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Review

Liver X receptors in cardiovascular and metabolic disease

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Abstract. Liver X receptors (LXRs) α and β are nuclear oxysterol receptors and metabolic sensors initially found to regulate cholesterol metabolism and lipid biosynthesis. Recent studies have elucidated the importance of LXR in the development of cardiovascular diseases and metabolic disorders. LXR agonists prevent development of atherosclerosis by modulation of metabolic as well as inflammatory gene expression in rodent models. Moreover, LXR activation inhibits hepatic gluconeogenesis and lowers serum glucose levels, indicating possible application of LXR activation in the treatment of diabetes mellitus. However, first-generation LXR agonists elevate hepatic and serum trigylceride levels, making subtype-specific agonists and selective LXR modulators rather than unselective LXR agonists a potential pharmacological strategy. This review summarizes the multiple physiological and pathophysiological implications of LXRs and observations that identify LXRs as potential targets for therapeutic interventions in human cardiovascular and metabolic disease.

Key words. Liver X receptor; macrophages; atherosclerosis; cardiovascular disease; diabetes mellitus; metabolic syndrome.

Introduction

Liver X receptors (LXRs) belong to the nuclear receptor superfamily of proteins. Many nuclear receptors are implicated in the regulation of genes involved in essential metabolic pathways and developmental processes [1–4]. Nuclear receptors are transcription factors that are activated by ligand binding. Many of them are transcriptionally active as heterodimers with the retinoid X receptor (RXR) and some of them – including the LXR/RXR heterodimer – are 'permissive' in that they can also be activated by an RXR ligand alone [5]. Several RXR heterodimer partners were cloned on the basis of sequence homology or biological function prior to discovery of their ligands and were therefore classified as orphan nuclear receptors [5]. Over the past several years, chemical, structural and genomic technologies have rapidly advanced our understanding of orphan nuclear receptors. To date, endogenous ligands have been identified for a number of them [6]. In many cases, ligands turned out to be intermediates or end products of metabolic pathways that are regulated by these receptors [7]. Putative endogenous ligands of LXRs are oxidized cholesterol derivatives (oxysterols). In contrast to nuclear hormone receptors but similar to other orphan nuclear receptors involved in metabolic regulation, oxysterol binding to LXR occurs at relatively low affinity, consistent with the higher physiological concentration of these ligands.

Cardiovascular diseases elicited by atherosclerosis and type 2 diabetes mellitus are leading causes of morbidity and mortality in industrialized countries. These diseases are associated with substantial abnormalities in metabolic and inflammatory pathways and are based on genetic as well as environmental factors [8]. Recent data indicate multiple implications of LXRs in metabolic and in-

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flammatory pathways that are involved in pathogenesis of cardiovascular and metabolic diseases.

This review focuses on the current understanding of physiological and pathophysiological roles of LXRs. Based on available data, LXR appears to be an interesting target for the development of sophisticated drugs with potential extraordinary actions in cardiovascular and metabolic disorders.

Nuclear receptors

Forty-eight members of the nuclear receptor superfamily exist in humans [9]. Most members of them are traditionally defined as ligand-activated transcription factors that contain several canonical structural components: an N-terminal region that often contains a ligand-independent activation function (AF-1), a central DNA-binding domain containing two zinc fingers, a hydrophobic C-terminal ligand domain that mediates ligand recognition and receptor dimerization, and a ligand-dependent transcriptional activation function (AF-2).

A subset of nuclear receptors and particularly several orphan receptors requires heterodimerization with the RXR for DNA binding [5]. Many RXR heterodimers are constitutively resident in the nucleus and do not have to shuttle between the cytoplasm and nucleus as do, e.g., steroid hormone receptors. The transcriptional activity of RXR heterodimers is induced by ligand binding, which changes the structure of RXR heterodimers, leading to displacement of corepressors and facilitating interaction with coactivator proteins, thereby promoting gene transcription [10]. Biochemical and expression cloning approaches have identified factors that interact with nuclear receptors in either a ligand-dependent or a ligand-independent manner as components of large multiprotein complexes. These coactivator complexes act in a sequential and/or combinatorial manner to reorganize chromatin templates and to modify and recruit several factors, such as DNA-dependent ATPases; histone acetyltransferases; and TFIID, the general transcription factor complex of TATA-binding protein and RNA polymerase II [11, 12]. Nuclear receptors primarily activate gene expression through direct association with specific DNA sequences known as hormone response elements (HREs) [13, 14].

