3965 (Figure 7), are commonly used in experimental studies. T0901317 activates both LXRα and LXRβ with an EC_{50} of about 20 nM [142]. GW3965 has a greater affinity toward LXRβ (EC_{50}=30 nM) than LXRα (EC_{50}=190 nM); however, the difference is too small to be useful in differentiating the two isoforms. T0901317 is not a completely selective LXR agonist since it activates also the pregnane X receptor (PXR) [145] and farnesoid X receptor [63]. The affinity of T0901317 for PXR and FXR is, however, much lower than for LXR (EC_{50}=4–7 μM).

Other synthetic LXR agonists are used occasionally. Acetylpodocarpic dimer (APD) is a derivative of podocarpic acid, which was isolated from the resin of a plant, *Podocarpus* [147]. APD activates LXRα and LXRβ in the low nanomolar range [153]. An indole alkaloid, paxilline, produced by a fungus, *Penicillium paxilli*, is the first natural nonoxysterol LXR agonist. Its affinity for LXR is similar to that of 22(R)-HC. However, paxilline is unsuitable for *in vivo* studies due to its toxicity. In addition, it is not LXR-specific since it is also an antagonist of the large-conductance Ca^{2+}-activated potassium channel [20]. Riccardin C is a natural nonsteroid compound isolated from liverworts which is an LXRα agonist and LXRβ antagonist [156]. The search for compounds with specific agonistic and antagonistic activities toward both LXR isoforms is essential for future research in this field.

Interestingly, the hepatitis C virus core protein facilitates LXR/RXR binding to the LXRE. Due to the potent lipogenic effect of LXR, it is suggested that this mechanism may contribute to the liver steatosis frequently observed in patients chronically infected with HCV [115].

Plant cells do not synthesize cholesterol but several other sterols with similar chemical structure, such as sitosterol, stigmasterol, campesterol, brasicasterol, and ergosterol. Plant sterols are poorly absorbed from the intestine and inhibit cholesterol absorption by displacing it from bile micelles. Supplementation of plant sterols may reduce plasma cholesterol level and is considered a potential antiatherosclerotic therapy [124]. It has been demonstrated that stigmasterol, but not sitosterol, is a potent LXR ligand due to the double bond between C22 and C23 in the side chain of its sterol moiety [177]. In contrast, Plat et al. [128] observed that both sitosterol and campesterol effectively stimulated LXR in cultured intestinal Caco-2 cells. Kaneko

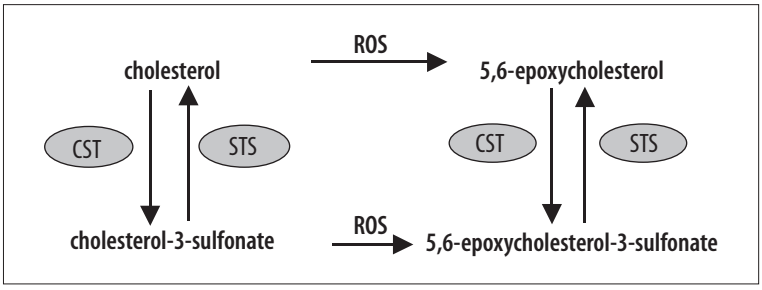

Figure 8. Metabolic pathways of cholesterol-3-sulfonate and 5,6-epoxycholesterol-3-sulfonate. CST – cholesterol sulfotransferase, STS – sterol sulfatase, ROS – reactive oxygen species

et al. [75] have shown that certain oxidized derivatives of phytosterols ("oxyphytosterols") are more effective LXR activators than 24(S),25-epoxycholesterol. Thus at least some exogenous phytosterols or their derivatives may be physiologically important LXR ligands.

4.4. Are all LXR agonists equivalent?

In most experimental systems, natural LXR ligands are less potent then synthetic agonists, which is usually interpreted as the result of the rapid metabolism of oxysterols to the inactive compounds. However, Albers et al. [2] recently demonstrated that 22(R)-HC is a partial agonist, i.e. it increases the affinity of LXR to both transcription co-activators and co-repressors, whereas T0901317 is a full agonist, i.e. it induces dissociation of co-repressors and binding of co-activators. The final effect of a given agonist depends on the relative availability of certain co-activators and co-repressors in a given cell and may thus be tissue and even gene specific [2].

4.5. LXR antagonists

Geranylgeranylpyrophosphate (GGPP), one of the products of the mevalonate cascade, inhibits the transcriptional activity of LXRα and LXRβ by antagonizing their interaction with nuclear co-activators [50,53]. In contrast, geranylpyrophosphate, farnesylpyrophosphate, and mevalonate have no effect on LXR. Polyunsaturated fatty acids (PUFAs) of the n-3 and n-6 family, such as arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and linoleic acid, are competitive antagonists of the interaction between LXRs and their ligands [122,180]. In contrast, saturated fatty acids possess no, and monounsaturated fatty acids have only minimal, antagonistic activity. There is no difference between the antagonistic potencies of n-3 and n-6 PUFAs, and the degree of unsaturation rather than spatial distribution of the double bonds seems to be the most important factor [179].

Song et al. [149] demonstrated that oxidized cholesterol sulfonate, 5α,6α-epoxycholesterol-3-sulfonate, normally detected in human plasma, inhibits the transcriptional activity of LXR. Interestingly, its enantiomer, 5β,6β-epoxycholesterol-3-sulfonate, is not an LXR antagonist. 7-ketocholesterol-3-sulfonate inhibits LXR with a potency similar to 5α,6α-epoxycholesterol-3-sulfonate. 5α,6α-epoxycholesterol-3-sulfonate is most likely a product of the autooxidation of cholesterol-3-sulfonate, the most abundant sulfonated sterol in plasma and tissues, or it originates by the sulfonation of 5α,6α-epoxycholesterol by cholesterol sulfotransferase. Overexpression of cholesterol sulfotransferase inactivates LXR signaling and reduces the expression of LXR target genes both *in vitro* and *in vivo* [38]. These data suggest that the relative abundance of oxysterols and their 3-sulfonates, determined by the activities of cholesterol sulfotransferase and sulfatase, may be important in the regulation of LXR signaling (Figure 8). Currently it is not clear if sulfonated derivatives of enzymatically formed oxysterols are present in tissues and if they are also LXR antagonists.

5. Regulation of LXR Expression and Activity

5.1. Regulation of LXR expression

Apart from agonists and antagonists, LXR signaling may be modulated by changes in receptor expression (Table 1). Several studies have shown that stimulation of the peroxisome proliferator activated receptors α and γ (PPARα and PPARγ) increases LXR expression. Tobin et al. [159] have shown that the PPARα agonists Wy14643 and gemfibrozil as well as the PPARγ agonist rosiglitazone increase LXRα mRNA and protein levels in the rat liver. *In vitro*, PPARα agonists increase LXRα expression in isolated rat hepatocytes through the peroxisome proliferator response element (PPRE) contained in the 5'-flanking region of the LXRα gene [159]. The human LXRα gene also contains PPRE [88], and four-month administration of fenofibrate resulted in the up-regulation of LXRα in the peripheral blood monocytes of patients with type 2 diabetes [49]. Because fatty acids are endogenous PPARα agonists, they may also increase LXRα expression. Indeed, high-fat diet or fasting, both associated with elevation of the plasma fatty acid level, up-regulated hepatic LXRα in the rat [159]. Various saturated and unsaturated fatty acids increased LXRα expression also in cultured rat hepatoma cells *in vitro* [159]. Thus, whereas in the short run unsaturated fatty acids are LXR antagonists, long-term elevation of their level results in the up-regulation of LXRα gene expression.

