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Review

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Liver X receptors (LXRs). Part II: Non-lipid effects, role in pathology, and therapeutic implications

Receptory wątrobowe X (LXR). Część II: Działania niezwiązane z gospodarką lipidową, znaczenie w patologii i implikacje terapeutyczne

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Summary

Liver X receptors (LXRs) α and β belong to a group of nuclear receptors which, after ligand binding, regulate gene transcription. Their natural agonists are oxidized cholesterol derivatives (oxysterols). The main function of LX receptors is the regulation of cholesterol metabolism. In the first part of this work we discussed the structure and mechanism of action of LXRs, their agonists and antagonists, the regulation of LXR expression, and their role in cholesterol and lipid metabolism. In the present article we describe other roles of LXRs. Agonists of these receptors increase insulin sensitivity and stimulate insulin secretion. Activation of LXRs inhibits inflammation and autoimmune reactions. Moreover, pharmacological studies and genetic manipulations indicate that these receptors inhibit atherogenesis. LX receptors are also involved in the regulation of renin secretion, inhibit the formation of amyloid β in the central nervous system, regulate gonadal function and steroidogenesis both in gonads and in adrenals, influence the proliferation and differentiation of keratinocytes, and inhibit the proliferation of tumor cells. Changes in the expression of these receptors and in the level of their agonists are observed in many diseases. Taking into account the multiple roles of LX receptors, their agonists may be applied in the future in the treatment of many disorders, including diabetes, inflammatory diseases, atherosclerosis, Alzheimer's disease, and hypogonadism. However, possible side effects should be taken into account, including enhancement of lipogenesis, hypertriglyceridemia, and liver steatosis. The function of LX receptors is also modulated by many currently used drugs such as statins, fibrates, and thazolidinedione derivatives.

Key words: **liver X receptor • oxysterols • diabetes mellitus • inflammation • atherosclerosis • Alzheimer's disease • statins • fibrates**

Streszczenie

Receptory wątrobowe X (LXR) typu α i β należą do grupy receptorów jądrowych, które po związaniu ligandu regulują transkrypcję genów. Naturalnymi agonistami tych receptorów są utlenione pochodne cholesterolu (oksysterole). Podstawową funkcją receptorów LXR jest regulacja metabolizmu cholesterolu. W pierwszej części pracy omówiono budowę i mechanizm działania receptorów LXR, ich agonistów i antagonistów, regulację ekspresji tych receptorów oraz ich udział w regulacji metabolizmu cholesterolu i lipidów. W niniejszym artykule opisano znaczenie re-

ceptorów LXR w innych procesach. Agoniści tych receptorów zwiększają wrażliwość na insulinę oraz pobudzają jej wydzielanie. Pobudzenie receptorów LXR hamuje proces zapalny i reakcje autoimmunologiczne. Ponadto, badania farmakologiczne i manipulacje genetyczne wskazują na przeciwmiażdżycowe działanie tych receptorów. Receptory LXR biorą udział w regulacji wydzielania reniny, hamują powstawanie amyloidu β w ośrodkowym układzie nerwowym, regulują funkcję gonad, a także proces steroidogenezy zarówno w gonadach, jak i w nadnerczach, wpływają na proliferację i różnicowanie komórek naskórka oraz hamują proliferację komórek nowotworowych. Zmiany w ekspresji tych receptorów oraz zmiany poziomu ich agonistów – oksysteroli, występują w wielu chorobach. Biorąc pod uwagę wielorakie znaczenie receptorów LXR, ich agoniści mogą w przyszłości znaleźć zastosowanie w leczeniu wielu chorób, w tym cukrzycy, chorób zapalnych, miażdżycy, choroby Alzheimera, hipogonadyzmu, itp. Należy jednak brać pod uwagę możliwe działania niepożądane, w tym wzrost lipogenezy, hipertriglicerydemię i stłuszczenie wątroby. Na funkcję receptorów LXR wpływa też wiele stosowanych obecnie leków, takich jak statyny, fibraty i pochodne tiazolidinedionu.

Słowa kluczowe: **receptory wątrobowe X • oksysterole • cukrzyca • zapalenie • miażdżyca • choroba Alzheimera • statyny • fibraty**

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Abbreviations: **9cRA** – *9-cis* retinoic acid; **24(S),25-EC** – 24(S),25-epoxycholesterol; **24(S)-HC** – 24(S)-hydroxycholesterol; **25-HC** – 25-hydroxycholesterol; **27-HC** – 27-hydroxycholesterol; **ACC** – acetyl-CoA carboxylase; **ABC** – ATP-binding cassette transporters; **Aβ** – amyloid beta protein; **AD** – Alzheimer's disease; **Angptl3** – angiopoietin-like protein-3; **APP** – amyloid precursor protein; **CAR** – constitutive androstane receptor; **CETP** – cholesterol ester transfer protein; **COX** – cyclooxygenase; **CRP** – C-reactive protein; **CYP** – cytochrome P450; **FAS** – fatty acid synthase; **FF-MAS** – follicular fluid meiosis-activating sterol; **G6Pase** – glucose 6-phosphatase; **G-CSF** – granulocyte colony-stimulating factor; **GLUT** – glucose transporter; **GM-CSF** – granulocyte and macrophage colony-stimulating factor; **HDL** – high-density lipoproteins; **HMG-CoA** – 3-hydroxy-3-methyloglutaryl-coenzyme A; **HSD** – hydroxysteroid dehydrogenase; **ICAM** – intercellular adhesion molecule; **IL-1β** – interleukin-1β; **IL-6** – interleukin-6; **iNOS** – inducible nitric oxide synthase; **IRS** – insulin receptor substrate; **LDL** – low-density lipoproteins; **LPL** – lipoprotein lipase; **LPS** – lipopolysaccharide; **LXR** – liver X receptor; **LXRE** – liver X receptor response element; **MCP** – monocyte chemoattractant protein; **M-CSF** – macrophage colony-stimulating factor; **MMP** – matrix metalloproteinases; **MPO** – myeloperoxidase; **MS** – multiple sclerosis, **NF-κB** – nuclear factor-κB; **OPN** – osteopontin; **PDGF** – platelet-derived growth factor; **PEPCK** – phosphoenolpyruvate carboxykinase; **PGES** – prostaglandin E synthase; **PKB** – protein kinase B; **PPAR** – peroxisome proliferator-activated receptors; **PXR** – pregnane X receptor; **RXR** – retinoid X receptors; **siRNA** – small interfering RNA; **SAP** – serum amyloid P protein; **SCD** – stearoyl-CoA desaturase; **SREBP** – sterol response element binding protein; **StAR** – steroidogenic acute regulatory protein; **TLR** – Toll-like receptors; **TNF-α** – tumor necrosis factor-α; **VEGF** – vascular endothelial growth factor.

1. INTRODUCTION

Liver X receptors (LXRs) are nuclear transcription factors which dimerize with the retinoid X receptor (RXR) and, upon ligand binding, regulate the expression of target genes. Oxygenated oxysterol derivatives (oxysterols) such as 22(R)-hydroxycholesterol (22(R)-HC), 24(S),25-epoxycholesterol (24(S),25-EC), 24(S)-hydroxycholesterol (24(S)-HC), 25-hydroxycholesterol (25-HC), and 27-hydroxycholesterol (27-HC), which are either intermediates in cholesterol biosynthesis or originate by the oxidation of cholesterol by various cytochrome P450 (CYP) isoforms, are natural LXR ligands. Recent data suggest that these receptors may also be activated by glucose. Upon stimula-

tion, LXRs exert many effects protecting from cholesterol overload, including stimulation of cholesterol efflux from the cell to plasma high-density lipoproteins (HDLs) through several ATP-binding cassette (ABC) transporters, its transport to the liver, conversion to bile acids, and excretion. The undesirable effect of LXR agonists is stimulation of lipogenesis, which leads to hypertriglyceridemia and liver steatosis. However, LXRs have widespread distribution in most tissues and are involved in the regulation of multiple physiological processes. In the first part of this review, we described the structure and function of LXRs, their endogenous and synthetic agonists, and their role in the regulation of cholesterol and plasma lipoprotein metabolism. In this article we address other functions of LXRs, including their effect on carbohydrate metabolism, inflammatory reaction, atherogenesis, adrenal and gonadal function, and their role in the central nervous system. In addition, we characterize alterations of LXR signaling in pathological conditions, potential therapeutic applications of LXR agonists, and the effect of currently used therapies on LXR signaling.

2. Role of LXRs in Carbohydrate Metabolism

2.1. Antidiabetic effect of LXR agonists

Several studies have demonstrated that LXR agonists reduce plasma glucose concentration and increase insulin sensitivity in different animal models of diabetes and insulin resistance. For example, administration of the LXR agonist T0901317 for seven days reduced plasma glucose concentration in db/db mice and Zucker diabetic fatty rats, two rodent models of type 2 diabetes associated with severe obesity and insulin resistance [24,104]. In contrast, T0901317 had no effect on plasma glucose in control animals. Another LXR agonist, GW3965, markedly improved glucose tolerance in mice with dietary-induced obesity [92]. Similarly, GW3965 reduced plasma glucose and insulin concentrations as well as increased insulin sensitivity in leptin-deficient diabetic ob/ob mice [62].

2.2 Effect of LXR agonists on glucose metabolism and insulin sensitivity

The mechanism through which LXR agonists improve carbohydrate metabolism is probably complex, but it is mainly associated with the improvement of insulin sensitivity. Indeed, T0901317 had no effect on plasma insulin, but it reduced glucose concentration in db/db mice and Zucker diabetic fatty rats [24], which suggests an increase in insulin sensitivity. It has been demonstrated that T0901317 and GW3965 reduce the expression of key enzymes involved in hepatic gluconeogenesis and glucose output, including pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase, and glucose 6-phosphatase (G6Pase) [92, 150]. In rat hepatoma cells, T0901317 or adenoviral overexpression of either the *LXRα* or the *LXRβ* gene decreased the expression and activity of G6Pase [63]. *In vitro*, T0901317 reduced glucose output from hepatic slices isolated from Zucker rats [24]. In addition, GW3965 increased hepatic expression of glucokinase and glucose 6-phosphate dehydrogenase (the first enzyme of the pentose phosphate shunt) in mice with dietary-induced obesity, thus enhancing glucose uptake and metabolism in the liver [92].

In cultured 3T3-L1 adipocytes, LXR agonists stimulate basal (GLUT1-dependent), but not insulin-stimulated (GLUT4-dependent), glucose uptake [132]. In contrast, Dalen et al. [40] observed stimulation of GLUT4 expression and insulin-induced glucose uptake by T0901317 in mouse 3T3-L1 adipocytes and in cultured human adipocytes. LXRE was identified within the promoter regions of mice and human *Glut4* genes, and it was demonstrated that the LXR/RXR heterodimer binds to this LXRE and stimulates transcription [40]. *In vivo*, both GW3965 [92] and T0901317 [40] increased GLUT4 expression in epididymal adipose tissue of healthy mice. In addition, a minor but significant increase in GLUT4 expression by T0901317 was also observed in skeletal muscle [40]. Taken together, these data suggest that LXRs may stimulate peripheral glucose uptake by insulin-sensitive tissues.

Tumor necrosis factor-α (TNF-α) is synthesized in adipocytes and its production is markedly increased in obese animals and humans. Many studies have demonstrated that TNF-α impairs insulin signaling and thus contributes to obesity-associated insulin resistance. It has been shown that T0901317, GW3965, and 22(R)-HC restore insulin-induced glucose uptake impaired by TNF-α in rat fetal brown adipocytes. In contrast, LXR agonists have no effect on glucose uptake in the absence of TNF-α [47]. TNF-α impairs key steps of insulin signaling, including tyrosine phosphorylation of insulin receptor substrates 1 and 2 (IRS-1 and IRS-2) as well as phosphorylation and activity of protein kinase B/Akt. These abnormalities were corrected by T0901317. Indeed, this LXR agonist reduced the expression and activity of protein tyrosine phosphatase-1B, an enzyme dephosphorylating IRS tyrosine residues, which is a TNF-α-activated negative modulator of insulin signaling [47].

2.3. Effect of LXR agonists on insulin secretion

Efanov et al. [45] demonstrated that T0901317 stimulates glucose-induced insulin secretion by isolated pancreatic β cells. Although T0901317 increased insulin content in the cell, the ratio between insulin secretion and content also increased, indicating that LXRs regulate the insulin secretory mechanism independently of changes in hormone synthesis. When glucose enters pancreatic β cells, it is metabolized through the glycolytic pathway, and the ATP which is generated blocks ATP-sensitive K^+ channels, leading to cell membrane depolarization, Ca^{2+} influx, and insulin release. In pancreatic β cells, T0901317 stimulates the expression of the glucose transporter GLUT2 [197] as well as of glucokinase, the first and rate-limiting enzyme of the glycolytic pathway [45]. Collectively, these effects of T0901317 facilitate glucose metabolism in insulin-producing cells and trigger the mechanism of glucose-induced insulin output.

Although glucose has no effect on LXRα expression in islets, it modulates the intracellular distribution of this receptor. Under normoglycemic conditions, LXRα is localized mainly in the cytosol, but raising glucose to 8 mM induces translocation of LXRα to the nucleus [69]. This effect may result from the recently described stimulation of LXR by glucose [113].

Gerin et al. [58] and Zitzer et al. [197] observed that $LXR\beta^{-/-}$ mice, despite having less adipose tissue, are glu-

cose intolerant due to impaired basal, glucose-induced, and K^+-induced insulin secretion. The expression of ABCA1 and ABCG1 is reduced in the islets of LXRβ$^{-/-}$ mice, which results in overaccumulation of cholesterol esters in β cells and ultimately causes their damage. Moreover, T0901317 stimulates the expression of the pancreatic duodenal homeobox-1 (PDX-1) gene, which is critical for maintaining the differentiated phenotype of β cells. Consequently, β cells isolated from LXRβ$^{-/-}$ mice exhibit reduced expression of PDX-1 and insulin genes [197]. These alterations were not observed in LXRα$^{-/-}$ mice. Thus, intact LXRβ is mandatory for maintaining β-cell structure and function, at least in mice.