LXR activation

The LXR subfamily of nuclear receptors contains two members, LXRα (NR1H3) [15] and LXRβ (NR1H2) [16–19]. LXRα and β share the canonical nuclear receptor structure [15, 20], and LXR/RXR heterodimers bind to response elements (LXREs) containing the hexameric sequence (AGGTCA) separated by four nucleotides (DR4 element) [15, 16, 20–22]. LXR/RXR heterodimers are characterized by the ability to be activated by either ligand in an independent manner. Thus LXR/RXR heterodimers are activated by the RXR ligand, e.g., 9-cis retinoic acid, the LXR ligands, e.g. oxysterols, or are activated synergistically in the presence of ligands for both receptors [20] (fig. 1a). Whereas the two LXRs share sequence homology and appear to respond to the same ligands, their tissue distribution differs considerably. LXRα is highly expressed in liver, adipose tissue and macrophages, whereas LXRβ is expressed in essentially all tissues examined [7].

Putative endogenous activators of LXRs are oxidized cholesterol derivates (oxysterols). The most potent natural activators are 22-(R)-, 20-(S)-, 24-(S)-hydroxycholesterol and 24-(S),25-Epoxycholesterol, which induce LXR transcriptional activity at physiological concentrations [23, 24]. 24-(S),25-Epoxycholesterol is particularly abundant in the liver, where both cholesterol metabolism and LXR expression are high. However, the oxysterols mentioned above appear not to be present in cholesterol-loaded human macrophages in which LXR action is of particular importance with respect to atherogenesis [25]. In these cells sterol 27-hydroxylase (CYP27) abundantly generates 27-hydroxycholesterol, representing the putative endogenous LXR ligand in these cells [25]. Thus, depending on their relative concentrations, different oxysterols may represent the most important physiological LXR ligands in a particular cell or pathology [26]. Most endogenous LXR ligands identified so far activate both LXRα and β with the exception of 5,6 24-(S), 25-diepoxycholesterol and 6α-hydroxy bile acids [27], which are somewhat selective for LXRα. In addition to endogenous ligands, a number of synthetic LXR ligands have been developed. The compounds T0901317 and GW3965 activate both LXRα and LXRβ [28, 29]. Coactivators implicated in transactivation by LXRs include Grip1, a p160 coactivator [30, 31], transcription domain-associated protein (TRRAP) [32] and peroxisome-proliferator-activated receptor (PPAR)γ coactivator 1α (PGC-1α) [33]. PGC-1α is not only a key regulator of hepatic gluconeogenesis [34] but serves as a coactivator for the LXRα and is hence also implicated in the regulation of cellular cholesterol homoeostasis [33]. Interestingly, LXR agonist treatment decreased expression of PGC-1α in murine liver, resulting in decreased expression of gluconeogenic enzymes [35]. Additional work will be required to decipher the relationship between LXR and PGC-1α. In the absence of ligands, nuclear receptors may repress gene transcription by recruiting corepressor proteins such as the nuclear receptor corepressor (NCoR) [36] and the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) [37–39] (fig. 1b). Transrepression by LXRs depend on their interaction with NCoR and SMRT in macrophages and human hepatocyte cell lines [38, 39]. Enhanced basal