Chineti et al. [40] demonstrated that PPARα and PPARγ agonists increase the expression of LXRα, but not of LXRβ, in primary human and murine macrophages. In contrast, Chawla et al. [33] observed that only PPARγ, but not PPARα, agonists increase LXRα gene expression in cultured human THP-1 macrophages. The PPARγ agonist rosiglitazone increased the level of LXRα mRNA in cultured 3T3-L1 adipocytes, but not in skeletal muscle cells *in vitro*, whereas another PPARγ ligand, darglitazone, stimulated LXRα expression in rat epididymal adipose tissue *in vivo* [74]. Two PPARγ agonists, troglitazone and rosiglitazone, administered orally, increased LXRα expression

Table 1. Regulation of LXR signaling

Natural ligands	
Agonists	**Antagonists**
22(R)-HC 24(S),25-EC 24(S)-HC 25-HC 27-HC FF-MAS/T-MAS HCA, HDCA* Glucose	GGPP PUFAs Oxysterol 3-sulfonates
LXR expression	
Increase	**Decrease**
PPARα agonists PPARγ agonists Insulin IL-10 LXR agonists** Lipid peroxidation products (by stimulating PPARγ)	17β-estradiol Lipopolysaccharide Proinflammatory cytokines (IL-1β, TNF-α) Oxidative stress
Posttranslational modulation of LXR activity	
Increase	**Decrease**
cAMP (stimulates binding to CNRE)	Phosphorylation by protein kinases • cAMP-protein kinase A (inhibits binding to LXRE) • ALK-1*** Other nuclear receptors which compete with LXR for a common pool of RXR (PPARα, PPARγ, VDR, TRβ, FXR) Non-functional complex with PPARα
Modulation of LXRE-containing genes by other nuclear receptors	
Increase	**Decrease**
Binding of TRβ1 to LXRE in the presence of T_3	Binding of TRβ1 to LXRE in the absence of T_3

* Hyocholic acid (HCA) and hyodeoxycholic acid (HDCA) are specific agonists of LXRα; ** only LXRα expression is upregulated by LXR agonists; ***ALK-1 phosphorylates only LXRβ

in rabbit renal glomeruli [172]. Pioglitazone administered for three weeks at a dose of 30 mg/day increased LXRα expression in subcutaneous adipose tissue in non-diabetic insulin-resistant non-obese subjects [59].

Insulin increases LXRα expression in the liver both *in vitro* and *in vivo* and it is suggested that the stimulatory effect of insulin on hepatic lipogenesis may be partially mediated via LXRs [160]. 17β-estradiol reduces LXRα expression in cultured human THP-1 macrophages *in vitro* [84]. In addition, a single dose of estradiol decreased LXRα (but not LXRβ) mRNA level in mice liver and white adipose tissue [100].

Several studies have demonstrated that the inflammatory reaction induced by bacterial lipopolysaccharide (LPS) reduces the expression of LXRα and its heterodimeric partner, RXRα, in the liver, kidney, and adipose tissue both *in vitro* and *in vivo* [11,47,79,80,97,169]. This effect is reproduced by proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). In contrast, interleukin-10, an anti-inflammatory and atheroprotective cytokine, stimulates LXRα in cultured THP-1 macrophages [107].

In addition, LXRα expression is controlled by an auto-regulatory mechanism. The human LXRα gene promoter contains three functional LXREs, one of which is strongly activated by both LXRα and LXRβ [93]. Both natural and synthetic LXR agonists increase LXRα, but not LXRβ, gene expression in human macrophages, adipocytes, and hepatocytes [170]. This autoregulatory mechanism may be responsible for the strong up-regulation of LXRα induced by lipid loading of macrophages. Resting macrophages contain mainly LXRβ, whereas in lipid-loaded cells the expression of LXRα markedly increases while the level of LXRβ remains stable, presumably due to the up-regulation of LXRα gene expression by cholesterol-derived LXR agonists [88]. Initially it was suggested that the autoregulation of LXRα is specific to humans. However,

Ulven et al. [161] observed that T0901317 increased the amount of LXRα mRNA in mouse white adipose tissue. Nevertheless, murine LXRα is not autoregulated in other cell types, such as macrophages and hepatocytes.

5.2. Posttranslational regulation of LXR activity

Chen et al. [37] recently demonstrated that LXRα is phosphorylated at Ser198 by mitogen-activated protein kinases (MAPK). The functional significance of this phosphorylation is unclear since the phopshorylated and non-phosphorylated receptors have identical transcriptional activities. However, it should be mentioned that the MAPK consensus phosphorylation site within the LXRα molecule is conserved among species and is also contained in the LXRβ, which suggests that phosphorylation may have some functional implications.

Cyclic AMP stimulates the binding of LXRα to CNRE (see above); however, the mechanism of this effect is unclear. In particular, it is unknown if cAMP affects the conformation of LXRα (directly or by stimulating its phosphorylation by protein kinase A) or acts on CNRE, increasing its affinity for LXRα. On the other hand, some data suggest that cAMP synthesized in response to dopamine D1-like receptor stimulation inhibits classical LXR/RXR-LXRE-mediated signaling in neurons [141]. Recently it was demonstrated that protein kinase A phosphorylates LXRα at two sites, Ser^{195}–Ser^{196} and Thr^{200}–Ser^{201}, which impairs its dimerization with RXR, DNA-binding, and transcriptional activity [175].

Other nuclear receptors which form heterodimers with RXR can inhibit LXR signaling by competing with LXRs for the limited pool of their common dimeric partner. For example, vitamin D receptor (VDR) antagonizes the transcriptional activity of LXRα when stimulated with its ligand, 1,25-dihydroxycholecalciferol [71]. Recent studies indicate that apart from 1,25-dihydroxycholecalciferol, vitamin D receptor may be activated by litocholic acid [105]. Since LXR stimulates the formation of bile acids, inhibiting its activity through the litocholic acid-VDR pathway may be a negative feedback mechanism which prevents the overproduction of potentially toxic bile acids. Triiodothyronine receptor, TRβ, and farnesoid X receptor (FXR) also suppress LXR-mediated signaling by quenching RXR [178].

Activin receptor-like kinase (ALK-1) is a plasma membrane-bound serine/threonine protein kinase belonging to a transforming growth factor-β (TGF-β) receptor family, which is specifically expressed in endothelial cells. Activated ALK-1 phosphorylates LXRβ, but not LXRα, and induces its translocation from the nucleus to the cytosol. In addition, LXRβ inhibits ALK-1-dependent signaling. These data suggest that significant cross-talk between LXRβ and the TGF-β receptor superfamily exists in vascular tissue [114].

The major thyroid hormone receptor expressed in the liver, TRβ1, may bind not only to its cognate sequence, thyroid hormone response element (TRE), but also to LXRE within the promoter region of at least two LXR target genes, sterol regulatory element binding protein-1c (SREBP-1c) [76] and cholesterol 7α-hydroxylase (CYP7A1) [60]. In the absence of triiodothyronine (T3), TRβ1/RXR heterodimer binds to LXRE and preferentially associates with co-repressors, thus suppressing LXR-stimulated transcription. In the presence of T_3, TRβ1 stimulates the transcription of *CYP7A1* gene. However, the concentration of T_3 required to stimulate the LXR target gene by TRβ1 is higher than that required to stimulate transcription through the TRβ1-TRE-dependent mechanism [60,76]. Neither unliganded nor oxysterol-bound LXRα has any effect on T3-induced gene expression. Moreover, LXR is unable to cross-bind with the TRE [76].