T0901317 increased the expression of SREBP-1c in pancreatic β cells [45]. In the short run, SREBP-1c is essential for augmenting glucose-induced insulin secretion by LXR agonists. Indeed, SREBP-1c-specific small interfering RNA (siRNA) attenuates the T0901317-induced increase in the expression of insulin, GLUT2 and PDX-1 genes, and insulin secretion [197]. Glucose stimulates the processing of the SREBP-1c precursor to its mature active form, while LXR agonist increases SREBP-1c synthesis. Thus, glucose and LXR may cooperate in increasing SREBP-1c signaling in insulin-producing cells [197]. It is suggested that the role of SREBP-1c in insulin secretion is associated with cataplerosis, i.e. export of mitochondrial metabolic intermediates, in particular acetyl-CoA, to the cytosol, where it is converted by ACC to malonyl-CoA and subsequently by FAS to fatty acids (stimulators of insulin secretion); both ACC and FAS are SREBP-1c target genes. Indeed, blocking ACC or FAS attenuates the stimulatory effect of T0901317 on insulin secretion [197].

2.4. Effect of LXR agonists on hormones antagonistic for insulin

Glucocorticoids have a well-known deleterious effect on insulin sensitivity and glucose tolerance by stimulating hepatic gluconeogenesis. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) is expressed in adipose tissue and the liver and converts inactive cortisone to active cortisol in humans and inactive 11-dehydrocorticosterone to active corticosterone in mice. Increased expression of 11β-HSD-1 in the adipose tissue of obese animals and humans contributes to a local excess of glucocorticoids, which impair insulin sensitivity. Stulnig et al. [149] showed that natural (22(R)-HC and 20(S)-HC) and synthetic (T0901317) LXR agonists reduce 11β-HSD-1 expression in 3T3-L1 adipocytes. In addition, T0901317 administered for seven days decreased 11β-HSD-1 expression in the liver and adipose tissue *in vivo* both in nondiabetic [149] and in diabetic (db/db) mice [104]. In addition, the expression of glucocorticoid receptor (GR) is increased in the liver of db/db mice, and T0901317 reduces its level to values observed in wild-type animals [101]. Thus, LXR agonists inhibit gluconeogenesis and increase insulin sensitivity partially by ameliorating the local generation and action of glucocorticoids in the liver and adipose tissue.

Loffler et al. [104] demonstrated that T0901317 reduces the expression of growth hormone (GH) receptor and the synthesis of its downstream mediator, insulin-like growth factor-1 (IGF-1), in the liver. Since augmented activity of the GH/IGF-1 axis may contribute to insulin resistance, this is an additional mechanism through which LXRs modulate carbohydrate metabolism.

2.5. LXR agonists and lipotoxicity

The plasma concentration of nonesterified fatty acids is increased in patients with obesity and/or diabetes mellitus, and overaccumulation of triglycerides in cells other than adipocytes has many deleterious consequences, referred to as lipotoxicity. For example, ectopic triglyceride storage impairs insulin sensitivity of skeletal muscles and the liver and may induce apoptosis of pancreatic β cells. The lipogenic effect of LXR agonists raises concerns about possible lipotoxicity if they are administered for a long time. Indeed, LXR agonists increased fatty acid uptake and lipid accumulation in cultured skeletal muscle cells [116]. Unexpectedly, however, the insulin sensitivity of these cells was simultaneously increased [36]. Similarly, although in ob/ob mice GW3965 induced marked liver steatosis, a condition expected to enhance insulin resistance, it had in fact no effect on the suppression of hepatic glucogenogenesis by insulin [62], and other studies even demonstrated improvement of insulin-induced hepatic glucose disposal after treatment with LXR agonists [92]. These data indicate that, despite inducing lipogenesis, LXRs have no negative effect on insulin sensitivity in the liver or skeletal muscle. Thus any potentially unfavorable effect of triglyceride accumulation is probably counteracted by the direct mechanisms through which LXRs improve insulin sensitivity. However, two recent studies [32,182] indicate that prolonged treatment with LXR agonists induces apoptosis of pancreatic β cells, an effect which results most likely from enhanced lipogenesis, lipid overaccumulation, and lipotoxicity.

3. Adipose Tissue and Energy Balance

The role of LXRs in the regulation of adipocyte differentiation and triglyceride storage is controversial. For example, Juvet et al. [82] observed that T0901317 and 22(R)-HC increased the size of lipid droplets in 3T3-L1 adipocytes by stimulating the expression of proteins involved in fatty acid uptake from plasma lipoproteins, i.e. fatty acid translocase (CD36) and fatty acid binding protein (FABP). Hummasti et al. [73] demonstrated that although the expression of LXR increases during adipogenesis, synthetic LXR agonists have no effect on the differentiation of preadipocytes to adipocytes, but stimulate triglyceride accumulation in mature fat cells. In contrast, Seo et al. [139] observed that LXR agonists stimulated adipocyte differentiation by increasing the expression of PPARγ. Recently, Darimont et al. [41] demonstrated that LXRs specifically stimulate *de novo* fatty acid synthesis in human preadipocytes, but have no effect on fatty acid uptake from plasma lipoproteins.

Adipose tissue mass is reduced in LXRα/LXRβ double-knockout mice; however, this is observed only in aging (18-month-old) but not in young animals [92]. Gerin et al. [58] found that neither LXRα nor LXRβ was necessary for the normal differentiation of mouse preradipocytes; however, LXRβ, but not LXRα, was required for the increase in adipocyte size associated with aging or dietary-induced obesity. Despite similar food intake and oxygen

consumption, LXRβ$^{-/-}$ mice accumulate less triglycerides in adipose tissue when placed on a high-calorie diet than wild-type control animals [58]. Taken together, these data suggest that LXRs, in particular LXRβ, have an anabolic effect in adipose tissue.

Interestingly, Kalaany et al. [83] demonstrated that LXRα/LXRβ double-knockout mice are resistant to the development of obesity induced by a high-fat "Western-type" diet, but only when this diet contains also excess cholesterol. If the high-fat diet is not supplemented with cholesterol, body weight increases to a similar extent in wild-type and LXRα/LXRβ-knockout animals. Food intake and fat absorption from the gastrointestinal tract are similar in both groups, whereas peripheral fat metabolism is accelerated in LXRα/LXRβ-knockout mice, partially due to the increased activity of iodothyronine deiodinase type 2, the enzyme which generates active thyroid hormone, triiodothyronine (T_3), from its inactive precursor, thyroxine (T_4), in peripheral tissues. Although plasma T_4 and T_3 levels are similar in wild-type and knockout animals, increased local T_3 concentration stimulates energy expenditure in knockout mice by inducing ectopic expression of uncoupling protein-1 (UCP-1) in skeletal muscle and white adipose tissue. UCP-1 uncouples the mitochondrial respiratory chain from ATP synthesis and increases energy dissipation. The mechanism of stimulation of type 2 deiodinase by high-fat high-cholesterol diet in LXR-knockout mice is unclear.

In contrast to these data, Ross et al. [132] demonstrated that LXR agonists have no effect on fat storage, but they increase the release of nonesterified fatty acids from 3T3-L1 adipocytes, which suggests that LXR ligands stimulate lipolysis. Interestingly, release of glycerol was not stimulated because LXR agonists increased the expression of glycerol kinase, which phosphorylates glycerol for further metabolism. *In vivo*, specific overexpression of LXRα in adipose tissue reduced adipogenesis, whereas administration of T0901317 increased the plasma concentration of nonesterified fatty acids, evidence of enhanced lipolysis in adipose tissue. Taken together, these results [132] suggest a catabolic role of LXRα in adipose tissue.

4. Inflammation and Immune Response

4.1. Effect of LXR agonists on inflammatory cells

In 2003, Joseph et al. [80] first demonstrated that T0901317 and GW3965 reduced LPS-induced expression of several proinflammatory proteins, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), interleukin-1β (IL-1β), granulocyte colony-stimulating factor (G-CSF), and monocyte chemoattractant proteins 1 and 3 (MCP-1 and MCP-3) in mouse peritoneal macrophages. LPS injection induced a greater increase in hepatic synthesis of TNF-α, IL-1β, and iNOS and a higher circulating IL-6 level in LXRα/LXRβ double-knockout than in wild-type mice. It was suggested that the anti-inflammatory effect of LXRs is important when macrophages phagocytose apoptotic cells and cell debris. Large amounts of oxysterols derived from ingested material activate LXRs, thus increasing cholesterol efflux and protecting the phagocyte from cholesterol overload. Inflammatory reaction is concomitantly inhibited, because it is not desired when apoptotic cells are phagocytozed. On the other hand, when macrophages phagocytose bacteria or other pathogens which contain little or no cholesterol, LXRs are not stimulated, which allows the inflammatory reaction to develop; this reaction is desirable to protect against pathogens [80]. The mechanism through which LXR agonists suppress the transcription of proinflammatory genes is not clear, but may include inhibition of proinflammatory transcription factors such as nuclear factor-κB (NF-κB) or activator protein-1 (AP-1) [190,194].

Many subsequent studies demonstrated that other inflammation-related genes are also targeted by LXR agonists. Apart from COX-2, LXR agonists reduce the expression of microsomal prostaglandin E synthase-1 (PGES-1) [118]. PGES-1 is an inducible enzyme up-regulated during inflammation which contributes, together with COX-2, to enhanced eicosanoid production. In contrast, LXR agonists have no effect on macrophage COX-1, microsomal PGES-2, and cytosolic PGES, which are constitutive enzymes maintaining a continuous low-level PGE_2 synthesis for housekeeping functions. Thus, LXR agonists may target COX-2 while not affecting COX-1; such a high level of specificity cannot be achieved by pharmacological COX inhibitors.

Myeloperoxidase (MPO) is expressed in phagocytes and catalyzes the reaction between H_2O_2 and Cl^- to form a potent bactericidal and prooxidant agent, hypochlorite ($HClO^-$). Reynolds et al. [129] demonstrated that T0901317 reduces the expression of *MPO* gene in human macrophages. Human *MPO* gene contains a specific regulatory sequence called AluRRE consisting of four AGGTCA half-sites separated by 2, 4, and 2 nucleotides. The second and third half-sites create a canonical LXRE, the first and second half-sites bind the estrogen receptor, and the third and fourth half-sites form a PPAR response element. Both LXR and PPARα ligands suppress *MPO* gene transcription by inducing the binding of these receptors to respective parts of AluRRE. Interestingly, AluRRE is specific to primate, but not rodent *MPO* gene; consequently, the mouse *MPO* gene is not repressed by either LXR or PPAR ligands [129]. *MPO* gene is the first recognized gene whose transciption is suppressed directly by binding of the LXR/RXR dimer to its LXRE sequence. Suppressing MPO may lead to an antioxidant effect of LXR agonists, at least during the inflammatory reaction. In addition, because MPO-mediated oxidation of HDLs impairs cholesterol efflux [141], this effect may be essential for maintaining the stimulatory influence of LXRs on reverse cholesterol transport.

Osteopontin (OPN) is an extracellular matrix protein and a proinflammatory cytokine which stimulates monocyte migration, adhesion and activation. Ogawa et al. [119] demonstrated that GW3965 and T0901317 suppress cytokine-induced *OPN* gene expression in various murine and human macrophage cell lines. Mice treated with T0901317 exhibited a lower level of OPN mRNA in peritoneal macrophages than vehicle-treated controls [119].

Arginase decomposes L-arginine to ornithine and urea; the former is subsequently metabolized to polyamines. Arginase I is constitutively expressed only in the liver and initiates

the urea cycle, whereas arginase II is ubiquitously expressed and is up-regulated during inflammation. Arginase II interferes with iNOS by depleting their common substrate, L-arginine. In addition, polyamines stimulate tissue repair and contribute to the resolution of inflammation. In primary and cultured murine macrophages, LXR agonists increase arginase II mRNA and protein levels in a dose-dependent manner. The regulatory region of *arginase II* gene contains a classical LXRE sequence [108]. Because the adipose tissue-derived protein adiponectin inhibits the inflammatory reaction, the stimulatory effect of T0901317 on adiponectin receptor expression in human monocyte-derived macrophages [30] may also contribute to the anti-inflammatory activity of LXR agonists.

However, macrophages are not the sole target for the anti-inflammatory effect of LXRs. For example, GW3965 dose-dependently attenuated LPS-induced release of TNF-α and PGE_2 by hepatic Kupffer cells [177]. Walcher et al. [174] showed that T0901317 reduced the secretion of interferon-γ (IFN-γ), TNF-α, and IL-2 by Th1 lymphocytes, while having no effect on IL-10 production by Th2 cells. Lee et al. [94] recently demonstrated that 22(R)-HC and synthetic LXR or RXR agonists suppress iNOS expression and NO release from LPS-stimulated rat brain astrocytes. Similarly, LXR agonists reduced LPS-stimulated production of NO, IL-1β, IL-6, and MCP-1 by mousee astrocytes and microglial cells [196]. Finally, T0901317 inhibits the production of various cytokines, including GM-CSF, G-CSF, MCP-1, IL-6, and RANTES as well as reduces COX-2 expression in human airway smooth muscle cells [44]. In addition, LXR agonists suppress PDGF-induced migration and proliferation of these cells.