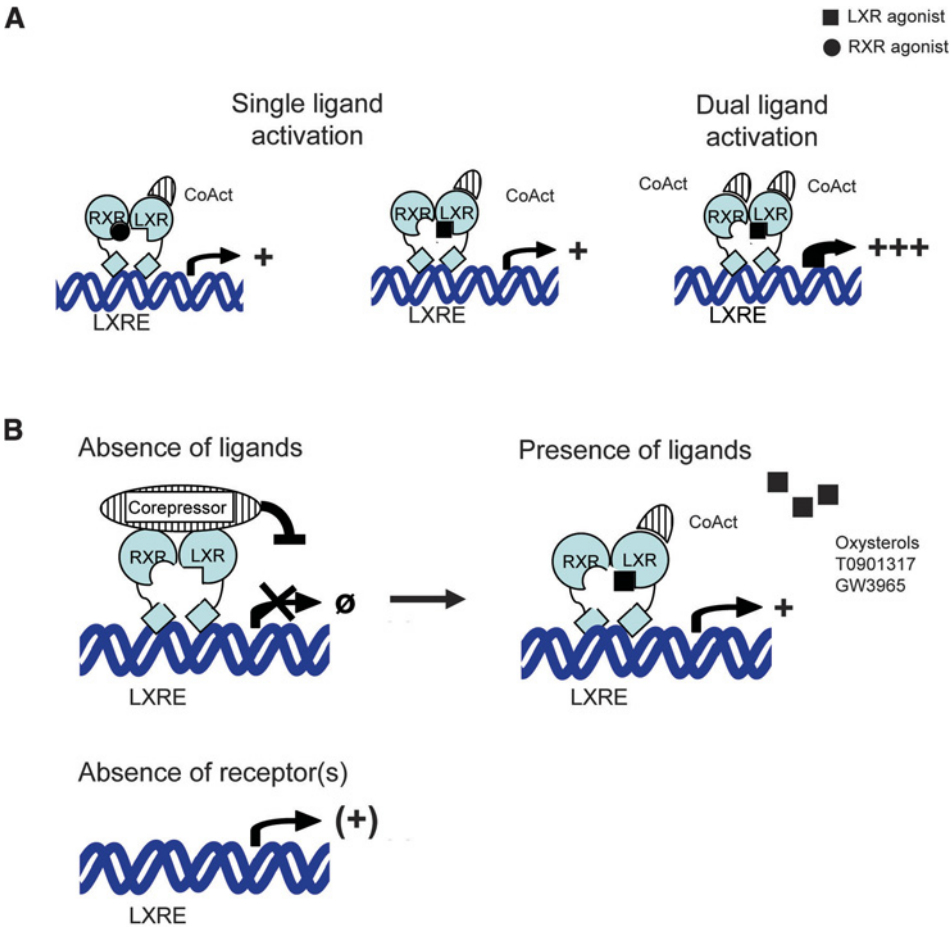

Figure 1. Transcriptional regulation mediated by LXRs. (*A*) LXR functions as an obligate RXR heterodimer that binds to its response element, the LXRE, within the promoter region of target genes. The LXR/RXR heterodimer is a permissive heterodimer and is hence activated by either LXR ligands such as oxysterols or synthetic ligands (filled rectangles), an RXR ligand such as 9-cis-retinoic acid (filled circles) or synergistically by ligands of both receptors. Upon ligand binding, the heterodimer undergoes a conformational change that recruits coactivators (CoAct) and results in the transactivation of LXR target genes. (*B*) Left: In the absence of ligands, corepressors may be recruited to LXR target genes and result in inhibition of target gene expression. Right: In the presence of ligands, corepressors are released and coactivators are recruited, leading to induction of LXR target genes. Bottom: In the absence of receptor(s), basal repressive effects of the corepressor complex bound to the LXR/RXR heterodimer might be absent depending on target gene and cell type.

expression of some LXR target genes in certain cells, including macrophages from LXR$\alpha^{-/-}\beta^{-/-}$ mice compared to wild-type animals, indicates that in the absence of receptor(s), basal suppressive effects of the corepressor complex bound to the LXR/RXR heterodimer might be absent [28, 38] (fig. 1b).

In contrast to oxidized cholesterol metabolites that enhance transcriptional activity of LXRs, geranylgeranyl-PP, an intermediate of cholesterol biosynthesis, inhibits LXRα and β by interfering with coactivator interaction [40, 41]. Similarly, unsaturated fatty acids antagonize LXR activation by competing with agonist binding, thus preventing the binding of LXR/RXR heterodimers to the LXRE [42, 43]. Moreover, human blood plasma contains distinct oxidized cholesterol 3-sulfates that could represent natural antagonists for LXRα and β [44, 45]. Thus, endogenous LXR antagonists could counteract LXR agonist action in cholesterol homeostasis and atherogenesis.

Regulation of LXRα expression

The expression of LXRα in liver, adipose tissue, muscle and macrophages is regulated by nuclear receptors, particularly PPARs and LXRα itself.

Several studies have demonstrated the existence of an autoregulatory loop controlling the expression of LXRα, but not LXRβ [46–48]. Interestingly, the LXRα autoregulatoryloop appears to be limited to human cells, and does not occur in murine macrophages or preadipocytes [46]. Autoregulatory upregulation of LXRα expression could be particularly important during lipid accumulation in human macrophages, when LXRα is dramatically up-

regulated and becomes the predominant LXR isoform, in contrast to resting macrophages [46].