5.3. LXR and PPAR

The interaction between LXR and PPAR is probably cell specific and, in general, very complex. First, as mentioned above, stimulation of PPARα and PPARγ may increase LXR gene expression in certain cell types. On the other hand, expression of PPAR may be induced by LXR through the LXRE contained in its promoter region. For example, Seo et al. [143] demonstrated that T0901317 increased PPARγ expression in cultured 3T3-L1 adipocytes *in vitro* as well as in adipose tissue and liver of T0901317-treated mice.

At the posttranslational level, PPAR may inhibit the LXR signaling and vice versa by competing for the limited pool of their common heterodimeric partner, RXR [110]. Transfection of human hepatoma HepG2 cells or human embryonic kidney HEK293 cells with PPARα or PPARγ inhibits LXR-mediated signaling even in the absence of PPAR ligands [178]. Stimulation of PPARα or PPARγ with Wy14643 or pioglitazone, respectively, enhanced this inhibitory effect, whereas overexpression of RXR abolished this inhibition. On the other hand, stimulation of LXR by T0901317 or 22(R)-HC inhibits PPARα-induced fatty acid oxidation through a similar mechanism [66]. In addition to competing with PPARα for a common pool of RXR, LXRα may inhibit PPARα signaling by forming a nonfunctional LXRα/PPARα heterodimer which is unable to bind to either LXRE or PPRE [113]. A unique mechanism of PPARα-LXRα interaction was described in the regulation of the murine cholesterol 7α-hydroxylase gene (*CYP7A1*). The *CYP7A1* promoter contains overlapping PPRE and LXRE sequences. Therefore, whereas stimulation of either of these receptors alone enhances gene expression, PPARα agonists inhibit LXR-induced transcription and vice versa by forming the LXRα-PPARα heterodimer which binds to this overlapping sequence and blocks the binding of either LXRα/RXR or PPARα/RXR heterodimer [54].

Despite these *in vitro* studies, the antagonism between PPAR and LXR *in vivo* remains elusive. Anderson et al. [5] demonstrated that treatment of mice with Wy14643 or T0901317 changed the expressions of 49 genes in the liver, but 48 of them were regulated in the same direction. In addition, of 64 genes downregulated in PPARα-null mice (i.e. constitutively stimulated by PPARα in wild-type animals), only one was downregulated by T0901317. These data question the functional antagonism between these receptors, at least in the liver, and suggest that PPARα and LXR may in fact have many overlapping activities.

5.4. Effect of oxidative stress on LXR signaling

Oxidatively modified plasma low-density lipoproteins (oxLDL) have a complex cell-specific effect on LXR si-

gnaling. For example, loading cultured macrophages with oxLDL stimulates LXR signaling because oxidized fatty acids activate PPARγ and increase LXRα expression [146]. However, in cultured human umbilical vein endothelial cells, oxLDL diminishes 27-HC production by reducing CYP27 activity and thus inhibits LXR signaling. In contrast, loading endothelial cells with native (non-oxidatively modified) LDL stimulates 27-HC formation and LXR signaling by providing more cholesterol substrate [186]. In addition, enhanced lipid peroxidation induced by iron and ascorbate reduced LXRα and LXRβ gene expression in cultured human THP-1 macrophages [107]. The main mechanisms regulating LXR expression and activity are summarized in Table 1.

6. Role of LXRs in Cholesterol Metabolism

LXRs sense excess cholesterol and trigger various adaptive mechanisms protecting the cells from cholesterol overload. Activation of LXRs results in: (1) stimulation of cholesterol removal from the cell, transport to the liver, and biliary excretion, processes referred to together as reverse cholesterol transport (RCT), (2) inhibition of intestinal cholesterol absorption, and (3) inhibition of cholesterol synthesis and uptake by the cells. The effects of LXRs on reverse cholesterol transport and excretion as well as on intestinal cholesterol absorption are presumably the major mechanisms, whereas those on cholesterol uptake and synthesis are weak and play only a minor role.

6.1. Reverse cholesterol transport

Reverse cholesterol transport (RCT) is cholesterol trafficking from peripheral tissues to the liver, where it is excreted in the bile as unchanged cholesterol or after conversion to bile acids. RCT is initiated by cholesterol removal from the cell to high-density lipoproteins (HDLs) or to lipid-free apolipoproteins such as apoA-I or apoE. Within HDLs, most of the free cholesterol is esterified by lecithin: cholesterol acyltransferase (LCAT), and cholesterol esters are then transported to the liver either in HDLs or in apoB-containing lipoproteins (VLDLs and LDLs), to which they are first transferred by cholesterol ester transporting protein (CETP).

Recently, Naik et al. [117] demonstrated that if mice are injected with macrophages previously loaded with titrated cholesterol and then are treated with GW3965 for 10 days, fecal excretion of cholesterol tracer is markedly higher and excretion of titrated bile acids tends to be higher than in vehicle-treated animals. These data clearly indicate that this LXR agonist stimulates cholesterol transport to the liver for subsequent excretion and/or metabolism *in vivo*.

Most, if not all, steps of RCT are stimulated by LXRs. First, LXRs up-regulate the expression of transporters involved in cholesterol removal from plasma membrane to extracellular acceptors, the ATP-binding cassette transporters A1 (ABCA1) and G1 (ABCG1). ABCA1 and ABCG1 are abundant in macrophages where they prevent foam cell formation by removing excess cholesterol, but they are also expressed in most other cell types, including hepatocytes, enterocytes, adipocytes, and skeletal muscle cells. ABCA1 is a full transporter, i.e. it operates as a single molecule and transfers both cholesterol and phospholipids from plasma membranes to small discoid-shape pre-HDL or to lipid-free apoA-I. Thus, apart from RCT, ABCA1 is also involved in the formation of nascent HDL particles in the liver and small intestine. Deficiency of ABCA1 is responsible for Tangier disease, a rare inherited disorder associated with a very low level of HDLs, overaccumulation of cholesterol in peripheral tissues, and premature atherosclerosis. In contrast, ABCG1 is a half-transporter, i.e. it operates as a homodimer and transfers cholesterol to HDLs but not to lipid-free apoA-I [29,86].

Multiple studies have demonstrated that LXR agonists increase the expression of ABCA1 and ABCG1 by binding to the LXREs within their genes, which is accompanied by enhanced cholesterol efflux from various cell types both *in vitro* and *in vivo* [42,78,153,162]. In particular, the expression of ABCG1 may be increased up to 1000-fold by natural and synthetic LXR agonists [139,162]. LXR agonists not only stimulate the expression of ABCG1, but also induce its redistribution from intracellular stores to the plasma membrane, further facilitating cholesterol efflux [167]. In peritoneal macrophages obtained from ABCG1-null mice, T0901317 normally stimulates cholesterol efflux to apoA-I, but fails to stimulate cholesterol efflux to HDLs [77]. In addition, ABCG1-specific small interfering RNA (siRNA) blocked HDL-mediated cholesterol efflux [167]. These data suggest that ABCG1 has a critical role in cholesterol efflux to HDLs, but not in the formation of these lipoproteins. Indeed, the plasma lipid profile is normal in ABCG1-null mice, but large amounts of cholesterol accumulate in macrophages/foam cells in various tissues of these animals [77]. In contrast, in ABCA1-null macrophages, T0901317 failed to stimulate cholesterol transport to apoA-1, but normally stimulated its efflux to HDLs.