C-reactive protein (CRP) is a prototypical acute-phase protein synthesized by hepatocytes in response to proinflammatory signals, in particular IL-1β and IL-6. Increased plasma CRP promotes atherogenesis and is an independent predictor of acute cardiovascular events. T0901317 and GW3965 reduce cytokine-induced CRP production by human hepatocytes [15]. IL-1β and IL-6 activate *CRP* gene transcription by inducing the dissociation of the nuclear co-repressor from its promoter. LXR prevents this effect, thus maintaining the *CRP* gene in a repressed state. *In vivo*, T0901317 administered for three days attenuated an LPS-induced increase in hepatic synthesis and plasma concentration of CRP and serum amyloid P (SAP) protein [15]. The mechanisms of the anti-inflammatory effect of LXR agonists are listed in Table 1.

Table 1. Anti-inflammatory effects of LXR agonists

Suppression of proinflammatory genes	
Cytokines	TNF-α IL-1β IL-6 G-CSF
Chemokines	MCP-1 MCP-3
Adhesion molecules	ICAM-1
Enzymes synthesizing inflammatory mediators	COX-2 PGES-1 iNOS MPO
Matrix metalloproteinases and extracellular matrix proteins	MMP-9 OPN
Acute-phase proteins	CRP SAP
Stimulation of anti-inflammatory genes	
Arginase II Adiponectin receptors	

4.2. Cross-talk between LXR and TLR signaling

The innate immune system recognizes conserved motifs found in microbes through the so-called pattern-recognition receptors that include the Toll-like receptor (TLR) family of proteins. For example, bacterial lipids activate TLR2, double-stranded RNA stimulates TLR3, LPS activates TLR4, and TLR9 recognizes DNA. Castrillo et al. [26] first demonstrated that the activation of macrophage TLR3 or TLR4 during bacterial (*E. coli*) or viral (influenza A virus) infection compromises the LXR-induced expression of ABCA1, ABCG1, and apolipoprotein E and impairs cholesterol efflux. In contrast to TLR3 and TLR4, ligands of TLR2 or TLR9 as well as the proinflammatory cytokines TNF-α and IL-1β had no effect on LXR signaling in murine peritoneal macrophages [26]. On the other hand, LXR agonists attenuate TLR2- or TLR4-induced NF-κB activation, suggesting that significant cross-talk exists between LXR and TLR signaling. One of the possible mechanisms of this cross-talk is competition between LXR and the TLR3-induced transcription factor interferon regulatory factor-3 (IRF-3) for the limited pool of the common co-activator p300/CBP [26]. Given the antiatherogenic role of LXR (see below), the inhibition of LXR signaling by bacterial infection may contribute to the proatherogenic effect of some pathogens such as *Chlamydia pneumoniae* or *Helicobacter pylori*.

4.3. Effect of LXR agonists on the inflammatory reaction *in vivo*

The anti-inflammatory effect of LXR agonists was also demonstrated in several *in vivo* models. T0901317, GW3965, 25-HC, and 22(R)-HC applied topically on the mouse ear attenuated inflammatory reaction in experimental irritant contact dermatitis induced by phorbol esters [51,80]. The effect of LXR agonists was evidenced by a lower increase in ear thickness and weight as well as less inflammatory infiltrate in the epidermis. LXR agonists failed to attenuate the inflammatory reaction in LXRα/LXRβ double-knockout mice. Interestingly, the efficacy of LXR agonists in irritant dermatitis is comparable to that of locally administered glucocorticoids. In a related model of allergic contact dermatitis induced by oxazoline, LXR agonists were slightly less effective, but also exerted significant protection [51].

Experimental autoimmune encephalitis (EAE) is a $CD4^+$ T cell-mediated immune response to central nervous system

autoantigens and is an animal model of multiple sclerosis. Hindinger et al. [70] demonstrated that T0901317 administered for two days before immunization reduced the clinical severity of EAE in mice, which was accompanied by less T-cell infiltrate in the brain and a lower degree of demyelinization. Moreover, the expressions of IFN-γ, MMP-9, intercellular adhesion molecule-1 (ICAM-1), and TNF-α were lower in mice treated with LXR agonist than in control animals. Interestingly, the expression of class II MHC antigens was induced in microglial cells in the EAE model, which makes these cells capable of presenting antigen to lymphocytes. T0901317 reduced the expression of class II MHC antigens by gilal cells and consequently weakened the immune response [70].

In the rat, GW3965 administered 30 min before LPS injection attenuated the increase in plasma levels of alanine aminotransferase and bilirubin (markers of liver injury/dysfunction) and reduced hepatocyte necrosis. This effect was associated with reduced infiltration of mast cells in the liver and reduced gene expression of the chemokines eotaxin 1 and 2. Plasma levels of TNF-α and PGE_2 were also reduced by GW3965 [177]. These data indicate that LXRs protect against inflammatory liver damage. Finally, T0901317 reduced clinical severity, inflammatory reaction, cartilage damage, and bone destruction in an experimental model of rheumatoid arthritis induced in mice by administration of chicken collagen [31].

4.4. LXRs and immunity

LXRs are also involved in the regulation of nonspecific and specific immune responses. LXRα$^{-/-}$ and LXRα/LXRβ double-knockout, but not LXRβ$^{-/-}$ mice, are more susceptible to the intracellular pathogen Listeria monocytogenes, suggesting that LXRα plays an important protective role against this infection [79]. Indeed, animals lacking LXRα develop more severe infection, as demonstrated by a greater amount of bacteria in macrophages, more neutrophil abscesses in the liver, and higher mortality. The susceptibility to Listeria is reduced by transplantation of bone marrow from wild-type animals, suggesting that macrophage LXRα is crucial for the host response to infection. It was demonstrated that GW3965 increases the expression of the antiapoptotic gene *Api6* in wild-type mice [79]. Api6 is also up-regulated in wild-type, but not in LXR-null, macrophages infected with *Listeria*. The expression of LXRα, but not LXRβ, is strongly stimulated in macrophages infected with intracellular pathogens such as *Listeria* and *Shigella flexneri*, but it is only weakly stimulated by extracellular pathogens such as *E. coli* or *Staphylococcus aureus* [79]. These data suggest that LXRα is involved in the increase in Api6 expression during infection. Lack of LXRα interferes with the infection-induced up-regulation of Api6, which results in premature macrophage apoptosis. Consequently, phagocytosed pathogens evade killing and may infect other cells. Subsequently, it was shown that T0901317, GW3965, and 24(S),25-EC reduce the rate of apoptosis of bone marrow-derived macrophages in response to various stimuli, including translation inhibitor, cycloheximide, deficiency of macrophage colony-stimulating factor (M-CSF), serum deprivation, and infection with different pathogens such as *Bacillus antracis*, *E. coli* and *Salmonella typhimurium* [168]. Api6 is also up-regulated in cholesterol-loaded macrophages [5], presumably due to accumulation of cholesterol-derived LXR-activating oxysterols. Taken together, these data indicate that macrophage LXRα is up-regulated during infection, enhances the expression of Api6, and thus protects these cells from apoptosis. It should be noted that LXR agonists are characterized by a unique profile of activity among other anti-inflammatory agents in that they inhibit inflammatory and autoimmune reactions, but simultaneously stimulate a nonspecific immune response, at least toward intracellular pathogens. In contrast, other anti-inflammatory medications, such as glucocorticoids and cytokine antagonists, in general impair all types of immunity.

5. Effect of LXR Agonists on Atherogenesis

5.1. *In vivo* studies

Several studies have shown that LXR agonists reduce atherosclerotic lesions in animal models. LDL-receptor-knockout mice and apolipoprotein E-knockout mice are commonly used models of atherosclerosis. LDL-R$^{-/-}$ mice develop hypercholesterolemia and atherosclerosis only if fed a high-cholesterol diet, whereas apoE$^{-/-}$ mice are hypercholesterolemic and develop vascular lesions even on a chow diet. Joseph et al. [81] demonstrated that GW3965 reduces the size of aortic atherosclerotic lesions in apoE$^{-/-}$ and in male LDL-R$^{-/-}$ mice by about 50% and in female LDL-R-null animals by 35%. Interestingly, GW3965 had differential effects on plasma lipids in both models, i.e. it slightly reduced total cholesterol and had no effect on triglycerides or HDLs in LDL-R$^{-/-}$ mice, but had no effect on total or HDL-cholesterol and increased triglycerides in apoE$^{-/-}$ mice. These data suggest that GW3965 protects against atherosclerosis independently of changes in plasma lipids, presumably through its direct effect on the vascular wall. Similarly, T0901317 administered to LDL-R$^{-/-}$ mice for eight weeks dose-dependently reduced atherosclerotic lesions by up to 70% [159]. In that study, no changes in total plasma cholesterol were observed, but T0901317 transiently increased plasma triglycerides and to some extent corrected the fall in HDLs induced by an atherogenic diet. Importantly, T0901317 given to LDL-R$^{-/-}$ mice with developed atherosclerosis induced the regression of established aortic plaques by up to 70%. These data indicate that LXR agonists may be effective not only during lesion development, but also in established atherosclerosis. Interestingly, treatment with T0901317 beneficially affected the structure of aortic plaques, i.e. reduced macrophage and increased collagen content, thus increasing plaque stability and reducing the risk of rupture [98].

Specific overexpression of LXRα in the liver improves the plasma lipid profile and reduces atherosclerotic lesions by about 60% in LDL receptor-knockout mice fed a high-fat high-cholesterol diet [95]. In addition, Schuster et al. [137] demonstrated that cholesterol-loaded macrophages accumulate in the arterial wall, liver, and spleen of older (>1-year-old) LXRα/LXRβ double-knockout mice. Although these animals do not develop gross atherosclerosis, these data suggest that a lack of LXRs may enable the formation of foam cells.

Transplantation of bone marrow from LXRα/LXRβ double-knockout mice to LDL-R$^{-/-}$ or apo-E$^{-/-}$ mice aggra-

vates atherosclerosis in these animals despite reducing the plasma cholesterol level. Transplanted animals lack LXRs in macrophages, but have intact LXR signaling in other tissues. These data confirm that LXRs inhibit atherogenesis mainly through their effect on macrophage cholesterol efflux and/or the intravascular inflammatory reaction rather than by modulating the lipid profile [157]. In addition, transplantation of LXR-null bone marrow to LDL-R$^{-/-}$ mice abolished the antiatherosclerotic effect of T0901317 otherwise observed in these animals [98]. These studies indicate that macrophage LXRs are essential for the antiatherosclerotic effect of LXR agonists.

5.2. Mechanisms through which LXR agonists suppress atherogenesis

Apart from stimulating reverse cholesterol transport from macrophages/foam cells contained in the vascular wall and inhibiting th einflammatory reaction, LXR agonists may inhibit atherogenesis through several other mechanisms. For example, T0901317 and GW3965 suppressed platelet-derived growth factor- or insulin-induced proliferation of vascular smooth muscle cells by inhibiting cell cycle progression from G_1 to S phase [14]. LXR agonists reduced the expression of cyclin D1 and cyclin A, which stimulate cyclin-dependent kinases. These kinases normally phosphorylate retinoblastoma protein (Rb), which dedicates the cell to the S phase. LXR agonists reduced the extent of Rb phosphorylation in human aortic smooth muscle cells [14]. The proliferation of smooth muscle cells plays an important role in the growth of atherosclerotic plaque and is particularly involved in the development of vascular restenosis after angioplasty. Indeed, T0901317 reduced the vascular neointima formation induced by balloon angioplasty in the rat common carotid artery [14].

Increased plasma fibrinogen level is an independent risk factor of acute cardiovascular events. Xia et al. [188] showed that oxysterols reduce basal and IL-6-induced fibrinogen synthesis and secretion by HepG2 cells, while having no effect on the secretion of α1-antitrypsin, another acute-phase protein. However, this effect was not reproduced by T0901317, suggesting that it may be LXR independent.

Tissue factor (TF) is a cell surface glycoprotein which is a major initiator of coagulation. TF is abundant in a lipid-rich core of atherosclerotic plaque, and plaque rupture induces coagulation by exposing TF to circulating blood. T0901317 and GW3965 attenuated LPS-, TNF-α-, or IL-1β-induced TF expression in murine and human macrophages. *In vivo*, LXR agonists abolished the LPS-induced increase in TF expression in kidney and lung. Moreover, T0901317 reduced the amount of TF mRNA in atherosclerotic lesions of LDL-R$^{-/-}$ mice [158].

Matrix metalloproteinases (MMPs) are expressed in atherosclerotic lesions. These enzymes, by degrading extracellular matrix, promote the migration of smooth muscle cells and thus facilitate the growth of atherosclerotic plaque. In addition, MMPs, by degrading the fibrous cap of atherosclerotic plaque, contribute to plaque rupture. GW3965 reduced the basal and cytokine-induced synthesis and secretion of MMP-9 while having no effect on MMP-12 and MMP-13 in murine bone marrow-derived macrophages [25]. In addition, basal and TNF-α-induced MMP-9 expression was greater in macrophages isolated from LXRα/LXRβ double-knockout mice than in wild-type animals.

Recently, Chang et al. [27] demonstrated that LXR agonists attenuate the stimulatory effect of homocysteine (Hcy) on immunoglobulin production by B lymphocytes. Homocysteine enhances the B-cell response to antigen by increasing the intracellular production of reactive oxygen species (ROS), which subsequently activate nuclear factor-κB. LXR agonists attenuate the effect of Hcy on ROS and NF-κB activity [27]. Although this study relates to a specific function of immune cells, its results are of great interest, taking into account an important role of immune response in atherogenesis as well as an involvement of ROS and Hcy in cardiovascular pathology.