In addition to LXR autoregulation, PPARs were shown to affect LXRα expression. Agonists of PPARα and γ induce LXRα expression in murine and human macrophages [49, 50]. Accordingly, oxidized low-density lipoproteins (LDLs), which include agonists of PPARα and γ [51, 52], have been found to increase LXRα expression [53]. Unsaturated fatty acids not only interfere with LXR activation as detailed above, but also induce LXRα expression, indicating a complex role of fatty acids in LXRα regulation [54]. Fatty acid induction of LXRα expression appears to involve PPARα at least in hepatocytes. Insulin increases hepatic LXRα messenger RNA (mRNA) levels both *in vitro* and vivo even though primarily by elevating its transcript half-life [55]. However, since insulin activates PPARα [56], it could be speculated that PPARα contributes to the insulin-mediated increased LXRα mRNA expression. Thus, the PPAR family of nuclear receptors appear to be involved in the regulation of LXRα expression [26].

LXR and metabolism

Cholesterol and lipid metabolism

LXR activation by oxysterols suggests a regulatory function in cholesterol homeostasis. The first known direct target gene for LXRs in mice was Cyp7a1. Cyp7a1 encodes the rate-limiting enzyme cholesterol 7-α-hydroxylase (CYP7a1) in hepatic bile acid synthesis and is upregulated in response to cholesterol-rich diet. Mangelsdorf and colleagues showed that LXR$\alpha^{-/-}$ mice fail to induce CYP7a1 expression in response to high cholesterol diet [57]. Analysis of LXR$\alpha^{-/-}\beta^{-/-}$ or LXR$\beta^{-/-}$ mice revealed that LXRα is the primary player in hepatic lipid metabolism, though a functional overlap could exist between both LXRs [58]. However, the LXRE in the Cyp7a1 gene is mutated in humans, so that a role of LXRs in human bile acid synthesis cannot be inferred [59, 60].

A number of LXR target genes are implicated in the regulation of reverse cholesterol transport by which excess cholesterol is transferred from peripheral tissues to the liver by use of HDL particles. For instance, LXR agonists upregulate the expression of the adenosine triphosphate-binding cassette (ABC) proteins A1 and G1 [61–66]. ABCA1 serves as free-cholesterol and phospholipid translocator, enabling cholesterol efflux from the cells to various acceptors, including nascent cholesterol-poor high-density lipoproteins (HDL) and circulating lipid-free apolipoprotein (apo)AI [60, 61, 64, 67–70]. In contrast, cholesterol efflux via ABCG1 uses HDL_3 as an acceptor [70] (fig. 2). LXR agonist treatment enhances ABCA1-dependent reverse cholesterol transport and elevates HDL serum concentrations [61, 63, 65, 71]. Homozygous mutations of ABCA1 in humans are the basis for Tangier's disease, which is characterized by cholesterol accumulation in peripheral tissues along with near total lack of cellular cholesterol efflux and plasma HDL. Patients with Tangier's disease have a four- to sixfold increased risk of atherosclerotic cardiovascular disease, compared with age-matched controls [72–76]. Even subjects heterozygous for ABCA1 deficiency have a threefold increased risk for coronary artery disease [77]. Accordingly, bone marrow transplantation studies have shown that selective inactivation of ABCA1 in macrophages increases atherosclerosis in mice without altering plasma cholesterol levels [78, 79]; however, ABCA1 knockout mice do not generally bear a higher risk for atherosclerosis [80].

LXR activation also upregulates expression of Niemann-Pick C proteins, leading to enhanced intracellular cholesterol trafficking to the plasma membrane in primary human macrophages [81]. Since cholesterol trafficking to the plasma membrane is an important prerequesite for cholesterol to become available for extracellular acceptors, this effect contributes to the overall action of LXR agonists to promote cholesterol efflux from macrophages.

Notably, other ABC transporters involved in cholesterol homeostasis are also targets of LXR regulation, namely ABCG5 and ABCG8. The induction of hepatic ABCG5 and ABCG8 promotes cholesterol excretion into bile [82, 83]. ABCG5 and ABCG8 expression is enhanced by LXR agonists in mice in a receptor-dependent manner [84]. In accordance with these changes, biliary cholesterol content is increased [28, 85]. Similar changes have been observed in transgenic mice overexpressing the human ABCG5 and ABCG8 genes [83]. Finally, the ability of LXR ligands to stimulate biliary cholesterol secretion is preserved in mice lacking ABCA1, consistent with an important role for ABCG5 and ABCG8 in this process [85]. Genetic deficiency of these transporters causes abnormal absorption of sitosterols (plant sterols) and a hyperabsorption of cholesterol, leading to sitosterolemia, a rare genetic disorder.