Cholesterol loading of the cell results in the increased formation of oxysterols, which stimulate LXRs and enhance cholesterol efflux through these transporters, thus providing the regulatory feedback mechanism maintaining a constant cholesterol content [94]. Engel et al. [46] demonstrated that 22(R)-HC and 9cRA increase the expression of ABCG4, a half-transporter homologous to ABCG1, in human monocyte-derived macrophages. The precise role of ABCG4 is not clear, but it is suggested that it forms a heterodimer with ABCG1. However, ABCG4 is not essential for LXR-stimulated cholesterol efflux since cholesterol export from ABCG4$^{-/-}$ macrophages is normal [167]. Recently, Wang et al. [166] reported that various ABC transporters may be involved in the efflux of cholesterol from different intracellular pools. In particular, cholesterol delivered by intact LDL through the LDL receptor-dependent pathway is subsequently removed by the ABCG1/ABCG4 heterodimer, whereas cholesterol derived from modified LDL, which entered the cell through the scavenger receptor, is exported by ABCA1. In contrast to ABCA1, ABCG1, and ABCG4, the expression of ABCG2 is not regulated by LXRs. Thus, ABCG2 seems to be a constitutive cholesterol exporter [46].

LXRs are also involved in the regulation of intracellular cholesterol traffic. Before efflux, cholesterol has to be transported from the endosomal compartment to the plasma membrane. This process is mediated by two carriers,

Niemann-Pick C1 (NPC1) and C2 (NPC2) proteins. LXR agonists increase the expression of NPC1 and NPC2 and thus stimulate the redistribution of cholesterol from the endosomal compartment to the plasma membrane, where it becomes available for efflux to extracellular acceptors. Indeed, the stimulation of cholesterol efflux by T0901317 or GW3965 was markedly suppressed by siRNA specific to either NPC1 or NPC2 [138]. Although LXR agonists have no effect on the expression of acyl-CoA: cholesterol acyltransferase-1 (ACAT-1), a major intracellular cholesterol-esterifying enzyme, they reduce the rate of cholesterol esterification by removing cholesterol from the intracellular pool available for ACAT-1 to the plasma membrane.

Scavenger receptor type B1 (SR-B1) is a specific HDL receptor expressed in the liver and, to a lesser extent, in other tissues. Hepatic SR-B1 is essential for the delivery of cholesterol esters from HDLs to hepatocytes. In human hepatoma HepG2 cells, 22(R)-HC increased SR-B1 mRNA and protein levels [106]. This effect resulted from the binding of the LXR/RXR dimer to the LXRE within the promoter region of *SR-B1* gene. Until now, no study has addressed the regulation of hepatic SR-B1 by LXR *in vivo*. Yu et al. [181] demonstrated that 25-HC reduces SR-B1 expression in human and murine macrophages and in human umbilical vein endothelial cells. Although this effect may be beneficial by decreasing the uptake of oxidized LDLs, it is preserved in cells lacking both LXR isoforms and thus is clearly LXR-independent. In contrast, neither T0901317 nor 24(S)-HC had any effect on SR-B1 expression in porcine brain capillary endothelial cells [123].

24(S)-hydroxycholesterol, an endogenous LXR agonist synthesized in the brain, stimulates cholesterol efflux from choroid plexus epithelial cells to extracellular apoE by increasing the expression of ABCA1, ABCG1, ABCG4, and ABCG5 [52]. Since this effect was observed only in the apical membrane directed toward the cerebrospinal fluid, which lacks HDLs, it may represent a unique HDL- and apoA-I-independent mechanism of cholesterol removal. In addition, 24(S)-HC and GW3965 increase the expression of ABCA1, ABCG1, and apoE in astrocytes, but not in neurons. These data suggest that 24(S)-HC of neuronal origin may stimulate apoE-mediated cholesterol efflux from the neighboring glial cells [1]. Although apoE-mediated cholesterol efflux is not involved in the net export of cholesterol from the brain, it may be important for the redistribution of cholesterol between various brain regions. This process is essential during development and neuronal injury, because cholesterol is required for neurite outgrowth and synaptogenesis.

6.2. Cholesterol metabolism to bile acids

The first and rate-limiting enzyme of hepatic bile acid synthesis is cholesterol 7α-hydroxylase (CYP7A1). Promoter regions of mouse and rat *CYP7A1* genes contain LXRE, and their transcription is potently up-regulated by natural and synthetic LXR agonists [92,111]. LXRE of *CYP7A1* gene binds LXRα more potently than LXRβ, and therefore LXRα$^{-/-}$ mice, but not LXRβ$^{-/-}$ animals, accumulate large amounts of cholesterol in the liver in response to a high-cholesterol diet. This is accompanied by hepatomegaly and severe liver damage. LXRα$^{-/-}$ mice do not exhibit any increase in CYP7A1 expression when switched to a cholesterol-rich diet nor are they able to increase bile acid synthesis and fecal excretion [126].

In contrast to rat and mice, human *CYP7A1* gene does not contain the LXRE and its transcription is not up-regulated by LXR agonists [39,55]. This species difference is responsible, at least in part, for much greater susceptibility of humans to dietary-induced hypercholesterolemia [111]. Indeed, cholesterol 7α-hydroxylase is not up-regulated in response to cholesterol feeding in mice expressing human CYP7A1, and these animals easily develop hypercholesterolemia when challenged with a high-cholesterol diet [35].

CYP7A1 is also a target for FXR, which inhibits its expression by stimulating the synthesis of a small heterodimeric partner (SHP) protein. FXR is a specific receptor for bile acids and regulates their formation in a negative feedback manner. FXR inhibits CYP7A1 expression in all known mammalian species. Goodwin et al. [55] demonstrated that the LXR/RXR heterodimer directly activates SHP expression in human hepatocytes, leading to the suppression of CYP7A1 synthesis in response to cholesterol loading. It is suggested that this mechanism evolved in humans to suppress cholesterol absorption from the intestine (by limiting the formation of bile acids and the emulsification of alimentary lipids). However, this mechanism becomes inefficient when humans are exposed to a high-cholesterol diet, which ultimately results in cholesterol overabsorption despite the down-regulation of the intestinal bile acid pool.

Although *in vitro* FXR agonists stimulate SHP and inhibit the transcription of *CYP7A1* gene in rat hepatocytes, feeding these animals a high-cholesterol diet results in the up-regulation of this enzyme, while hepatic expression of SHP does not change [55]. It has been shown that FXR is not activated by a high-cholesterol diet in the rat because the hepatic bile acid pool does not increase; although their synthesis is stimulated, this is balanced by increased excretion [174]. Therefore, although at the molecular level rat CYP7A1 is up-regulated by LXR and down-regulated by FXR, the former mechanism predominates *in vivo* when animals are exposed to a high-cholesterol diet. However, if the high-cholesterol diet is supplemented by exogenous cholic acid, FXR is activated, SHP expression is increased, and CYP7A1 expression is reduced, resulting in impaired cholesterol metabolism and hypercholesterolemia [174].