However, LXR agonists also have some effects which may be potentially proatherogenic. First, LXR agonists stimulate lipoprotein lipase (LPL) [195]. Although LPL metabolizes proatherogenic lipoproteins, its expression in macrophages may promote atherogenesis. In particular, LPL contained in the vascular wall is a bridging factor that links lipoprotein particles to the extracellular matrix of the subendothelial space and thus increases their residence time in the arterial wall, enabling their conversion into more atherogenic forms. Indeed, macrophage-specific overexpression of LPL aggravates atherogenesis [123]. Second, Davies et al. [42] demonstrated that LXR agonists stimulate lipogenesis in vascular smooth muscle cells by increasing SREBP-1c and FAS expression. Under prolonged LXR stimulation, vascular smooth muscle cells acquire the "adipocyte-like" phenotype and accumulate triglycerides and cholesterol esters originating from endogenous synthesis rather than from plasma lipoproteins [42]. LXR-activating oxysterols as well as 9cRA also stimulate the secretion of soluble phospholipase A_2 ($sPLA_2$) by rat aortic smooth muscle cells [4]. $sPLA_2$ is considered a proatherogenic factor since it generates proinflammatory mediators by hydrolyzing phospholipids contained in plasma lipoproteins.

Arai et al. [5] demonstrated that oxysterols contained in oxidized LDLs increase the expression of Api6 (also referred to as Spα or AIM from apoptosis inhibiting mediator) in cultured murine macrophages. *In vivo*, a high-cholesterol diet increases Api6 expression in the liver and atherosclerotic plaques of apoE$^{-/-}$ mice. However, this effect is not observed in mice tranplanted with LXR-null bone marrow. Stimulation of Api6 supports survival of macrophages. Knockout of the *Api6* gene in LDL-R-null mice increases the rate of apoptosis in atherosclerotic lesions and reduces lesion area [5]. Api6 is proatherogenic because the inhibition of apoptosis may accelerate the growth of early atherosclerotic plaque. However, the net effect of LXRs on macrophage apoptosis within the plaque is unclear, since Landis et al. [93] demonstrated that LXR agonists stimulate the synthesis and secretion of TNF-α, a major apoptosis-inducing cytokine, in human bone marrow-derived macrophages. Despite these uncertainties, the results of the previously mentioned *in vivo* studies clearly suggest that the net effect of LXRs is antiatherogenic. Moreover, inhibiting macrophage apoptosis in the developed lesion may be beneficial because it reduces the risk of plaque rupture.

Abundant expression of LXRα and LXRβ was observed in macrophages within human aortic atherosclerotic lesions, but not in the intact aortic wall [179]. Indeed, the expression of LXRα markedly increases during the differentiation of monocytes to macrophages. Thus, up-regulation of LXRs within atherosclerotic lesions may be an adaptive response aimed to limit cholesterol accumulation in foam cells and the inflammatory reaction in the vascular wall.

6. Other Roles of LXRs

6.1. Angiogenesis

Kaplan et al. [85] showed that natural and synthetic LXR agonists stimulate the expression of angiopoietin-like protein 3 (Angptl3). In addition, T0901317 or a high-cholesterol diet increased Angptl3 mRNA in the liver and plasma Angptl3 level in mice. Angptl3 may mediate, at least in part, the effect of LXR ligands on lipogenesis and plasma lipid profile (see part I of this review). Indeed, Angptl3-deficient KK/San mice are characterized by low plasma triglycerides and cholesterol levels despite being obese and diabetic, whereas overexpression of *Angptl3* gene or injection of Angptl3 protein increased plasma triglycerides [87]. Angptl3 increases plasma VLDLs mainly by inhibiting LPL activity [143], and the hypertriglyceridemic effect of LXR agonists is markedly reduced in Angptl3-knockout mice [75]. In addition to Angptl3, LXR agonists stimulate the expression of vascular endothelial growth factor (VEGF) in macrophages and adipose tissue [175].

6.2. LXRs and the kidney

LXRα is widely expressed along the nephron, at least in the rabbit [187]. LXR agonists increase ABCA1 expression and cholesterol efflux from rabbit glomerular mesangial cells [187]. Because overaccumulation of lipids in glomeruli is observed in many nephropathies, LXR agonists might have a nephroprotective effect, although this issue has not been directly addressed so far.

However, the most significant role of LXRs in the kidney seems to be their involvement in renin secretion. LXRα and LXRβ are abundantly expressed in renin-producing juxtaglomerular cells. As mentioned previously (see part I), cAMP increases renin gene expression by stimulating LXRα-CNRE binding [155,156]. More importantly, Morello et al. recently demonstrated that T0901317 and GW3965 increase renin mRNA in the kidney and plasma renin activity [114]. Although these agonists do not regulate LXR binding to CNRE [155], they may stimulate renin synthesis by up-regulating LXRα expression in the juxtaglomerular apparatus through an autoregulatory mechanism. In addition, the cAMP-dependent increase in renin synthesis induced by β-adrenergic stimulation is totally abolished in LXRα$^{-/-}$ and LXRα/LXRβ double-knockout, but not in LXRβ$^{-/-}$ mice [114], indicating that LXRα plays a pivotal role in the regulation of renin gene expression by the β-adrenergic system. In accordance with this, chronic administration of β-adrenergic agonists, a low-sodium diet, or clipping the renal artery, factors which stimulate the β-adrenergic system in the kidney, lead to the increased binding of LXRα to the renin gene promoter *in vivo* [114]. It has been demonstrated that in the absence of cAMP, a significant binding of LXRβ to renin CNRE occurs. In contrast, when the cAMP level is high, LXRβ dissociates from CNRE, whereas LXRα binds to it, leading to a more potent stimulation of renin synthesis. These data suggest that baseline and cAMP-induced renin synthesis are regulated by LXRβ and LXRα, respectively. Indeed, basal renin mRNA level in the kidney and plasma renin activity are lower in LXRβ$^{-/-}$ and in LXRα/β double-knockout mice than in wild-type or LXRα$^{-/-}$ mice [114].

In contrast to mice [114], GW3965 administered for 7–15 days had no effect on plasma renin activity in the rat [96]. In addition, this LXR agonist had no effect on blood pressure. However, GW3965 blunted the pressor response to acutely infused angiotensin II, and this effect was associated with lower expression of angiotensin II AT_1 and AT_2 receptors in the mesenteric artery [96].

6.3. Central nervous system

Both LXRα and LXRβ are expressed in the brain, although LXRβ is more abundant [183]. Several studies have demonstrated that LXR agonists increase the expression of its typical target genes, ABCA1, ABCG1, and apoE, in brain astrocytes, which suggests that LXRs may be essential for maintaining the brain cholesterol balance [54,99,183].

Abnormalities in cholesterol metabolism have been observed in patients with Alzheimer's disease (AD). In particular, hypercholesterolemia facilitates the processing of amyloid precursor protein (APP) by β- and γ-secretases to amyloid β (Aβ), the major component of amyloid plaques in patients with AD. Therefore, several groups addressed the effect of LXR agonists on Aβ formation and secretion, but the results of these studies are somewhat controversial. In Chinese hamster ovary (CHO) cells stably transfected with human APP as well as in H4 human neuroglioma cells, 22(R)-HC, and 9cRA reduced Aβ secretion [88]. T0901317 had a similar, but more potent, effect [89]. Similarly, 24(S)-HC and 27-HC reduced Aβ secretion from rat embryonic cortical neurons transfected with human APP [20]. T0901317 dose-dependently reduced Aβ secretion from mouse neuroblastoma Neuro2A cells expressing human APP [152]. In contrast, Fukumoto et al. [56] demonstrated that 22(R)-HC, T0901317, and 9cRA stimulate Aβ42 secretion by Neuro2A cells. These variable results may be associated with using different cell types and culture conditions; in particular, the presence of extracellular cholesterol acceptors such as apoA-I in the culture medium may be important, since the effect of LXR agonists on Aβ formation may be partially related to the stimulation of cholesterol efflux [89]. It has been observed that at low, nanomolar concentrations, T0901317 stimulates cholesterol efflux, but has no effect on Aβ formation [38]. At higher, micromolar concentrations, this LXR agonist increases the secretion of Aβ42, but has no effect on the other form, Aβ40. Interestingly, in a cell-free system, T0901317 inhibited Aβ production from APP by directly inhibiting γ-secretase [38]. Thus T0901317 may have some LXR-independent effects on amyloidogenesis.

Nevertheless, the majority of *in vitro* studies quoted above demonstrate the antiamyloidogenic effect of LXR agonists. Consistent with this conclusion, T0901317 administe-

red for six days reduced the amount of Aβ while increasing the amount of concurrent, α-secretase-derived, soluble, nonamyloidogenic Aα in mice expressing human APP [21,89]. Recently, Riddel et al. [130] demonstrated that T0901317 reduced $A\beta_{42}$ content selectively in the hippocampus, but not in other brain regions, and improved memory in a transgenic mouse model of Alzheimer's disease. Taken together, these data suggest that LXR agonists could provide a novel therapeutic option for patients with Alzheimer's disease.

Wang et al. [176] observed that severe neurodegeneration developed in the brain of aging (>one-year-old) LXRα/LXRβ double-knockout mice. In particular, the size of the lateral ventricles was markedly reduced in these animals due to periventricular accumulation of astrocytes and lipid deposition in choroid plexus epithelial cells. In addition, a significant loss of neurons was observed together with increased size and number of blood vessels, accumulation of lipofuscin in pericytes, microhemorrhages, and perivascular fibrosis. Subsequently, Anderson et al. [2] observed impaired motor coordination, loss of spinal cord motor neurons, and other features of amyotrophic lateral sclerosis (ALS) in seven-month-old male LXRβ$^{-/-}$ mice. The fact that neurodegeneration develops earlier in the life-course of LXR-deficient mice in the spinal cord than in the brain probably results from the higher rate of cholesterol synthesis in the spinal cord and the greater requirement for LXR-regulated cholesterol efflux. In addition, CYP46 (cholesterol 24-hydroxylase) expression is lower in the spinal cord, suggesting that other pathways of cholesterol removal, such as the classical LXR-stimulated ABCA1-dependent pathway, are more important here. These data indicate that LXRs are obligatory to maintain normal structure and function of the central nervous system.

6.4. LXRs and the reproductive system

Frenoux et al. [52] first noticed that male LXRβ$^{-/-}$ mice older than five-six months became less fertile than their wild-type controls. This was associated with significant structural abnormalities of the epididymis, including disruption of the epithelium, widening of the epididymal canaliculi, which became filled with an amorphous lipid-rich substance, and lipid loading of epithelial and interstitial cells. In addition, fewer spermatozoa were found in the epididymis of LXRβ$^{-/-}$ mice, and these spermatozoa exhibited structural and functional abnormalities. During transit through the normal epididymis, spermatozoa lose most of the cholesterol from their plasma membranes, which renders them capable of fusing with an oocyte. These data suggest that the LXR-driven cholesterol efflux from spermatozoa and/or epididymal epithelium is crucial for male fertility. These observations are consistent with reduced fertility of male ABCA1-null mice [138].

Subsequently it was demonstrated that large amounts of cholesterol esters accumulated in Sertoli cells of LXRβ$^{-/-}$ mice older than five months, which was accompanied by the degeneration of seminiferous epithelium. Similar abnormalities are observed in ABCA1-null and RXRβ-null mice as well as in some azoospermic males [109]. LXRα$^{-/-}$ mice exhibit small lipid droplets in Sertoli cells, but spermatogenesis and fertility are not impaired. However, the reproductive phenotype is more severe in LXRα/LXRβ double-knockout than in LXRβ single-knockout mice [131].

Interestingly, Frenous et al. [52] noted that the serum level of androgens was reduced in LXRα/LXRβ double-knockout mice. However, supplementation of androgens failed to improve epididymal histology and sperm quality, indicating that hypoandrogenism is not the sole factor responsible for compromised fertility. Although androgen-producing Leydig cells are histologically normal in LXRα/LXRβ double-knockout animals, testosterone production is reduced. Older double-knockout males exhibit hypergonadotropic hypogonadism, as evidenced by reduced serum testosterone and increased FSH and LH concentrations. The mechanism through which lack of LXRs impairs Leydig cell function is unclear. LXRs may regulate Leydig cell cholesterol balance and steroidogenesis directly. Otherwise, increased the corticosterone concentration observed in mice lacking LXRs might disrupt testosterone production [131]. Recently, Volle et al. [171] confirmed that hypoandrogenism exists in LXRα$^{-/-}$/LXRβ$^{-/-}$ double-knockout and in LXRα$^{-/-}$ single-knockout, but not in LXRβ$^{-/-}$ single-knockout mice, consistent with the expression of LXRα, but not LXRβ, in Leydig cells. However, in this study [171] hypoandrogenism was hypogonadotropic in nature, since the plasma level of LH as well as the expression of its β subunit in the pituitary were low. In addition, human chorionic gonadotropin (hCG) stimulated testosterone production by Leydig cells normally in mice lacking LXRβ, suggesting that the sensitivity of these cells to gonadotropin stimulation was intact. T0901317 administration increased testosterone content in the testes of wild-type mice, which was accompanied by increased expression of steroidogenic acute regulatory protein (StAR), which transports cholesterol to the inner mitochondrial membrane, and 3β-hydroxysteroid dehydrogenase-1 (3β-HSD-1), which converts pregnenolone to progesterone; both StAR and 3β-HSD-1 are key proteins in the early steps of steroidogenesis [171].

Apart from cholesterol efflux from Sertoli cells and/or maturing spermatozoa and androgen production, LXRs may regulate the balance between the proliferation and apoptosis of germ cells. In particular, the proliferation of germ cells is reduced in LXRβ$^{-/-}$ mice with a secondary reduction of apoptosis. In contrast, apoptosis is accelerated in LXRα$^{-/-}$ animals, but this is balanced by augmented proliferation [171]. Due to these adaptive changes, the ratio between proliferation and apoptosis is relatively intact in both single knockouts. In contrast, in double-knockout mice, proliferation is compromised and apoptosis is augmented due to the lack of LXRβ and LXRα, respectively, and these changes cannot be compensated, thus leading to severe impairment of spermatogenesis. This may explain why the reproductive phenotype is much more severe in double-knockout than in single-knockout males [171].