ApoE is another gene directly regulated by LXR that is involved in cholesterol homeostasis [62]. Notably, LXREs were found in macrophage and adipocyte enhancer regions that are responsible for tissue-specific expression [62]. ApoE was the first gene shown to be regulated by LXR/RXR heterodimers in a tissue-specific manner [62]. LXR mediates lipid-inducible expression of the apoE gene in adipose tissue and macrophages but not in liver. ApoE is a principal protein component of very low density lipoprotein (VLDL) and chylomicron remnants and involved in mediating their hepatic uptake. In addition, apoE can serve as extracellular acceptor for cholesterol in the ABCA1 efflux pathway [86]. Thus, LXRs not only induce cellular cholesterol export by inducing the expression of ABC transporters but also enhance apoE expression and hence

the availability of cholesterol acceptors, thereby promoting reverse cholesterol transport at two different levels.

In addition, LXRs regulate the expression of several lipoprotein-remodeling enzymes, including lipoprotein lipase (LPL), cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) [87–89]. LPL catalyzes the hydrolysis of lipoprotein triglycerides and is highly expressed in adipose tissue and muscle, and is also produced in macrophages [90]. LXR agonists induce expression of LPL only in liver and macrophages but not in adipose tissue [91]. This tissue specifity is important concerning its pro- or anti-atherogenic effects. Hydrolysis of triglyceride-rich lipoproteins and the concomitant promotion of HDL formation seem to be antiatherogenic, as revealed by overexpression of human LPL in animal models of atherosclerosis [92, 93]. On the other hand, overexpression of LPL in macrophages was shown to accelerate atherosclerosis in both apoE$^{-/-}$ and LDLR$^{-/-}$ mice [94, 95]. Thus, a clear-cut pro- or anti-atherogenic effect of LPL induction by LXR agonists cannot be inferred.

The remodeling enzyme CETP mediates the transfer of HDL cholesterol esters to apoB-containing particles for return to the liver. Triglycerides are transferred to HDL in exchange. HDL modification by CETP makes HDL more susceptible to hydrolysis by hepatic lipase, which is an important component of the regeneration of small HDL particles and free apoAI to recirculate in the reverse cholesterol transport pathway.

In addition, PLTP has been identified as a key modulator of HDL metabolism by its ability to remodel HDL particles into large α-HDL and small pre-β-HDL particle fractions, leading to lower plasma HDL levels [30, 96]. The pre-β-HDL particles are efficient acceptors of cholesterol from peripheral cells and are involved in reverse cholesterol transport [97–99]. Moreover, PLTP has recently been shown to regulate VLDL secretion from the liver. PLTP-deficient mice exhibit decreased levels of VLDL and LDL on an apoE-deficient or apoB-transgenic background [100]. Thus, the rise in plasma VLDL, and triglyceride concentrations by LXR agonist treatment, due to formation of larger triglyceride-rich VLDL particles [101], could probably involve PLTP induction in addition to their profound direct effects on lipogenesis and apoE expression. However, these larger TG-rich VLDL particles are considered to be more proatherogenic [101].

Lipogenesis

Besides cholesterol and lipoprotein metabolism, LXRs have also been implicated in the control of fatty acid metabolism. Mice carrying a targeted disruption in the LXRα gene were noted to be deficient in expression of sterol regulatory element-binding protein (SREBP)-1c, fatty acid synthase (FAS), stearoyl coenzyme A desaturase (SCD)-1 and acyl CoA carboxylase (ACC), in addition to defects in cholesterol metabolism [57]. The regulation of fatty acid biosynthesis by LXRs was mainly mediated by inducing the expression of SREBP-1c expression [102, 103]. SREBP-1c induces the transcription of many lipogenic genes, including FAS and ACC, two key enzymes of de novo fatty acid synthesis [104]. In addition to effects on SREBP-1c, direct actions of LXR on certain lipogenic genes such as FAS [105] and PLTP [87, 88] are also likely to contribute to the ability of LXR agonists to cause hypertriglyceridemia [105, 106].