Although rabbit CYP7A1 gene contains LXRE and its expression is stimulated by T0901317 and 22(R)-HC *in vitro* [144], high-cholesterol diet fails to induce CYP7A1 in the rabbit *in vivo*. Xu et al. [173] demonstrated that if rabbits are exposed to a high-cholesterol diet for only one day, hepatic CYP7A1 is up-regulated, presumably through the LXR-dependent mechanism, whereas the expression of the FXR target SHP does not change. However, if the high-cholesterol diet is applied for 10 days, SHP is up-regulated and the expression of CYP7A1 is reduced, which indicates that the positive LXR-mediated regulation is overridden by the inhibitory effect of FXR [144,173]. Therefore, hypercholesterolemia easily develops if rabbits are fed a high-cholesterol diet. The regulation of CYP7A1 by LXR and FXR in various mammalian species is summarized in Table 2.

Table 2. Regulation of hepatic *CYP7A1* gene expression in various mammalian species

Species	LXRE in the promoter region of CYP7A1 gene	Up-regulation by LXR agonists *in vitro*	Inhibition through bile acid-FXR-SHP pathway	Overall effect of high-cholesterol diet *in vivo*
Rat/mice	+	+	+	↑
Rabbit	+	+	+	↓
Human	–	–	+	↓

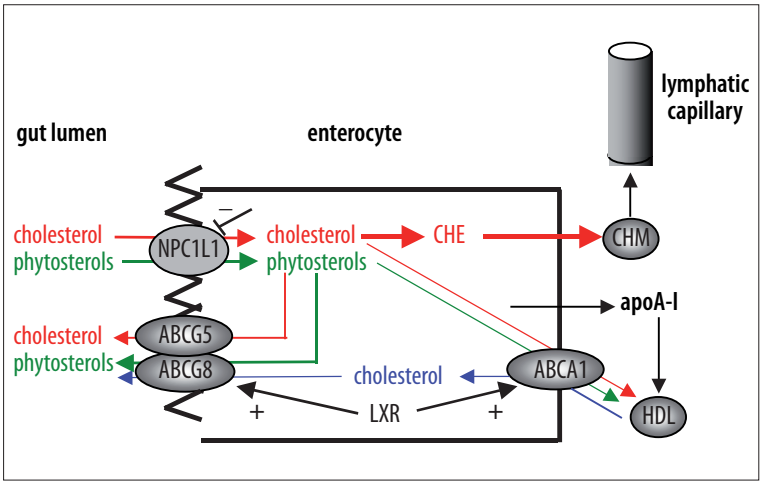

Figure 9. Intestinal cholesterol absorption (red arrows), intestinal phytosterol handling (green arrows), and the role of enterocytes in fecal excretion of cholesterol delivered from peripheral tissues by HDLs (i.e. alternative reverse cholesterol transport, blue arrows). Cholesterol is absorbed from bile micelles through the apical membrane NPC1L1 protein. Most of it is subsequently esterified by ACAT-2, and cholesteryl esters are incorporated into chylomicrons (CHM), which are exocytozed and enter the lymphatic capillaries. Some cholesterol is extruded to the intestinal lumen through the apical ABCG5/ABCG8 heterodimer and the minority of cholesterol is extruded through the basolateral ABCA1 to enter the HDL fraction, in part formed locally in the intestine from locally generated apoA-I. Alimentary phytosterols are absorbed by NPC1L1, but are not esterified; most of them are rapidly extruded to the gut lumen through the ABCG5/ABCG8 complex and some phytosterols are transferred to HDLs by basolateral ABCA1. In addition, the intestine may drive the removal of peripheral tissue-derived cholesterol from HDLs to the feces through the concerted action of basolateral ABCA1 and apical ABCG5/ABCG8. LXR agonists inhibit NPC1L1 and increase ABCA1, ABCG5, and ABCG8

6.3. Bile acid transport and metabolism

22(R)-HC stimulates the expression of the bile salt export pump (BSEP), which drives bile acid transport from hepatocytes to bile canaliculi in primary human hepatocytes and in Huh7 hepatoma cells. However, this effect is mediated by FXR rather than LXR. 22(R)-HC binds to a part of the FXR ligand-binding domain at a place distinct from its cognate ligands, bile acids. LXR agonists stimulate the expression of intestinal bile acid-binding protein (I-BABP), which is involved in the absorption of bile acids from intestinal lumen to the portal blood [90]. Interestingly, this effect is mediated by LXR binding to the FXRE in the promoter region of the *I-BABP* gene.

In human hepatocytes, T0901317 up-regulates UDP-glucuronyltransferase 1A3 (UGT1A3), which glucuronidates chemodeoxycholic and litocholic acids (CDCA and LCA) at the C-24 position, thus increasing their water solubility and allowing urinary excretion [163]. A similar effect is observed in transgenic mice expressing human UGT1A3. Apart from glucuronidation, CDCA and LCA may be hydroxylated at the 6α position to generate hyocholic acid (HCA) and hyodeoxycholic acid (HDCA), respectively. This reaction is inhibited by LXR agonists. Both HCA and HDCA are specific LXRα agonists and thus inhibit their own formation through a negative feedback mechanism. In summary, stimulation of LXR shifts CDCA and LCA metabolism toward 24-glucuronides by UGT1A3 at the expense of 6α-hydroxylation. In contrast, FXR stimulates CYP3A4 and UGT2B4, leading to predominant formation of 6α-glucuronides of HCA and HDCA.

6.4. Intestinal cholesterol absorption

Alimentary cholesterol is absorbed in the small intestine. An important role in this process is played by the Niemann-Pick C1-like 1 protein (NPC1L1) contained in the apical membranes of enterocytes. Next the majority of cholesterol is esterified inside the enterocyte by ACAT-2 and is incorporated into chylomicrons. Some cholesterol is exported through the ABCA1 contained in the basolateral membrane to apoA-I; the process is essential for intestinal HDL formation (Figure 9). In addition, a substantial part of the intracellular cholesterol is back-extruded from the enterocyte to the intestinal lumen through the ABCG5/ABCG8 heterodimer contained in the apical membrane [165].

In 2000, Repa et al. [137] first demonstrated that T0901317 reduced intestinal absorption of dietary cholesterol. Because this effect was accompanied by the increased expression of ABCA1 in the small intestine, it was initially sugge-

sted that this transporter drives cholesterol removal from the enterocytes to the intestinal lumen and thus reduces the net cholesterol absorption [137]. However, this possibility is unlikely since ABCA1 is contained mainly in the basolateral membrane of intestinal cells and the effect of LXR agonists on fecal cholesterol excretion is preserved in ABCA1 knockout mice [130]. Indeed, LXR agonists stimulate cholesterol recycling from the enterocyte to the intestinal lumen by up-regulating ABCG5 and ABCG8 [134]. Fecal cholesterol content is increased, whereas net intestinal cholesterol absorption is reduced in mice overexpressing ABCG5 and ABCG8, whereas the opposite is observed in ABCG5/ABCG8 double-knockout animals [182].

Apart from the intestine, ABCG5 and ABCG8 are abundantly expressed in the canalicular membrane of hepatocytes, where they drive cholesterol transport to the bile. Hepatic ABCG5 and ABCG8 are also stimulated by LXR agonists, and this results in enhanced biliary cholesterol excretion [182]. In contrast to wild-type animals, T0901317 does not stimulate biliary cholesterol excretion and fails to reduce fractional cholesterol absorption in ABCG5/ABCG8 double-knockout mice [182].