However, female LXR single- or double-knockout mice are also less fertile than their wild-type controls [148]. Among them, LXRβ$^{-/-}$ mice have the most severe phenotype, as evidenced by less frequent conception and lower number of pups per litter. In contrast to male animals, female LXRβ$^{-/-}$ mice exhibit no hormonal abnormalities, and the number of follicles in their ovaries is normal. However, LXRs seem to be essential for oocyte maturation. GW3965 stimulates the

resumption of meiosis of isolated oocytes, and the effect of FSH on oocyte maturation is impaired in LXRα/LXRβ double-knockout mice, although the expression of FSH receptors in their ovaries is normal [148]. In addition, FSH stimulates LXRα and LXRβ expression in the ovary. It is suggested that FSH acts on follicle cumulus cells and enhances the secretion of FF-MAS, which in turn stimulates oocyte meiosis by activating LXRs. According to this hypothesis, LXR-null oocytes are resistant to FF-MAS and thus cannot maturate. However, exogenous FF-MAS did stimulate meiosis of LXR-null oocytes (although less efficiently than of wild-type oocytes), suggesting that the effect of FF-MAS is not mediated exclusively by LXRs.

It should be noted that the lack of LXRs impairs fertility of aging, but not of young mice of both sexes. This closely resembles the frequently observed premature loss of fertility in humans and raises the important question if abnormal LXR signaling contributes to human infertility.

However, some data suggest that the role of LXRs in reproduction is not exclusively beneficial. In particular, 7-ketocholesterol and T0901317 inhibit the invasiveness of cultured cytotrophoblast cells *in vitro* [122]. In addition, LXRs mediate the inhibitory effect of oxidized LDLs on trophoblast invasiveness. If this effect is reproduced *in vivo*, LXR agonists might interfere with conceptus implantation. Moreover, inefficient trophoblast invasion may lead to subsequent impairment of placental perfusion, which is a main pathogenic factor in preeclampsia. Furthermore, LXR agonists reduce the synthesis and secretion of human chorionic gonadotropin (hCG) from trophoblast cells, which is mandatory for maintaining pregnancy in the first trimester [180]. The possible effect of LXR agonists on the effectiveness of implantation and the risk of abortion remains to be addressed in future studies; however, the previously discussed observations made in LXR-knockout mice strongly suggest that the net effect of LXRs on fertility is positive.

LXRβ$^{-/-}$ and LXRα$^{-/-}$/LXRβ$^{-/-}$, but not LXRα$^{-/-}$, mice accumulate cholesterol esters in uterine myometrial cells and exhibit reduced uterine contractility in response to oxytocin or luprostiol, a $PGF_{2\alpha}$ analogue. T0901317 stimulates ABCA1 and ABCG1 expression in the myometrium and increases cholesterol efflux from myometrial cells. Overaccumulation of cholesterol esters in the myometrium in mice lacking LXRβ results in impaired uterine contractility by disrupting the signaling from oxytocin and $PGF_{2\alpha}$ receptors, whereas the number of these receptors is not altered [115].

6.5. LXRs and adrenals

Both LXRα and LXRβ are expressed in the adrenal cortex. Moreover, 22(R)-HC, an intermediate in the conversion of cholesterol to pregnenolone, is present in adrenals at a relatively high concentration. LXRα$^{-/-}$ single-knockout and LXRα$^{-/-}$/LXRβ$^{-/-}$ double-knockout mice are characterized by adrenomegaly due to the overaccumulation of cholesterol esters in the adrenal cortex. In addition, the plasma corticosterone concentration is twofold higher in animals lacking LXRα, whereas aldosterone and ACTH levels are normal. *In vitro*, adrenocortical cells isolated from LXRα$^{-/-}$ mice secrete more corticosterone [37]. The adrenal cortex of LXR-null animals expresses less ABCA1 and ABCG1, but it contains increased amounts of three proteins essential for steroidogenesis: 1) CYP11A1 (cholesterol side-chain cleavage enzyme), which converts cholesterol to pregnenolone, 2) 3β-HSD1, which converts pregnenolone to progesterone, and 3) steroidogenic acute regulatory protein (StAR), responsible for cholesterol transport from the outer to the inner mitochondrial membrane, where CYP11A1 is localized. These data suggest that cholesterol efflux from adrenocortical cells is impaired in LXR-null mice, and this cholesterol is shifted toward the steroidogenic pathway; this results in the overproduction of corticosterone, the major glucocorticoid in rodents.

In wild-type mice, T0901317 increases the expressions of ABCA1, ABCG1, StAR, CYP11A1, and 3β-HSD1 in the adrenals. These data suggest that steroidogenic proteins are repressed under baseline conditions by unliganded LXRs and are up-regulated by both LXR-knockout and LXR agonists. Consequently, T0901317 increases plasma corticosterone in wild-type mice. Thus, LXRs protect adrenals from cholesterol overload by stimulating both cholesterol efflux and steroidogenesis [37].

In contrast to this study [37], Nilsson et al. [117] recently demonstrated that GW3965 reduces StAR and CYP11A1 expression in human adrenocortical and ovarian cells and decreases the production of pregnenolone, dehydroepiandrosterone, and androstenedione, suggesting a suppressive effect of LXRs on steroidogenesis. Moreover, these authors observed that GW3965 and T0901317 increase ACTH production by cultured anterior pituitary cells *in vitro* and increase plasma ACTH in wild-type mice *in vivo*, although baseline plasma ACTH is higher in LXRα$^{-/-}$/LXRβ$^{-/-}$ mice than in wild-type controls. These findings suggest that the increase in plasma corticosterone in both LXRα$^{-/-}$/LXRβ$^{-/-}$ and T0901317-treated wild-type mice reported earlier [37] may be accounted for by changes in ACTH level rather than by the direct effect on adrenals. Moreover, Nilsson et al. [117] showed that T0901317 stimulates ACTH production by suppressing 11β-HSD1 in the pituitary. This enzyme converts inactive cortisone to active cortisol in pituitary cells, which is necessary for the feedback inhibition of ACTH secretion. It is suggested that adrenal and pituitary LXRs play opposite roles in the regulation of adrenal steroidogenesis. Within the adrenals, when steroidogenesis is activated, 22(R)-HC is generated in greater amounts and stimulates LXRs, thus inhibiting steroidogenesis in a classical negative feedback manner. In contrast, activation of pituitary LXRs up-regulates ACTH, which then stimulates adrenal steroidogenesis through a cAMP-dependent mechanism. *In vivo*, the latter mechanism predominates since cAMP-dependent stimulation overrides the LXR-dependent inhibition of steroidogenesis in the adrenocortical cells [117].

6.6. LXRs and the skin

LXRβ and, to a lesser extent, LXRα are expressed in the epidermis. Human keratinocytes express CYP27 and thus may produce 27-HC. In addition, oxysterols may be formed in the skin by cholesterol oxidation initiated by ultraviolet light or air pollutants [68]. Thus all the components of LXR signaling exist in the skin.

22(R)-HC and 24(S),25-EC applied topically stimulate differentiation and reduce proliferation of keratinocytes [90]. LXR agonists inhibit DNA synthesis in proliferating keratinocytes and induce the expression of proteins involved in epidermal maturation, such as involucrin [136]. These data suggest that locally administered LXR agonists may be potentially useful in the treatment of skin diseases associated with hyperproliferation and impaired differentiation of epidermis, such as psoriasis.

In addition, LXR ligands accelerate epidermal barrier repair after disruption of the epidermis by acetone or tape stripping [136]. Oxysterols exert these effects in wild-type and in LXR$\alpha^{-/-}$ mice, but not in LXR$\beta^{-/-}$ mice, suggesting that LXRβ plays a predominant role in this context. In addition, LXR agonists stimulate the secretion of extracellular lipids which are key components of lamellar bodies, the major constituent of the stratum corneum of the epidermis [107]. Furthermore, LXR ligands accelerate the maturation of these extracellular lipids by up-regulating β-glucocerebrosidase, required for the conversion of glucosylceramides to ceramide, an essential step in the formation of a functional epidermal barrier.

Cholesterol-3-sulfonate is found in large amounts in the epidermis. It is formed in the basal and spinous layers, reaches its highest concentration in the granular layer, and then decreases in the stratum corneum. This "epidermal cholesterol sulfonate cycle" results from the concerted actions of two enzymes: cytosolic sulfotransferase type 2B1b (SULT2B1b), also referred to as cholesterol sulfotransferase (CST), and sterol sulfatase (STS), also called arylsulfatase C. This cycle plays an important role in epidermal function since cholesterol sulfonate accelerates keratinocyte differentiation, but inhibits desquamation; degradation of cholesterol sulfonate in the more superficial epidermal layers is essential for desquamation of epidermal cells. Impaired activity of STS leads to inherited X-linked ichthyosis, characterized by very high concentrations of cholesterol-3-sulfonate in the skin and blood. 22(R)-HC and 25-HC increase the expression of SULT2B1b in keratinocytes. The mechanism of this effect is unclear since the *SULT2B1b* gene does not contain the LXRE [76]. Increased formation of cholesterol-3-sulfonate may thus contribute to the enhancing effect of LXR agonists on keratinocyte differentiation. In addition, due to the observation that certain oxysterol sulfonates are LXR antagonists [146], one cannot exclude that such compounds are formed in the epidermis from the abundant cholesterol sulfonate by enzymatic and/or ROS-mediated hydroxylation.

Hanley et al. [67] demonstrated that 22(R)-HC injected intra-amniotically accelerates the maturation of the fetal epidermal barrier in the rat. Fluhr et al. [48] showed that 22(R)-HC, T0901317 and GW3965 also exert this effect if applied topically in rat pups and that this effect is associated, at least in part, with the acceleration of epidermal acidification, which occurs in the postnatal period [48]. Premature infants have an immature skin barrier and are therefore susceptible to dehydration and hypothermia. These studies [48,67] suggest that LXR agonists might be useful in the management of the epidermopathy of prematurity.

6.7. Cell proliferation

T0901317, 22(R)-HC, and 24(S)-HC inhibit the growth and cell cycle progression of cultured prostate cancer cells. Interestingly, androgen-insensitive cells (characteristic of more advanced stage of prostate cancer) are more sensitive to T0901317 than the more differentiated androgen-sensitive cells [55]. The expression of LXRα and LXRβ in prostate cancer cells decreases during tumor progression, which is accompanied by reduced ABCA1 expression and increased cholesterol content [34]. *In vivo*, T0901317 retards the progression of prostate tumor induced in castrated mice inoculated with prostate cancer cells [34]. In addition, T0901317 inhibited *in vitro* the proliferation of several other cancer cell lines, including breast cancer, lung cancer, osteoblastic tumor, epidermoid carcinoma, squamous cell carcinoma, cervical cancer, and hepatoma cells [35]. It should be noted that a part of the beneficial effect of T0901317 on prostate cancer cells may be LXR independent, since this compound is also a competitive antagonist of androgen receptors [33].

6.8. Detoxification and drug metabolism

Various endogenous (e.g. steroid hormones) and exogenous (e.g. drugs) hydrophobic compounds are hydroxylated in the liver by multiple cytochrome P450 isoforms. The expression of many of these enzymes is enhanced by the corresponding substrate through several xenobiotic-sensitive nuclear receptors such as constitutive androstane receptor (CAR) and pregnane X receptor (PXR). For example, phenobarbital up-regulates CYP2B2 expression in a CAR-dependent manner, whereas CYP3A is stimulated by rifampicin and dexamethasone, which are PXR ligands. Handschin et al. [66] demonstrated that LXR-activating oxysterols impaired phenobarbital-induced expression of CYP2B2 in hepatocytes due to the competition between the LXR/RXR and CAR/RXR heterodimers for a common DR-4 DNA sequence in the promoter region of the *CYP2B2* gene [10]. On the other hand, 24(S),25-EC, and T0901317 increased the expression of CYP3A in rat and mouse hepatocytes; however, this effect is mediated by PXR rather than LXR [142].

Aldo-keto reductase 1-B7 (AKR1-B7) is expressed in the liver, adrenals, and the small intestine. In the adrenal cortex, AKR1-B7 reduces isocaproaldehyde formed from the side-chain of cholesterol during its cleavage by CYP11A1 in the first step of steroidogenesis. It is suggested that intestinal AKR1-B7 contributes to the detoxification of alimentary lipid peroxidation products such as 4-hydroxynonenal to less toxic compounds. Orally administered T0901317 increases AKR1-B7 mRNA and protein levels in the mouse small intestine, which is accompanied by a decrease in lipid peroxidation products in the intestinal wall [172]. This suggests that LXRs may control the lipid peroxidation process and may thus contribute to antioxidant protection. In contrast, LXR agonists have no effect on hepatic AKR1-B7 under baseline conditions, but they attenuate the stimulatory effect of growth hormone on its expression. The effect of LXR agonists on adrenal AKR1-B7 has not been studied yet.

The role of LXRs in the detoxification processes is not confined to exogenous compounds. Indeed, activation of LXR protects the liver from damage induced by high levels of

Table 2. Changes in LXR signaling in pathological conditions

Expression of LXR	
Increased	Decreased
Metabolic syndrome/Diabetes mellitus • ↑ LXRα and LXRβ in pancreatic β cells in various animal models of diabetes [32,182] • ↑ LXRα in adipose tissue of severely obese humans [39] Atherosclerosis [179]	Metabolic syndrome/Diabetes mellitus • ↓ LXRα and LXRβ in the kidney of mouse models of T1DM [125] • ↓ LXRα in epididymal fat of Zucker rat [82] • ↓ LXRα in peripheral blood monocytes of T2DM patients [49]
Multiple sclerosis [59]	Multiple sclerosis [100]
Ageing	
Level of endogenous LXR ligands	
Agonists	
Disease	Changes in LXR ligands
Smith-Lemli-Opitz syndrome (SLOS)	↑ 27-HC in plasma ↑ 24(S)-HC in plasma ↑↑ 27-hydroxy-7-dehydrocholesterol*
Niemann-Pick type C disease	↓ oxysterol formation
Alzheimer's disease	↑ plasma 24(S)-HC (early phase) ↓ plasma 24(S)-HC (late phase)
Multiple sclerosis	↑ plasma 24(S)-HC (early phase) ↓ plasma 24(S)-HC (late phase)
Cholestasis	↑ plasma and urinary 24(S)-HC
Atherosclerosis	↑ plasma 27-HC
Antagonists	
Disease	Changes in LXR ligands
Obesity/diabetes mellitus	↑ non-esterified fatty acids in plasma
Diabetic ketoacidosis	↑ acetoacetate

* Only trace amounts of this compound are present in healthy subjects. T1(2)DM – type 1(2) diabetes mellitus. References are provided only for controversial findings.

bile acids by inducing sulfonation and urinary excretion of these toxic compounds [167].