New findings are revealing that regulation of hepatic lipogenesis by LXRs is considerably more complex. LXR agonists increase the mRNA expression of angiopoietin-like protein 3 (Angptl3) in human hepatoma cells and wild-type mice [107, 108]. Since in wild-type mice LXR agonist treatment increased triglyceride content in liver and plasma but failed to do so in Angptl3$^{-/-}$ mice, Angptl3 was suggested to be a main mediator of LXR-induced lipogenesis [107]. Moreover, selective hepatic overexpression of LXRα is able to modulate the labile balance between triglyceride synthesis, uptake and metabolism [109]. Increased hepatic LXRα expression worsened serum lipid profiles when mice were fed a low-fat diet, but improved serum lipid profiles on a high-fat diet (Western diet). However, the benefits of increased hepatic LXRα were reversed by treatment with a synthetic LXRα agonist. These results reveal a complex gene-environment interaction, in which a specific genetic alteration (increased hepatic LXRα gene expression) has opposite phenotypic effects (improved or worsened serum lipid profiles) depending on the nutritional enviroment (Western or low-fat diets) [109]. These fundamental differences between endogenous and pharmacological pure LXR agonists underscore the need for selective LXRα modulators. Moreover, recent data revealed that regulation of lipid homeostasis by LXRs cannot be separated from their action in cholesterol homeostasis. LXR$^{-/-}$ mice are defective in hepatic lipid metabolism and are resistant to obesity when challenged with a diet containing both high fat and cholesterol [110]. However, this phenotype depends on the presence of dietary cholesterol and is accompanied by aberrant activation of thyroid hormone in liver. Defective hepatic lipogenesis and resistance to obesity of LXR$^{-/-}$ mice is due to abnormal energy dissipation resulting from uncoupled oxidative phosphorylation and ectopic expression of uncoupling proteins in muscle and white adipose tissue. These studies indicate an interaction between dietary cholesterol and lipid homeostasis in that LXRs selectively sense the cholesterol component of a lipid-rich diet to control the balance between storage and oxidtion of dietary fat [110].

LXRs are also involved in the insulin-mediated induction of lipogenesis [55]. After insulin stimulation, mRNA expression and half-life of LXRα were increased in primary cultures of hepatocytes *in vitro* as well as *in vivo* in rodent

liver. Accordingly, induction of lipogenic genes such as SREBP-1c by insulin is largely abrogated in LXR$\alpha^{-/-}\beta^{-/-}$ mice, implying that LXR functions as an essential regulatory component in insulin regulation of triglyceride metabolism [55]. However, a recent study showed that LXR agonists and insulin induce SREBP-1c transcription in rat hepatocytes in different ways. Whereas the generation of the mature and transcriptionally active form of SREBP-1c requires insulin, LXR induces solely the expression of the SREBP-1c precursor protein, which still has to be processed by site-specific proteases to be released from the endoplasmic reticulum and to act as a transcription factor [111–113]. Beyond their effects via SREBP-1c, LXRs also directly upregulate certain lipogenic genes, such as FAS [105]. Hence, direct and indirect mechanisms contribute to the lipogenic actions of LXR agonists. Clearly, induction of lipogenesis by LXR agonists driving severe hepatic steatosis [113] represents a significant barrier to their clinical application.

Carbohydrate metabolism

LXR agonist treatment inhibits expression of gluconeogenic enzymes such as phosphenolpyruvate carboxykinase (PEPCK) [114]. Accordingly, the LXR agonists T0901317 and GW3965 were shown to improve insulin sensitivity in insulin resistant zucker (fa/fa) rats and diet-induced obese mice, respectively [35, 115]. In addition, the insulin-sensitive glucose transporter GLUT4 was shown to be a direct LXR target gene [35, 116]. However, LXR agonists only slightly improve insulin sensitivity in diabetic mice despite considerable reduction in blood glucose concentrations, indicating that mechanisms other than direct effects on glucose metabolism could underlie their anti-diabetic action [117]. In addition to reduced expression of PEPCK, reduced expression of G6Pase, an enzyme which controls the flux of glucose-6-phosphate (G6P) towards glucose, might also be suppressed upon LXR activation [117]. However, although these enzymes are inhibited by LXR activation, gluconeogenesis and flux through G6Pase, measured with stable isotopes, were not affected in mice [117]. Notably, LXR agonists enhance insulin secretion by pancreatic β-cells [118], and LXR agonist treatment of diabetic mice is accompanied by a considerable increase in circulating insulin concentrations, indicating that LXR effects on β-cells are highly relevant for lowering blood glucose in diabetes ([117] and own unpublished observations).