However, the increase in fecal cholesterol by LXR agonists is not solely dependent on biliary cholesterol excretion. Indeed, GW3965 does not stimulate biliary cholesterol content, but increases fecal cholesterol loss in mice lacking the multidrug resistance protein-2 (Mdr-2, ABCB4) involved in the export of phospholipids from hepatocytes to bile canaliculi [85]. Interestingly, GW3965 also effectively increases the excretion of radiolabeled cholesterol from plasma to the feces in these animals. It is suggested that apart from the liver, the intestine is an alternative organ involved in reverse cholesterol transport and excretion. According to this hypothesis, HDLs may deliver cholesterol from peripheral tissues directly to the intestine, where it is removed to the gut lumen through the concerted action of basolateral ABCA1 and apical ABCG5/ABCG8 heterodimer (Figure 9) [85].

Recently, Duval et al. [44] demonstrated that T0901317 and GW3965 decrease the expression of NPC1L1 protein in mouse intestine *in vivo* and in human enterocyte cell culture *in vitro*, thus completing a complex mechanism through which LXRs reduce cholesterol absorption (Figure 9).

6.5. Effect of LXRs on intestinal HDL formation

Apart from the liver, the small intestine is a major HDL-generating organ in the body. Although LXR agonists inhibit cholesterol absorption in an ABCA1-independent manner, they do stimulate this transporter in enterocytes and enhance intestinal HDL formation. Indeed, LXR agonists stimulate apoA-I-mediated cholesterol efflux from the basolateral, but not from the apical membrane of enterocytes, and have no effect on the formation of chylomicrons [116,121]. Intestine-specific deletion of ABCA1 results in an about 30% reduction of plasma HDL [23]. Tissue-specific stimulation of intestinal ABCA1 by LXR agonists raises the plasma HDL level. Indeed, GW3965 increases plasma HDL in wild-type mice as well as in animals lacking hepatic ABCA1, but fails to do so in mice lacking ABCA1 selectively in the small intestine [24].

6.6. Effect of LXRs on plant sterol absorption

Although plant sterols are almost as abundant as cholesterol in food, only a small fraction of them is absorbed in the intestine for two reasons. First, plant sterols are much more effectively exported by the ABCG5/ABCG8 heterodimer. In addition, plant sterols are very poor substrates for esterification by intestinal ACAT-2. Thus, most of plant sterols reaching the enterocyte are rapidly removed to the intestinal lumen (Figure 9). Any absorbed plant sterols are also rapidly excreted to the bile by hepatic ABCG5/ABCG8 complex. Therefore, the plasma concentration of plant sterols is normally very low [62]. Mutation of either the *ABCG5* or *ABCG8* gene results in sitosterolemia (phytosterolemia), a rare inherited recessive disease characterized by a very high level of plant sterols in plasma and tissues, moderate hypercholesterolemia, and accelerated atherosclerosis, presumably related to deposition of plant sterols in the arterial wall.

Under normal conditions, LXR agonists limit intestinal absorption of plant sterols and increase their biliary excretion by stimulating ABCG5 and ABCG8. However, in ABCG5 knockout mice [129] and in ABCG5/ABCG8 double-knockout mice [182], LXR agonists paradoxically increase the plasma concentration of phytosterols. This is most likely associated with the unopposed stimulation of ABCA1-driven basolateral phytosterol efflux from the enterocyte to HDLs (Figure 9). Indeed, the plasma level of plant sterols is lower in ABCA1-deficient mice, and it was demonstrated that T0901317, 22(R)-HC, and 9cRA stimulate basolateral apoA-I-mediated efflux of β-sitosterol from cultured intestinal epithelial cells [48]. ABCA1 does not discriminate between cholesterol and plant sterols. Because plant sterols are not esterified by ACAT-2 and are not incorporated into chylomicrons, they are absorbed exclusively via a ABCA1-dependent route. These data suggest that LXR agonists could have a paradoxical, undesirable effect on plasma plant sterols in patients with sitosterolemia, and possibly also in the much more frequent asymptomatic ABCG5 or ABCG8 mutant heterozygotes.

6.7. Cholesterol synthesis and uptake

Cellular cholesterol content depends on the balance of three processes: 1) intracellular synthesis, 2) uptake from plasma lipoproteins, mainly LDLs, and 3) removal from the cell to plasma lipoproteins, mainly HDLs. Cholesterol synthesis is precisely regulated to maintain a constant cholesterol content and is thus adjusted to the remaining two processes. Due to the potent effect of LXR agonists on cholesterol removal from the cell, it is difficult to separate any direct effect of these receptors on cholesterol synthesis from adaptor changes induced by altered uptake and/or removal.

Several key enzymes in the cholesterol biosynthesis pathway, including 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, are stimulated by the sterol regulatory element binding protein-2 (SREBP-2). SREBPs are transcription factors synthesized as the inactive precursors which reside in the endoplasmic reticulum and are subsequently activated by proteolysis, move to the nucleus, and stimulate the expression of target genes. Three isoforms of SREBPs were identified; two of them (SREBP-1a and

SREBP-1c) are encoded by a single gene, whereas the third, SREBP-2, is encoded by a separate gene. SREBP-1a stimulates the expression of genes involved in both cholesterol and fatty acid synthesis, SREBP-1c stimulates lipogenesis, and SREBP-2 stimulates cholesterol-synthesizing enzymes and LDL receptor. SREBP-2 is activated in response to cholesterol depletion and stimulates compensatory up-regulation of cholesterol uptake and synthesis [31].

LXRα-null mice are characterized by a higher expression of SREBP-2 as well as of several of its target genes, including HMG-CoA synthase and reductase, farnesylpyrophosphate synthase, and squalene synthase in the liver [126]. The similar, although much smaller, up-regulation of cholesterol-synthesizing enzymes is observed in LXRβ-null mice [3]. In addition, administration of T0901317 reduces the hepatic expression of HMG-CoA synthase and squalene synthase in wild-type mice [142]. These data suggest that LXRs inhibit cholesterol synthesis. However, it should be kept in mind that natural LXR agonists, oxysterols, may affect cholesterol synthesis in an LXR-independent manner, e.g. by inhibiting the cleavage of SREBP-2. In addition, stimulation of cholesterol efflux by the LXR agonists may cause a compensatory up-regulation of cholesterol synthesis in certain cell types, such as macrophages. For example, T0901317 and GW3965 enhance cholesterol synthesis as well as increase LDL-receptor expression in human hepatoma HepG2 cells, probably by stimulating cholesterol efflux and activating the SREBP-2-dependent pathway. In contrast, natural agonists have no (22(R)-HC) or slight inhibitory (24(S),25-EC) effect on cholesterol production, most likely by directly suppressing SREBP-2 processing [6]. Therefore, the interpretation of data about the role of LXRs in the regulation of cholesterol synthesis is difficult. In general, it is assumed that the effect on cholesterol synthesis plays only a minor role in the regulation of cellular sterol homeostasis by LXR ligands. Recently, Ishimoto et al. [67] demonstrated that T0901317 increases the expression of LDL-receptor in the human hepatoblastoma Huh-7 cell line through the LXRE contained in the promoter of its gene. However, it is unclear if this effect also occurs in the intact liver or in extrahepatic tissues.

7. Other Effects on Lipid Metabolism

7.1. Lipogenesis

In 2000, Schultz et al. [142] first demonstrated that treatment with T0901317 markedly increased hepatic triglyceride content and plasma triglyceride concentration in mice and hamsters. Although the effect on plasma triglycerides was transient, that on hepatic triglycerides was persistent and lead to severe liver steatosis and dysfunction. In addition, plasma triglycerides are about fourfold lower in LXRα/LXRβ double-knockout mice than in wild-type animals.