6.9. LXR, ABCD2, and adrenoleukodystrophy

X-linked adrenoleukodystrophy (ALD) is an inherited disease characterized by the accumulation of very-long-chain fatty acids, which are not correctly oxidized in peroxisomes. Adrenoleukodystrophy results from a mutation of the *ALD* gene encoding an ABCD1 transporter involved in fatty acid transport to peroxisomes. Overexpression of a related transporter, ABCD2, also referred to as ALD-related protein (ALDRP), can compensate for ABCD1 deficiency in experimental studies. Cholesterol depletion stimulates *ABCD2* gene expression through an SREBP-2-dependent mechanism. In the promoter region of the *ABCD2* gene, SRE overlaps with the LXRE sequence. In the presence of LXR ligands, the LXR/RXR heterodimer binds to this sequence and blocks the stimulatory effect of SREBP-2. Therefore, ABCD2 expression is slightly increased in LXRα$^{-/-}$/LXRβ$^{-/-}$ double-knockout mice, whereas in wild-type animals it is suppressed by LXR agonists. These data suggest that LXR antagonists, by increasing the ABCD2 level, might be useful in the treatment of adrenoleukodystrophy [181].

7. Alterations of LXR Signaling in Pathological Conditions

Despite the many biological effects of LXR agonists described so far, little is known about changes in endogenous LXR signaling in pathological conditions. In particular, only a few studies have addressed LXR expression in human pathology or in respective experimental animal models of certain diseases. Relatively more data are available about alterations of endogenous oxysterols in various pathological states (Table 2).

7.1. Inherited disorders of cholesterol metabolism

Smith-Lemli-Opitz syndrome (SLOS) is a severe debilitating disease resulting from an inborn metabolic error in

the final step of cholesterol synthesis, the conversion of 7-dehydrocholesterol to cholesterol by 7-dehydrocholesterol 7α-reductase. Plasma and tissue concentrations of cholesterol are extremely low in SLOS patients. Bjorkhem et al. [13] demonstrated that plasma 27-HC is higher and 24(S)-HC is lower in patients with SLOS than in healthy controls. The increase in 27-HC probably results from its reduced metabolism by CYP7B1, whereas the rate of production and the metabolism to cholestenoic acid are normal. The mechanism of 24(S)-HC deficiency is less clear, but may involve reduced availability of cholesterol in the brain. Interestingly, the 7-dehydrocholesterol which accumulates in SLOS patients may be converted to 27-hydroxy-7-dehydrocholesterol by CYP27. Indeed, the plasma concentration of 27-hydroxy-7-dehydrocholesterol in SLOS patients is 0.01–0.25 μM vs. picomolar concentrations in healthy subjects. 27-hydroxy-7-dehydrocholesterol is a selective agonist of LXRα [178]. It remains to be established if these changes in oxysterol levels have any LXR-related implications for SLOS pathophysiology.

Niemann-Pick type C disease is a severe inherited disorder associated with cholesterol storage in the lysosomal compartment which results from the deficiency of either of the two proteins Niemann-Pick C1 (NPC1) or C2 (NPC2) involved in cholesterol trafficking to the endoplasmic reticulum (ER) from the late endosomal and lysosomal compartments [28]. Consequently, fewer oxysterols are formed in the ER of cells lacking NPC1 or NPC2 [53]. Apart from the overaccumulation of cholesterol in various tissues, Niemann-Pick type C disease is characterized by reduced plasma HDL-cholesterol and impaired ABCA1- and ABCG1-driven cholesterol efflux. Recently it was demonstrated that oxysterols or T0901317 stimulate cholesterol efflux from $NPC1^{-/-}$ fibroblasts, facilitate HDL formation, and deplete intracellular cholesterol stores [16]. These data indicate that impaired intracellular cholesterol trafficking results in reduced formation of endogenous LXR ligands in $NPC1^{-/-}$ cells and suggest that LXR agonists might be helpful in the treatment of Niemann-Pick type C disease.

7.2. Nervous system diseases

Due to exclusive production of 24(S)-HC in the brain, it is not surprising that its level is altered in neurological diseases. Lutjohann et al. [105] first demonstrated that plasma 24(S)-HC is moderately elevated in patients with Alzheimer's disease (AD) and non-Alzheimer's vascular dementia. However, later studies revealed that 24(S)-HC level exhibits time-dependent changes; it is transiently elevated in an early phase of the disease, reflecting enhanced brain cholesterol turnover, but is substantially reduced in the later phase, possibly due to progressive loss of neurons [19,121]. During the progression of AD, the amount of CYP46 in neurons decreases, which is partially compensated by its expression in glial cells [18]. A similar transition from elevated to depressed 24(S)-HC level is observed in patients with multiple sclerosis [97,160]. Liu et al. [100] demonstrated that the amounts of LXRα and LXRβ mRNAs were lower in monocytes of patients with multiple sclerosis than in a control group, but only in the population of Stockholm and not in the population of Sardinia. These data suggest that impaired anti-inflammatory LXR signaling may contribute to the development of multiple sclerosis in some ethnic groups. In contrast to this study [100], Giorelli et al. [59] observed increased expression of LXRβ in peripheral blood mononuclear cells of MS patients. However, the sensitivity to T0901317 was not enhanced, suggesting that a postreceptor defect in LXR signaling may exist in multiple sclerosis. The reason for these discrepant results [59,100] is not clear. However, it was suggested that increased expression of LXR may be an adaptive response to a reduced availability of its ligand, 24(S)-HC, in patients with multiple sclerosis [59]. It has also been shown that four single-nucleotide polymorphisms in the *LXRβ* gene correlated with the risk of late-onset Alzheimer's disease [1].

7.3. Obesity, diabetes mellitus, and metabolic syndrome

Uehara et al. [166] demonstrated that nonestrified fatty acids (NEFAs) and acetylacetate competitively inhibit 22(R)-HC-induced ABCA1 expression in cultured hepatocytes and macrophages. They suggested that increased concentration of NEFAs in patients with the metabolic syndrome and/or type 2 diabetes, by antagonizing LXRs, compromises cholesterol efflux from macrophages, ultimately leading to impaired HDL formation and reverse cholesterol transport. In addition, elevated acetoacetate in patients with diabetic ketoacidosis might have a similar effect.

Data concerning the level of LXR expression in animals and humans with diabetes and/or obesity are conflicting (Table 2). For example, Proctor et al. [125] observed reduced expressions of LXRα and LXRβ in the kidney in two mouse models of type 1 diabetes mellitus. Similarly, Juvet et al. [82] demonstrated that LXRα mRNA is reduced in epididymal fat of Zucker obese rats. Forcheron et al. [49] observed that the LXRα mRNA level in peripheral blood monocytes was lower in patients with type 2 diabetes than in healthy controls. On the other hand, the expressions of LXRα and LXRβ in skeletal muscle cells were similar in control and type 2 diabetic patients [86]. Similarly, Auboeuf et al. found no difference in LXRα or LXRβ expression in subcutaneous adipose tissue between control and either obese or type 2 diabetic subjects [8]. In contrast, Dahlman et al. [39] observed that LXRα, but not LXRβ, mRNA level was higher in subcutaneous and abdominal adipose tissue of severely obese (body mass index BMI >40 kg/m^2) subjects than in control individuals. Interestingly, it was observed that the lipogenic effect of LXR agonists in the skeletal muscle of patients with type 2 diabetes is enhanced in comparison with healthy control subjects because, in contrast to healthy individuals, these agents stimulate fatty acid synthesis, but not oxidation, in diabetics [86]. Importantly, whereas T0901317 increased insulin sensitivity of skeletal muscle cells in healthy patients, as evidenced by increased IRS-1 and PKB phosphorylation, no effect on insulin sensitivity was observed in diabetic myocytes [86].

Wente et al. [182] and Choe et al. [32] observed increased expression of LXRα and LXRβ in pancreatic β cells in various rodent models of diabetes, including Zucker fa/fa rats, OLETF rats, and db/db mice. Due to a possible lipotoxic effect of LXR agonists (see paragraph 2.5), overexpression of LXR in diabetic islets may contribute to β-cell dysfunction and damage.

7.4. Other conditions

The concentration of 24(S)-HC is markedly increased in the serum and urine of children with cholestasis [111]. Plasma concentrations of 27-HC and cholestenoic acid are increased in patients with atherosclerosis, which may reflect enhanced formation of these oxysterols in lipid-loaded foam cells in the atherosclerotic lesions [9]. The plasma concentration of cholesterol sulfonate is elevated in patients with liver cirrhosis and hypercholesterolemia [154]. Taking into account that certain oxidized cholesterol sulfonates are LXR antagonists [146] and that hypercholesterolemia is associated with oxidative stress, it is possible that an increase in sulfonated oxysterols could compromise LXR signaling. However, plasma-oxidized cholesterol sulfonates have not been measured in any pathological conditions. Finally, ageing is associated with a decrease in LXRα expression in various brain regions in male and female rats [135].

8. Therapeutic Potential of LXR Agonists

Given the potent effect of LXR agonists on HDL formation, cholesterol efflux, and inflammatory reaction, these compounds would be expected to be very helpful in the prevention and/or treatment of atherosclerosis. There are also other potential therapeutic applications, depicted in Table 3. However, the possible side effects should also be kept in mind, the most important of them being lipogenesis and its consequences, i.e. liver steatosis and/or hypertriglyceridemia. In addition, because unsaturated fatty acids are LXR antagonists, enhanced lipogenesis may limit the stimulatory effect on cholesterol efflux. Indeed, Sun et al. [151] demonstrated that LXR agonists induce SREBP-1c and SCD-1 expression in cultured macrophages and increase the production of fatty acids, which partially antagonize LXR-mediated ABCA1 expression and cholesterol efflux. Thus, specific LXR modulators with little or no effect on lipogenesis would be greatly desirable.

Several strategies may be applied to generate LXR ligands devoid of lipogenic potential. First, most data suggest that the lipogenic effect of LXR agonists in the liver is mediated mainly by LXRα. In contrast, macrophages contain both isoforms and both are involved in cholesterol efflux. Therefore, specific LXRβ agonists might specifically target cholesterol balance. Quinet et al. [127] demonstrated that synthetic LXR agonists stimulate ABCA1 expression and cholesterol efflux from peritoneal macrophages obtained from wild-type, LXRα$^{-/-}$, and LXRβ$^{-/-}$ mice, confirming that both receptors may mediate cholesterol efflux. *In vivo*, LXR agonists were less effective in increasing hepatic *SREBP-1c* gene expression in LXRα$^{-/-}$ than in wild-type mice and failed to increase plasma triglycerides in LXRα$^{-/-}$ animals, whereas the increase in HDL-cholesterol was comparable in both groups. Thus, selective activation of LXRβ effectively stimulates HDL formation and cholesterol efflux, but not hepatic lipogenesis. However, LXR agonists failed to stimulate *CYP7A1* gene expression in the liver of LXRα$^{-/-}$ mice, suggesting that the metabolism of cholesterol to bile acids may be ineffective following the administration of LXRβ-specific ligands. In addition, agonists with considerable selectivity for LXRβ over LXRα are not currently available, and the synthesis of such compounds will be a challenge since the ligand-binding domains of both isoforms show almost 80% homology of their amino-acid sequences.

The second possibility to generate LXR agonists devoid of lipogenic properties arises from the observation that, in contrast to nonsterol agonists, steroid LXR activators such as oxysterols are less potent in stimulating lipogenesis because they also inactivate SREBP (see part I of this review). Unfortunately, all known oxysterols are rapidly metabolized and their *in vivo* effectiveness is very limited. However, it cannot be excluded that synthetic sterol LXR agonists resistant to degradation will be synthesized in the future.

The third approach is to obtain gene-specific LXR modulators which will stimulate ABCA1 but not SREBP-1c. Such a strategy is theoretically possible because the cooperation between LXR, co-activators, and co-repressors may regulate various genes in a different manner. For example, in the absence of ligand, LXR inhibits the expression of ABCA1 (by binding co-repressors), but slightly stimulates that of SREBP-1c [173]. Therefore, loss of LXR results in moderate elevation of ABCA1 and depression of SREBP-1c. In theory, an agonist which induces the dissociation of co-repressors but does not recruit co-activators should stimulate ABCA1, but not affect, or even reduce, SREBP-1c. Among the experimentally used LXR activators, T0901317 increased plasma and liver triglycerides and exerted a potent effect on hepatic SREBP-1c and FAS expression, whereas GW3965 failed to elevate triglycerides in C57B1/6 mice. However, both agonists induced a comparable stimulation of ABCA1 in the liver and small intestine and similarly increased plasma HDLs [112].