LXR and the immune system

Macrophages are playing a central role in innate immunity and specifically in host defence against infections. LXR activation inhibits the induction of inducible nitrite oxide synthase (iNOS), cyclooxygenase (COX)-2, interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1 and matrix metalloproteinase (MMP) 9 in response to bacterial pathogens including LPS [119] (fig. 2). The inhibition of inflammatory gene expression by LXRs is mediated by nuclear factor-kappa (NF-κ)B. The repression of inflammatory gene expression by LXR agonist treatment was mitigated in LXR$\alpha^{-/-}$, and LXR$\beta^{-/-}$ compared with wild-type macrophages, but was completely abolished in LXR$\alpha^{-/-}\beta^{-/-}$ mice, implying that both LXR paralogues are involved in mediating the anti-inflammatory activity of LXR agonists. Furthermore, intraperitoneal injection of LPS in LXR$\alpha^{-/-}\beta^{-/-}$ mice triggers an exacerbated systemic inflammatory response as measured by increased cytokine serum concentrations and hepatic expression of inflammatory genes [119]. LXR agonists display potent anti-inflammatory effects in irritant and allergic dermatitis [119, 120]. Moreover, downregulation of MMP9 by LXR agonists could contribute to the prevention of vascular pathology. [121]. On the other hand, LXR agonists enhance the production of the proapoptotic and proinflammatory cytokine tumor necrosis factor (TNF)-α [122]. However, since LXR activation appears not to increase other proinflammatory cytokines involved in the inflammatory immune response, it is possible that specific LXR-mediated stimulation of TNF-α expression may reduce atherosclerotic lesion size by the induction of apoptosis of proliferating smooth muscle cells, foam cells and/or infiltrating T cells [122].

LXR activation is implicated in the susceptibility to microbial infections. Some microbia evade killing by the immune system by inducing macrophage apoptosis. LXRs interfere with this strategy and prevent bacterial-induced macrophage apoptosis [123]. This effect involves upregulation of scavenger receptor expression that also promotes killing of bacteria [124] (fig. 2). Thus, LXR agonists do not merely act as anti-inflammatory agents but mediate an immunomodulation with reduced inflammation in parallel with enhanced activity against certain bacterial infections. Accordingly, mice devoid of both LXRs are more susceptible to *Listeria monocytogenes*, developing higher bacterial burdens with more frequent and accelerated demise following infection [124]. Interestingly, mice devoid of LXRα but not LXRβ mirror the susceptibility of LXR$\alpha^{-/-}\beta^{-/-}$ animals, indicating a predominant role of LXRα in this respect. Although inflammatory cytokines and other serum mediators were not appreciably different between LXR$\alpha^{-/-}\beta^{-/-}$ and wild-type animals, gene expression analysis identified Spα, also known as apoptosis inhibitor 6 (API6), a member of the scavenger receptor cystine-rich repeat family, as a candidate susceptibility gene induced in wild-type and LXR$\beta^{-/-}$ but not LXR$\alpha^{-/-}$ or LXR$\alpha^{-/-}\beta^{-/-}$ infected livers [124]. This example underlines the fact that various inflammatory pathways are regulated by different LXRs.