LXR agonists markedly stimulate fatty acid synthesis (lipogenesis) in hepatocytes. This effect is partially mediated by increased expression of SREBP-1c, which subsequently binds to the sterol response element (SRE) within the promoter region of genes encoding various lipogenic enzymes [135]. In rat hepatoma cells, baseline expression of SREBP-1c is maintained by the endogenous oxysterol-LXR pathway since inhibition of oxysterol formation reduces SREBP-1c synthesis [43]. Yoshikawa et al. [179] identified two LXREs within the *SREBP-1c* gene promoter and demonstrated that LXR as well as RXR agonists increase its transcriptional activity. In addition, LXR directly (in a SREBP-1c independent manner) regulates the expression of several lipogenic enzymes, including acetyl-CoA carboxylase (ACC) [155], fatty acid synthase (FAS) [73], and stearoyl-CoA desaturase-1 (SCD-1) [168], by binding to the LXREs in the regulatory regions of *ACC*, *FAS*, and *SCD-1* genes, respectively. SCD-1 forms the double bond at the n-9 position of saturated fatty acids, thus converting saturated stearoyl-CoA and palmitoyl-CoA to monounsaturated oleoyl-CoA and palmitoleoyl-CoA, respectively. Indeed, LXR agonists increase the ratio of monounsaturated to saturated fatty acids ("desaturation index") in cellular lipids and plasma lipoproteins [185]. Chu et al. [41] demonstrated that SCD-1 deficiency protects against LXR-induced increase in plasma triglyceride and HDL-cholesterol levels. Thus, SCD-1 is crucial for the lipogenic effect of LXRs.

Treating wild-type mice with T0901317 increases *SREBP-1c*, *ACC*, *FAS*, and *SCD-1* gene expression in the liver [142]. This effect is preserved in LXRβ$^{-/-}$, but not in LXRα$^{-/-}$ mice. In addition, basal levels of SREBP-1c, ACC, FAS, and SCD-1 are reduced in LXRα$^{-/-}$ mice. These data suggest that LXRα is a major isoform responsible for hepatic lipogenesis.

Grefhorst et al. [56] demonstrated that LXR agonists stimulate the production of triglyceride-rich, large VLDL particles in the liver. Although the number of VLDL particles formed does not change, their diameter increases due to the higher amount of triglycerides per particle. In animals treated with LXR agonists, the plasma triglyceride concentration increases only transiently because VLDL metabolism is simultaneously stimulated, presumably due to the increase in lipoprotein lipase (see below). In addition, T0901317 stimulates peroxisomal β-oxidation of very-long-chain fatty acids in the liver [64].

Although enhanced lipogenesis is clearly undesirable if one considers the potential therapeutic application of LXR agonists, it is a logical component of a complex response to cholesterol excess because it enables the conversion of more toxic free cholesterol to less toxic cholesterol esters. Indeed, monounsaturated fatty acids generated by SCD-1 are preferable substrates for ACAT. In addition, lipogenesis may provide components essential for cholesterol removal from the cell, such as triglycerides required for VLDL assembly in the liver or phospholipids required for HDL formation.

It should be emphasized that synthetic LXR agonists induce lipogenesis much more potently than cholesterol excess. Although cholesterol excess, ultimately resulting in increased oxysterols, stimulates SREBP-1c synthesis through the LXR-dependent mechanism, cholesterol itself as well as certain oxysterols inhibit proteolytic processing of the inactive SREBP-1c precursor to its mature active form. Due to these two opposing effects, cholesterol does not stimulate lipogenesis dramatically. In contrast, synthetic nonsteroid LXR agonists increase SREBP-1c synthesis while having no inhibitory effect on its processing, which results in supraphysiological stimulation of fatty acid synthesis.

Table 3. Mechanisms involved in lipogenic effect of LXR agonists

Step of triglyceride/VLDL synthesis and metabolism	Specific mechanisms of action
Effect on substrates for lipogenesis	Stimulation of uridine phosphorylase and β-alanine production
Direct stimulation of lipogenic enzymes through the LXREs in their regulatory regions	ACC FAS SCD-1
Effect on prolipogenic transcription factors	SREBP-1c ChREBP
Effect on VLDL assembly in the liver	Increase in PLTP expression
Effect on other mediators involved in the regulation of lipogenesis	Reduction of adiponectin R1 receptors in the liver
Effects on VLDL metabolism*	Reduction of apoA-V Stimulation of Angptl3 Stimulation of LPL Stimulation of apoC-II

* The effect of LXR agonists on VLDL clearance is controversial; reduction of apoA-V and stimulation of Angptl3 (angiopoietin-like protein 3) impair VLDL metabolism, whereas increased expression of lipoprotein lipase (LPL) and its activator, apoC-II, tend to promote VLDL clearance

Carbohydrate response element binding protein (ChREBP) is a glucose-sensitive transcription factor which stimulates the expression of lipogenic enzymes when the glucose concentration is high and thus promotes the conversion of excess glucose to lipids. Recently it was demonstrated that LXR agonists stimulate the expression of ChREBP in the liver both *in vitro* and *in vivo* [30]. This effect results from the binding of LXR/RXR to the two LXREs in the promoter region of the *ChREBP* gene. It was demonstrated that the stimulatory effects of T0901317 on FAS, ACC, and SCD-1 were attenuated in ChREBP-knockout mice. In addition to inducing ChREBP expression, T0901317 stimulates its binding to the cognate DNA sequence, carbohydrate response element (CRE). ChREBP-CRE binding is inhibited by the active phosphorylated form of AMP-stimulated protein kinase (AMPK), and T0901317 reduces the rate of AMPK phosphorylation, thus removing its inhibitory influence on ChREBP activity [30].

Adiponectin is secreted in large amounts by adipocytes and exerts many beneficial metabolic effects, such as increased insulin sensitivity and stimulation of mitochondrial fatty acid oxidation. Adiponectin reduces hepatic lipogenesis and prevents hepatic steatosis by reducing SREBP-1c expression. Interestingly, 25-HC reduces the expression of adiponectin receptor, AdipoR1, in primary hepatocytes and in cultured human HepG2 cell line [118]. It is unclear if this effect is mediated by LXRs and if it also operates *in vivo*, but if so, downregulation of adiponectin signaling in the liver could contribute to enhanced lipogenesis.

Zhang et al. [184] demonstrated that LXR agonists stimulate the expression of liver-specific uridine phosphorylase, the rate-limiting enzyme in uridine catabolism. A major metabolic product of uridine catabolism in the liver is β-alanine, which serves as a precursor for fatty acid synthesis. Thus, the effect on uridine phosphorylase could be involved in LXR-induced lipogenesis. The currently known mechanisms of the lipogenic effect of LXRs are summarized in Table 3.

Because insulin stimulates LXRα expression in the liver, LXRs may be involved in insulin-induced lipogenesis. Indeed, insulin increases the expression of SREPB-1c and its target lipogenic enzymes. Moreover, the induction of SREBP-1c by insulin requires an intact LXRE in the *SREBP-1c* gene promoter [34] and is largely abrogated in LXRα/LXRβ double-knockout mice [160]. Interestingly, whereas LXR agonists increase the expression of the SREPB-1c precursor, insulin additionally stimulates its cleavage to the mature, transcriptionally active form. Thus LXR agonists and insulin may act synergistically to increase fatty acid synthesis [61]. *In vivo*, T0901317 stimulates SREBP-1c expression and increases the amount of active mature protein because endogenous insulin is present [61]. Because insulin secretion is proportional to glucose concentration, the net lipogenic effect of LXR agonists depends on glucose availability.