The fourth potential approach to obtain non-lipogenic LXR agonists is to generate tissue-selective compounds which will act on macrophages, but not on hepatocytes. In particular, tissue selectivity may result from the pharmacokinetics of a specific compound. Plant sterols have been used for a long time to suppress the absorption of alimentary cholesterol. In general, it is believed that their effect results mainly from competition with cholesterol for intestinal absorption mechanisms. Taking into account that certain oxidized phytosterols activate LXRs and that such oxidized derivatives are detected in the plasma of healthy humans [61], one cannot exclude the possibility that phytosterols exert their beneficial effect in part by stimulating LXRs in the intestine or even in other tissues [23]. Kaneko et al. [84] demonstrated that the oxidized derivative of ergosterol YT-32 effectively inhibits cholesterol absorption by up-regulating intestinal ABCG5 and ABCG8, but does not induce hypertriglyceridemia or hepatic steatosis when given orally. This selectivity results from the fact that, like native phytosterols, YT-32 is rapidly removed from enterocytes to the gut lumen and its net absorption is very poor; thus the effect of YT-32 in the liver is limited. In contrast, nonsteroid LXR agonists such as T0901317 are not transported by ABCG5 and ABCG8. Although these results may suggest a novel strategy to search for nonlipogenic LXR agonists, it should be expected that the effect of compounds like YT-32 on macrophage cholesterol efflux will also be very limited.

Song and Liao [147] demonstrated that a cholestenoic acid derivative, hypocholamide, exerts a desirable effect on cho-

lesterol metabolism, i.e. it reduces total plasma cholesterol and hepatic cholesterol content without affecting plasma triglycerides. It is suggested that this selectivity is accounted for by a rapid glucuronidation of hypocholamide in the liver, which limits its effect on hepatic SREBP-1c while allowing stimulation of reverse cholesterol transport at the level of peripheral tissues. Interestingly, *in vitro*, hypocholamide is a relatively specific LXRα activator, which indicates that LXRβ selectivity is not mandatory for a beneficial pharmacological profile.

Quinet et al. [126] described a synthetic oxysterol, N,N-dimethyl-3β-hydroxycholamide (DMHCH), which effectively stimulated the expressions of hepatic and macrophage ABCA1 and ABCG1 while having only a moderate effect on SREBP-1c. Although this gene selectivity could be accounted for by its oxysterol structure (see above), DMHCH also antagonized the stimulatory effect of GW3965 on SREBP-1c expression, suggesting that it behaves as a mixed LXR agonist-antagonist. *In vivo*, DMHCH raised HDLs, but had no effect on plasma triglycerides, not only due to its gene selectivity, but also because its absorption from the intestine is limited. Indeed, orally administered DMHCH increased intestinal ABCA1 expression and HDL formation, but had little effect on hepatic ABCA1.

9. Effect of Currently Used Drugs on the LXR Signaling System

Whereas the application of specific LXR agonists in therapy is a matter of the future, LXR signaling may also be modulated by currently used drugs. Below are briefly characterized the effects of commonly used drugs on LXR signaling.

9.1. Statins

Statins are competitive inhibitors of HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis, which converts HMG-CoA to mevalonate. Initially introduced as cholesterol-lowering agents, statins exhibit many so-called "pleiotropic" effects associated with inhibiting the formation of other sterols as well as nonsteroid isoprenoids (see Figure 3 in part I of this review). Statins are in general well tolerated, but in some patients severe side effects are observed such as myopathy, hepatotoxicity, etc. Statins may inhibit the formation of various mevalonate derivatives, including geranylgeranylpyrophosphate, an LXR antagonist [50], and of LXR-activating oxysterols, and the overall effect of them on LXR signaling is thus difficult to predict.

Several *in vivo* studies addressed the effect of statins on plasma and/or cerebrospinal fluid (CSF) level of 24(S)-HC in humans, although none of them was specifically designed to address the statin-LXR relationship. Simvastatin (80 mg/day) administered for 6–24 weeks reduced the plasma 24(S)-HC concentration and the 24(S)-HC/total cholesterol ratio in 18 hypercholesterolemic patients [103]. In a larger group of hypercholesterolemic patients (n=150), different statins decreased plasma 24(S)-HC, but did not change the ratio between 24(S)-HC and total cholesterol [46]. In another study [104], simvastatin administered at 40 or 80 mg/day slightly reduced 24(S)-HC in the CSF, but had no effect on the ratio between 24(S)-HC and cholesterol in 44 patients with Alzheimer's disease. Similarly, various statins reduced absolute 24(S)-HC but not the 24(S)-HC/cholesterol ratio in the plasma of 31 patients with Alzheimer's disease [169]. Hoglund et al. [72] reported that simvastatin (20 mg/day for 12 months) had no effect on the absolute 24(S)-HC level in the CSF in patients with Alzheimer's disease, but increased the 24(S)-HC/cholesterol ratio. Thelen et al. [162] demonstrated that pravastatin administered for 6 months at 40 mg/day had no significant effect on plasma 24(S)-HC despite reducing total cholesterol, which resulted in a 15% elevation of the 24(S)-HC/cholesterol ratio. This may be associated with the hydrophilicity of pravastatin, which fails to permeate the blood-brain barrier and is thus unable to block cholesterol synthesis in the brain, which is a precursor of 24(S)-HC [163]. In contrast to 24(S)-HC, pravastatin slightly reduced plasma 27-HC but, due to a greater reduction of cholesterol, still elevated the 27-HC/cholesterol ratio [162]. In addition, atorvastatin (40 mg/day) or simvastatin (80 mg/day) administered for 2 months reduced absolute plasma 24(S)-HC and 27-HC but did not change their concentrations per unit of total cholesterol [161]. In patients with type 2 diabetes and hyperlipidemia, fluvastatin (20 mg/day for 12 weeks) reduced the total plasma oxysterol level by about 30%. Specific analysis of certain oxysterols revealed that most of them, including 25-HC, 27-HC, 7β-HC, 24(S),25-EC, and 7-ketocholesterol, were reduced by fluvastatin therapy [164]. Because fluvastatin reduced not only enzymatically, but also nonenzymatically formed oxysterols, a part of its effect could have resulted from the inhibition of oxidative stress.

Taken together, these results suggest that statins have only a minor reducing effect on endogenous oxysterols in plasma; this effect results most likely from the inhibition of cholesterol synthesis or the depletion of plasma lipoproteins which carry most of the oxysterols in the circulation. However, because oxysterols are formed intracellularly, the effect of statins on tissue oxysterol level is of greater significance. In the guinea pig, pravastatin (250 mg/day for 3 weeks) reduced 24(S)-HC concentration in the brain only by 17%, whereas simvastatin (150 mg/day for 3 weeks) had no effect [106]. In the rat, a high dose of simvastatin (100 mg/kg for 3 days), although effectively reducing brain cholesterol synthesis, had no effect on 24(S)-HC in the brain tissue, suggesting that cholesterol availability is not a rate-limiting factor for the formation of this oxysterol [163].

It is unclear if these minor, if any, effects of statin on endogenous oxysterols have any consequences for LXR signaling. Orally administered pravastatin had no effect on either hepatic or leukocyte ABCA1 in mice [3]. Moreover, Thelen et al. [163] observed that simvastatin elevated ABCA1 level in the rat brain; the findings are inconsistent with any impairment of LXR signaling. In addition, although CYP46A1 (cholesterol 24-hydroxylase)-knockout mice demonstrate learning impairment and abnormal long-term synaptic potentiation in the hippocampus, these abnormalities are not corrected by LXR agonist, suggesting that severe impairment of 24(S)-HC production in the brain does not cause functionally significant consequences of LXR hypofunction. Instead, signs of CYP46A1 knocko-

ut were corrected by geranylgeraniol, which indicates that feedback inhibition of the mevalonate cascade (due to impaired cholesterol efflux from the brain) and compromised geranylgeranylation of proteins was mainly responsible for these abnormalities [91]. The findings that statins have no deleterious effect on ABCA1-mediated cholesterol efflux may be interpreted in at least three ways: 1) statins, despite reducing cholesterol synthesis, have a negligible effect on oxysterols because a high cholesterol level is not a rate limiting factor for oxysterol formation, 2) statins reduce oxysterols slightly, but the physiological level of endogenous oxysterols is high enough to be not rate-limiting for LXR signaling, (3) the negative consequences of oxysterol depletion are balanced by another, opposite mechanism beneficial for LXR signaling, such as reduction of geranylgeraniol.

However, several *in vitro* studies have demonstrated a considerable impairment of LXR signaling by statins. Forman et al. [50] first demonstrated that mevastatin or lovastatin reduced the transcriptional activity of the LXR/RXR heterodimer in cultured cells and that this effect was reversed by mevalonate and by LXR-activating oxysterols, but not by farnesol or geranylgeraniol. These results suggest that, despite the bidirectional regulation of LXRs by geranylgeraniol and oxysterols, the depletion of oxysterols in statin-treated cells has a dominant effect [50]. In rat hepatoma cells, mevastatin (compactin) reduced *SREBP-1c* gene expression and this effect was reversed by mevalonate, T0901317, or 22(R)-HC [43]. Gouedard et al. [60] demonstrated that statins reduce the expression of paraoxonase-1 (PON1), an antioxidant and atheroprotective enzyme, in cultured human hepatocytes and that 22(R)-HC normalized PON1 synthesis, secretion, and activity. In murine RAW264.7 macrophages, pravastatin reduced the expressions of ABCA1 and ABCG1 and their levels were restored by mevalonate and 22(R)-HC [3]. However, in the same study, pravastatin had no effect on ABCA1 in human HepG2 hepatocytes, suggesting that sensitivity to statin-induced oxysterol depletion may be cell specific. Consistent with this notion, Sone et al. [145] observed that atorvastatin, fluvastatin, simvastatin, and lovastatin reduced the ABCA1 mRNA level by almost 90% in various human and murine macrophage cell lines, had no effect on ABCA1 in Swiss 3T3 fibroblasts and human embryonic kidney HEK293 cells, and increased ABCA1 by more than twofold in HepG2 cells. The inhibitory effect on ABCA1 in macrophages was reversed by mevalonate or 22(R)-HC, similarly to previous studies [145]. Recently it was demonstrated that atorvastatin suppresses not only LXR target genes, ABCA1, and apo-E, but also CYP27 in THP-1 macrophages [102]. Zanotti et al. [193] observed that compactin and pitavastatin impaired cholesterol and phospholipid efflux from murine peritoneal macrophages, which was accompanied by reduced ABCA1 expression, and that mevalonate as well as a mixture of 22(R)-HC and 9cRA reversed this effect. Reduction of ABCA1 by statins was also observed in cultured human keratinocytes [77]. Huq et al. [74] demonstrated that pravastatin reduced the expression of LXR target genes, retinal dehydrogenase-1 and -2, in cultured liver cells and in the liver *in vivo*.

It should be noted that statins may downregulate ABCA1 also in an LXR-independent manner. In particular, statins, by depleting intracellular cholesterol, activate SREBP-2, which is a negative regulator of ABCA1 [184]. Recently it was demonstrated [186] that statins at pharmacologically relevant concentrations downregulate ABCA1 and ABCG1 expression in human macrophages when the cholesterol content in these cells is low, but not when the cholesterol content is high. This inhibitory effect of statins was abolished by natural and synthetic LXR agonists [186]. This study may explain, at least in part, the discrepant results of previous experiments, which might have resulted from using macrophages with different degrees of cholesterol load.

While the effect of statins on cholesterol-derived oxysterols such as 24(S)-HC, 25-HC, or 27-HC is controversial, a more consistent effect of HMG-CoA reductase inhibitors should be expected on 24(S),25-EC, a pre-cholesterol product of the mevalonate cascade (see Figure 3 in part I of this review), since the rate of synthesis of this compound closely parallels the rate of cholesterol production rather than the steady-state cholesterol concentration. Wong et al. [185] showed that statins reduce the 24(S),25-EC level in primary and cultured macrophages, which is accompanied by decreases in ABCA1 and ABCG1 mRNA and protein levels and impaired apoA-I-mediated cholesterol efflux. The effect of statins on cholesterol efflux pumps was reversed by mevalonate as well as by exogenous 24(S),25-EC [3,185].

Argmann et al. [7] showed that atorvastatin stimulated apoA-I or HDL-dependent cholesterol efflux despite reducing 24(S),25-EC synthesis in human THP-1 macrophages. This effect was abolished by mevalonate, farnesyl pyrophosphate, and also by geranylgeranylpyrophosphate (which is not converted to cholesterol). In addition, protein prenyltransferase inhibitors mimicked the effect of atorvastatin. These data suggest that atorvastatin stimulates cholesterol efflux by attenuating isoprenylation of proteins. Specifically, the effect of atorvastatin was abolished by inhibitors of geranylgeranylated Rho proteins and Rho-dependent protein kinase (ROCK). Interestingly, PPARγ or LXR antagonists also abolished the beneficial effect of atorvastatin. Further studies revealed that atorvastatin, by reducing geranylgeranylpyrophosphate availability, reduces geranylgeranylation of RhoA protein, leading to decreased ROCK-dependent phosphorylation of PPARγ. Dephosphorylated PPARγ has greater activity and stimulates LXR expression, ultimately leading to up-regulation of ABCA1 [7]. These data indicate that statins may modulate LXR independently of affecting oxysterol production. Interestingly, atorvastatin administered for three weeks prevented age-related reduction of LXRα expression and increased its transcriptional activity in the rat liver [134]. Various mechanisms through which statins may regulate the LXR-ABCA1 pathway are presented on Figure 1.