7.2. Apolipoproteins

LXR agonists stimulate the synthesis of apolipoprotein-E in macrophages and in adipose tissue both *in vitro* and *in vivo* [89]. In contrast, apoE expression in the liver is not regulated by LXRs. In fact, apoE was the first identified LXR-responsive gene which is regulated in a tissue-specific manner. The stimulatory effect on apoE is greater in human than in murine macrophages [88]. In addition to apoA-I, apoE is an alternative extracellular cholesterol acceptor and is thus involved in LXR-stimulated cholesterol efflux. Apart from apoE, LXR agonists stimulate the expression of apolipoproteins C-I, C-II, and C-IV, which also operate as cholesterol acceptors [104].

LXR agonists increase the expression of apolipoprotein D in adipocytes [65]. ApoD is a 29-kDa glycoprotein that is primarily associated with plasma HDL. It is synthesized in many tissues, including spleen, testes, and brain. The physiological function of apoD is unclear and it is not known if apoD is also regulated by LXRs outside the adipose tissue.

The next apolipoprotein regulated by LXRs is apoA-IV. ApoA-IV is synthesized in the intestine and, to a lesser extent, in the liver, and is detected mainly in chylomicrons and in lower amounts in HDLs, as well as a free plasma protein. In HDLs, apoA-IV stimulates LCAT, whereas free plasma apoA-IV facilitates cholesterol efflux from the cells and exerts antioxidant activity. Transgenic overexpression of apoA-IV reduces atherosclerosis in the mouse models, and plasma apoA-IV is inversely correlated with atherosclerosis in humans [132,154]. Although the apoA-IV gene contains the LXRE, LXR agonists stimulate its expression only in hepatocytes, but not in the intestine. *In vivo*, T0901317 increases HDL-associated apoA-IV, which is presumably of hepatic origin, but has no effect on intestine-derived chylomicron-associated apoprotein. Interestingly, apoA-IV acts on the hypothalamic appetite center to reduce food intake. Although LXR agonists have no reported effect on food intake in wild-type mice, T0901317 decreases food intake in Zucker diabetic fatty (ZDF) rat [27]. However, an anorectic effect is exerted by apoA-IV synthesized locally in the brain, and it is unclear if LXR agonists regulate central apoA-IV production.

Apolipoprotein A-V is the only known apolipoprotein downregulated by LXR agonists both *in vitro* and *in vivo* [68]. ApoA-V is synthesized in the liver and is incorporated into the HDL and VLDL fractions. Overexpression of apoA-V reduces plasma triglycerides in mice, whereas deletion of apoA-V gene induces hypertriglyceridemia [133]. The hypolipidemic effect of apoA-V is most likely mediated by stimulation of lipoprotein lipase. T0901317 reduces apoA-V mRNA level in cultured hepatocytes; however, this is an indirect effect mediated by SREBP-1c rather than by direct binding of LXR/RXR to the *apoA-V* gene [68].

7.3. Plasma lipoprotein-remodeling transporters

LXR agonists stimulate the expression of two plasma lipoprotein-remodeling proteins, CETP and phospholipid transfer protein (PLTP). CETP is synthesized in the liver and circulates in the HDL fraction. CETP transfers cholesterol esters from HDLs to VLDLs and LDLs in the exchange for triglycerides. Thus, CETP may facilitate cholesterol transport to the liver. However, the role of CETP in plasma lipoprotein metabolism and atherogenesis is controversial, because it also contributes to high LDL-cholesterol level in humans and rabbits, whereas species lacking CETP, such as rats and mice, have very low levels of LDLs [82].

LXR agonists increase hepatic CETP synthesis and plasma CETP concentrations [101]. Consistent with the role of CETP outlined above, LXR agonists markedly elevate plasma HDL-cholesterol in species lacking CETP, but have only a weak or no effect on HDLs in CETP-positive animals. In addition, in two CETP-positive species, Syrian hamster and cynomolgus monkey, LXR agonists significantly elevate LDL-cholesterol [58]. In addition, transgenic mice expressing human CETP demonstrate elevation of LDLs and no change in HDLs in response to LXR agonists [72,109].

Phospholipid transfer protein (PLTP) mediates the transport of phospholipids from VLDLs and chylomicron remnants to HDLs or to lipid-poor apoA-I. In addition, PLTP mediates phospholipid transfer between different HDL subfractions, thus generating lipid-poor small pre-β HDLs which are better cholesterol acceptors than large HDL particles. T0901317 increases PLTP activity in plasma as well as PLTP mRNA in the liver, adipose tissue, and intestine, which is accompanied by an increase in phospholipid content in the HDL fraction [26,87]. Stimulation of PLTP may contribute to LXR-induced VLDL production because PLTP is involved in the assembly of VLDLs in hepatocytes [56]. Indeed, the hypertriglyceridemic effect of T0901317 is markedly reduced in PLTP-deficient mice [26]. In addition to the liver, natural and synthetic LXR agonists increase PLTP expression in human and murine macrophages [87,103].

7.4. Enzymes involved in plasma lipoprotein metabolism

Triglycerides and/or phospholipids contained in plasma lipoproteins are hydrolyzed by three lipases expressed on the surface of endothelial cells: lipoprotein lipase (LPL), hepatic lipase (HPL), and endothelial lipase (EL). LPL has a high affinity for triglycerides and hydrolyzes triglyceride-rich lipoproteins such as VLDLs and chylomicrons. Mice and human *LPL* genes contain LXRE and are direct targets for LXRα, but not for LXRβ [183]. Interestingly, the stimulatory effect of LXR on LPL expression is restricted to the liver and, to a lesser extent, macrophages. In contrast, LXR agonists have no effect on LPL in adipose tissue.

Endothelial lipase (EL) hydrolyzes plasma phospholipids, including those associated with HDLs. Sovic et al. [152] demonstrated that 24(S)-HC reduces EL mRNA and protein levels in brain capillary endothelial cells. This effect may contribute to the LXR-induced increase in HDL fraction. However, Norata et al. [119] observed increased EL expression in human umbilical vein endothelial cells, human fibroblasts, and HepG2 cells treated with T0901317, but not with 22(R)-HC. Thus the effect of LXRs on EL may be cell specific and remains controversial. Until now, the role of LXRs in the regulation of hepatic lipase, the third enzyme involved in intravascular lipolysis, which is active toward both triglycerides and phospholipids, has not been studied.

Conclusions

Liver X receptors (LXRs) are nuclear receptors which form heterodimers with the retinoid X receptor and regulate gene expression. LXRs are activated by oxysterols, which are enzymatically or nonenzymatically formed cholesterol derivatives, as well as by glucose. T0901317 and GW3965 are two nonsteroid synthetic LXR agonists used in experimental studies that are more potent that endogenous ones. LXR signaling may also be modulated by changes in receptor expression. LXRs protect the cells from cholesterol overload by stimulating reverse cholesterol transport from peripheral tissues to the liver and its excretion in the bile. In addition, LXR agonists activate lipogenesis by stimulating the expression of a lipogenic transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c), leading to hypertriglyceridemia and liver steatosis. Other effects of LXRs include stimulation of several apolipoproteins (apo-E, apo-D, apo-AIV) as well as of cholesterol ester transporting protein (CETP) and phospholipid transfer protein (PLTP).

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