Taking into account all these considerations, it seems that a combination of statins and LXR agonists might be especially useful in atheroprotection. Statins markedly reduce intracellular cholesterol synthesis and plasma LDL-cholesterol level and although they may raise HDL to some extent, this effect is rather limited. On the other hand, LXR agonists markedly increase HDL-cholesterol and, at the cellular level, stimulate cholesterol efflux. Such a combination may

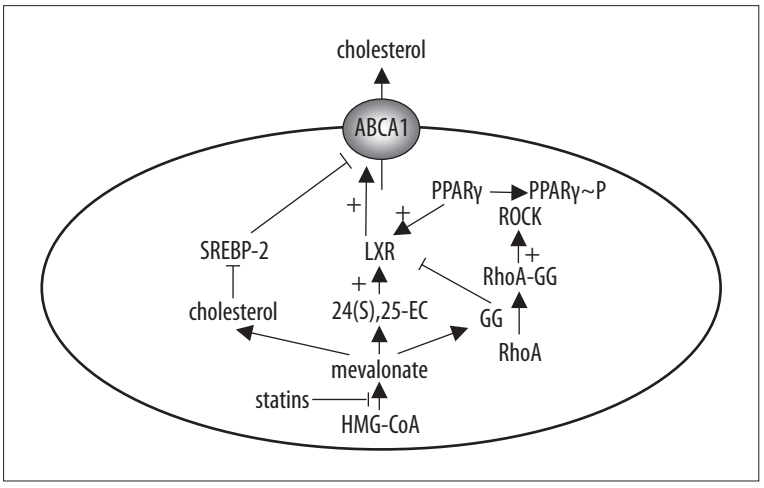

Figure 1. Mechanisms through which statins may modulate ABCA1 and cholesterol efflux in macrophages/foam cells. The expression of ABCA1 is stimulated by oxysterols acting on LXRs. Statins inhibit HMG-CoA reductase, which converts HMG-CoA to mevalonate, and thus reduce the availability of several mevalonate derivatives, including cholesterol, 24(S),25-epoxycholesterol (24(S),25-EC), and geranylgeraniol. Cholesterol inhibits the activation of sterol regulatory element-binding protein-2 (SREBP-2), which suppresses ABCA1 expression. Consequently, cholesterol stimulates ABCA1 and statins, by reducing cholesterol, tend to decrease it. In addition, statins tend to reduce LXR activity and ABCA1 expression by decreasing 24(S),25-EC concentration. On the other hand, statins tend to stimulate LXR by decreasing GG, an LXR antagonists. Moreover, reduction of GG interferes with the geranylgeranylation of RhoA, an activator of ROCK-dependent kinase (ROCK), which phosphorylates and inactivates PPARγ. Because active nonphosphorylated PPARγ stimulates *LXRα* gene expression, this latter effect of statins also favors the stimulation of LXR signaling and ABCA1 expression. The overall effect of statins results from the balance between at least these two stimulatory and two inhibitory mechanisms and depends on many factors, such as cell type, experimental conditions, pretreatment intracellular concentrations of relevant mevalonate derivatives, etc.

thus have a potentially very beneficial effect on the lipid profile and intracellular cholesterol balance. In addition, LXR agonists may prevent possible side effects of statins associated with depletion of the oxysterol pool.

Interestingly, although oxidosqalene: lanosterol cyclase (OSC) is involved in both the regular and shunt pathways of cholesterol synthesis (see Figure 3 in the part I of this review), it preferentially cyclizes dioxidosqalene. Therefore, at moderate concentrations, selective OSC inhibitor reduces cholesterol synthesis, but increases 24(S),25-EC synthesis, consequently enhancing ABCA1- and ABCG1-mediated cholesterol efflux in an LXR-dependent manner [133,185]. Thus OSC inhibitors may have very favorable pharmacological activity, simultaneously inhibiting cholesterol synthesis and stimulating the 24(S),25-EC/LXR pathway [11,170].

9.2. Fibrates

Fibrates are specific agonists of PPARα and are commonly used in the treatment of hyperlipidemia. Fibrates markedly reduce plasma triglycerides, slightly decrease LDL-cholesterol and elevate HDL-cholesterol. Taking into account the complex interactions between LXR and PPARα, it might be expected that fibrates will modulate LXR signaling. Apart from increasing LXR expression [165] and inducing a competition between PPARα and LXR for RXR [110], several other mechanisms of fibrates' action must be considered.

Very interesting data about the fibrate-LXR relationship were provided by Thomas et al. [164]. These authors demonstrated that fenofibrate, which is used in the form of an ester, is a potent LXR antagonist. Fenofibrate binds to the ligand-binding domain of LXR and displaces oxysterol ligands. Fenofibrate antagonizes the stimulatory effect of T0901317 on SREBP-1c and FAS in hepatocytes and reduces the basal levels of these proteins by antagonizing the effect of endogenous oxysterols. Interestingly, fenofibrate ester binds LXRs with greater affinity than PPARα [164]. *In vivo*, fenofibrate is rapidly hydrolyzed to fenofibric acid, which has no LXR-antagonistic activity. However, fenofibrate ester is detected in the liver of mice treated with fenofibrate, suggesting that LXR blockade may contribute to some of the pharmacological effects of this drug (e.g. reducing plasma triglycerides). Interestingly, fenofibrate has no effect on LXR-induced ABCA1 expression in the liver or macrophages, suggesting that its effect is gene specific. In contrast to fenofibrate, other fibrates are used as free acids which are inactive toward LXRs [164].

Johnson et al. [78] demonstrated that clofibrate and a nonfibrate PPARα agonist, Wy14643, markedly decreased LXR activity in cultured cells by reducing the formation of endogenous LXR ligands. The precise site of action was not identified, but it was downstream to HMG-CoA reductase since supplementation of mevalonate failed to reverse fibrates' effect [78]. Ciprofibrate reduces the expression of CYP7A1 and CYP27 in the liver both *in vitro* and *in vivo* [124]. The expression of the *CYP7A1* gene is inhibited because fibrates induce the formation of atypical PPARα/LXRα heterodimer, which binds to the specific LXRα/PPARα response element (LPRE) consisting of two adjacent hexameric nucleotide sequences within the regulatory region of *CYP7A1* gene and inhibits the stimulation of gene transcription by LXR agonists alone [57].

In contrast, Guan et al. [65] showed that clofibrate administered orally increased the concentrations of various oxysterols, including 7β-hydroxycholesterol, 5,6-epoxycholesterol, 7-ketocholesterol, 25-HC, and 27-HC, in rat hepatic

microsomes. Clofibrate also increased LXRα and ABCA1 expression in the liver. Because these effects of clofibrate were abolished by cytochrome P450 inhibitor, ketokonazole, it was suggested that the increase in oxysterols resulted from CYP-dependent oxidation of cholesterol. Indeed, CYP3A is induced by clofibrate in the liver [65]. Hepatic expression of CYP7A1 was reduced, presumably due to PPARα-dependent suppression of the *CYP7A1* gene promoter [57], but the level of its product, 7α-hydroxycholesterol, remained unchanged [124].

The overall effect of PPARα agonists on LXR signaling may thus be variable depending on the level of LXRs and PPARα and their coactivators in a given cell, the type, dose, and time of administration of PPARα agonist, and the target gene(s) of interest. For example, at least two studies [6,29] have demonstrated that PPARα agonists stimulate ABCA1 expression in cultured macrophages. On the other hand, the observation that fenofibrate blocks the effect of T0901317 on SREBP-1c, but not on ABCA1 [164], suggests that coadministration of fenofibrate might be useful in preventing lipogenesis induced by LXR agonists. Indeed, cotreatment with fenofibrate or Wy14643 and T0901317 caused a synergistic elevation of HDLs, but attenuated T0901317-induced hypertriglyceridemia and hepatic steatosis. This effect was partially mediated by PPARα-induced oxidation of fatty acids in liver peroxisomes [12]. Thus a dual LXR/PPARα agonsits may have a more beneficial pharmacological profile than pure LXR stimulators.

9.3. Thiazolidinediones

Thiazolidinediones (rosiglitazone, pioglitazone, and ciglitazone) are specific agonists of PPARγ and are used to improve insulin sensitivity in patients with type 2 diabetes. Similarly to PPARα, the relationship between PPARγ and LXR signaling is complex and the overall effect of PPARγ agonists on LXR target genes may be variable. However, most studies suggest that thiazolidinediones stimulate LXR signaling and a part of their beneficial effects may thus be mediated by an LXR-dependent pathway. First, PPARγ agonists increase the expression of LXRα both *in vitro* [71] and *in vivo* [187]. Consequently, pioglitazone and rosiglitazone increased ABCA1 expression and cholesterol efflux from cultured THP-1 macrophages [71]. Apart from modulating LXR expression, PPARγ agonists may influence oxysterol formation. Rosiglitazone and pioglitazone upregulated CYP27 and increased 27-HC and cholestenoic acid formation in primary and cultured human macrophages [102,128,153]. Rosiglitazone increased the expression of the LXR target genes ABCA1 and ABCG1 and this effect was not observed in cells obtained from patients with cerebrotendinous xanthomatosis, suggesting that it resulted from stimulation of LXR by CYP27-derived 27-HC [153]. The effect of thiazolidinediones on other LXR-activating oxysterols has not been studied so far.

Thiazolidinediones induce sodium and water retention in some patients. In addition, increased plasma renin activity and aldosterone level were reported in patients treated with these drugs [192]. Given the stimulatory effect of PPARγ on LXR signaling, it would be of interest to examine if LXR-induced renin secretion is involved in the fluid-retaining effect of thiazolidinediones.

9.4. Other drugs

The observations that n-3 and n-6 polyunsaturated fatty acids are LXR antagonists [120,191] suggest that a well-known hypotriglyceridemic effect of unsaturated fatty acids may be associated with inhibiting LXR-induced lipogenesis. This hypothesis is supported by the observation that n-3 PUFAs have no effect on HDLs, while most triglyceride-lowering therapies, such as weight loss, physical exercise, or fibrates, increase HDLs. It is possible that in the case of n-3 PUFAs, the tendency toward increased HDLs associated with lowering triglycerides is counterbalanced by inhibition of LXR signaling, which tends to reduce the HDL level.

Resveratrol, one of the most common flavonoids in red wine, increases LXRα expression dose- and time-dependently in cultured human macrophages, which is accompanied by stimulation of ABCA1, ABCG1, and apo-E levels [140]. Recently, Xia et al. [189] demonstrated that anthocyanins, pigments naturally occurring in the plant kingdom, increase the expression of LXRα by stimulating PPARγ in mouse peritoneal macrophages.

CYP3A4, involved in the formation of 4β-hydroxycholesterol, is a drug-induced CYP isoform. It was demonstrated that treatment with certain antiepileptic drugs such as carbamazepine, phenobarbital, or phenytoin, increased the plasma concentration of 4β-hydroxycholesterol up to 20-fold, whereas these drugs had no effect on nonenzymatically formed 4α-hydroxycholesterol [17]. In contrast, valproic acid, which does not activate CYP3A4, had no effect on 4β-hydroxycholesterol level. Interestingly, ursodeoxycholic acid, which is used in the treatment of gallstones, also induces hepatic CYP3A4 and increases plasma 4β-hydroxycholesterol by about 50% [17]. It remains to be established if the changes in 4β-hydroxycholesterol induced by these drugs cause overactivation of LXR. However, some studies indicate that chronic treatment with CYP3A-inducing antiepileptics results in the elevation of plasma triglyceride and HDL-cholesterol levels [22].

10. Conclusions and Future Perspectives

The identification of LXRs allowed us to understand many aspects of cholesterol metabolism. However, despite multiple experimental studies performed during the last decade, many issues still remain elusive. First, it is unclear what the specific targets for LXRα and LXRβ are and what the physiological role of such receptor redundancy is. Synthetic agonists selective for each isoform may be helpful in resolving this problem. However, synthetic LXR agonists differ from natural ones in that they are more potent and do not have some LXR-independent effects characteristic of oxysterols. Therefore, results obtained with compounds such as T0901317 may be misleading. Unfortunately, *in vivo* studies with natural LXR ligands are extremely difficult because oxysterols are rapidly metabolized and at high concentrations have many toxic effects.

Although there are many potential applications for LXR ligands as therapeutic agents (Table 3), no studies so far have addressed the effect of such agonists in humans. Most experiments were performed in mice and were relatively short lasting; agonists such as T0901317 and GW3965 were

Table 3. Therapeutic implications of LXR ligands

Diseases in which LXR agonists may be helpful	Metabolic disorders • Low plasma HDL level • Diabetes mellitus* • Niemann-Pick type C disease Cardiovascular diseases • Atherosclerosis* • Restenosis after angioplasty Inflammatory and autoimmune disorders • Rheumatoid arthritis* • Asthma, COPD • Multiple sclerosis* • Sepsis* Skin diseases • Allergic dermatitis* • Epidermopathy of prematurity* • Psoriasis Other conditions • Infertility • Impaired uterine contractility • Alzheimer's disease* • Cancer* • Hepatoprotection in severe cholestasis*
Potential side effects of LXR agonists	• Hypertriglyceridemia and elevation of LDL • Liver steatosis and damage • ↑ Fat storage in adipose tissue ? • Lipotoxicity • Pancreatic β-cell damage • Increased renin synthesis • Reduced invasiveness of cytotrophoblast cells (? impaired conceptus implantation, reduced placental perfusion, preeclampsia) • Reduced hCG production (? ↑ risk of abortion)
Diseases in which LXR antagonists may be helpful	• Hypertriglyceridemia • Liver steatosis • Adrenoleukodystrophy

* Diseases, in which the beneficial effect was demonstrated *in vivo* in experimental animals.

rarely administered longer than a week. Therefore, the potential long-term side effects remain to be recognized. The widespread expression of LXRs in most tissues suggests that such effects are very likely (Table 3). Moreover, there are multiple differences between LXR targets in mice and humans. In contrast to mice, human CYP7A1 is not regulated by LXR. On the other hand, human, but not mouse, LXRα is autoregulated by its own agonists; therefore, any effect of such agonists will be amplified in humans. Human CETP is up-regulated by LXR, whereas mice do not produce CETP at all. These and other problems in the research of this field make a potential therapeutic application of LXR agonists a matter for the future. However, unraveling LXR signaling helps to understand the mechanisms of action of some currently used drugs such as statins, fibrates, and thiazolidinediones. Finally, in some disorders such as adrenoleukodystrophy and hypertriglyceridemia, LXR antagonists may be a therapeutic option.

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