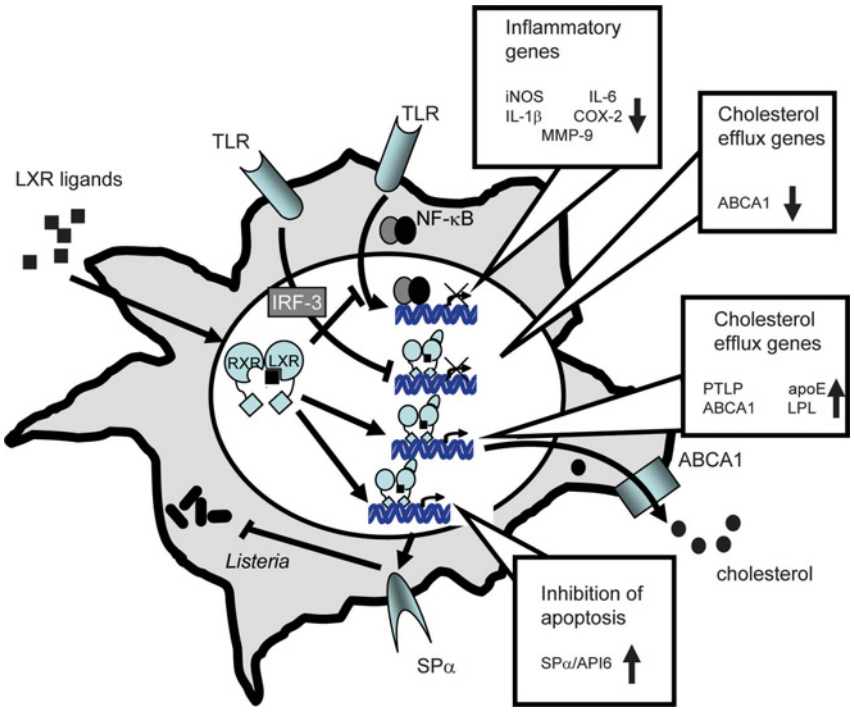

Figure 2. Role of LXRs in cholesterol efflux, inflammation, macrophage apoptosis, and bacterial killing in macrophages. LXRs regulate diverse aspects of macrophage biology. LXRs bind oxysterol metabolites and other LXR ligands and upregulate expression of cholesterol efflux genes such as ABCA1. LXRs also suppress NF-κB signaling, which may be initiated by TLRs, mitigating inflammation. Vice versa, activation of TLRs inhibits LXR-induced cholesterol efflux from macrophages by inhibition of ABCA1 gene expression. The inhibition of the transcriptional activity of LXR on its target promoters is mediated by IRF3. Uniquely, LXRα induces expression of SPα/API6, a scavenger receptor that inhibits macrophage apoptosis and promotes the killing of *Listeria.*

Not only do LXRs interfere with activation of the innate immune system, but LXR-mediated gene expression is also subject to interference by inflammatory pathways. The innate immune system recognizes conserved motifs found in microbes by so-called pattern recognition receptors, including those of the Toll-like receptor (TLR) family [125]. Potent agonists of TLR3 and TLR4 selectively inhibit LXR agonist-induced synthesis of ABCA1 and other mediators of cellular cholesterol efflux mediators. This crosstalk between the inflammatory TLR pathways and LXRs is independent of MyD88 and NF-κB, but mediated by another transcription factor, interferon regulatory factor (IRF)3, that is also implicated in the interferon response [126]. These findings highlight a common mechanism whereby various microbial pathogens could modulate macrophage cholesterol metabolism and perhaps even cardiovascular disease through interference with LXR signaling [127].

LXR and atherosclerosis

Atheriosclerosis is a major health problem worldwide, and cardiovascular disease is the predominant cause of death in industrialized countries. Lethal cardiovascular disease is mainly mediated by atherosclerotic alterations in large and medium-sized arteries. Atherosclerosis involves complex metabolic and inflammatory processes. Retention and modification of lipids in the vascular wall as well as infiltration with inflammatory cells, particularly macrophages that transform into foam cells by lipid accumulation, are hallmarks of this disease [128–130]. Hence local lipid metabolism as well as inflammatory reactions are potential targets for prevention and/or treatment of atherosclerosis. Numerous epidemiological studies and clinical trials have identified decreased HDL cholesterol and increased LDL cholesterol as major contributors to atherogenesis [128, 131]. Macrophages play a central role in atherogenesis through the accumulation of oxidized low-density lipoproteins (oxLDLs), and the production of inflammatory mediators, cytokines and extracellular matrix degrading enzymes. The first direct evidence for a protective role of LXRs for atherogenesis came from LXR agonist treatment in mouse models of atherosclerosis [132]. In particular, lesion area was reduced by the synthetic LXR agonist GW3965 in both apoE$^{-/-}$ and LDLR$^{-/-}$ mice. Bone marrow transplantation studies revealed that deletion of LXR specifically from the hematopoetic compartment provoked a significant increase in atherosclerotic lesion formation in apoE and

LDLR knockout recipient mice [133]. Thus, LXRs exert profound antiatherosclerotic effects at least in mice that are based on their expression in macrophages rather than cells of the vessel wall. The antiantherosclerotic effect of LXR could be mediated by two principally different actions on macrophage metabolism and inflammatory response, respectively.

Antiatherogenic effect of LXR mediated by metabolic regulation

LXR agonist treatment reduces the transformation of macrophages into foam cells by increasing cellular cholesterol efflux. Induction of ABCA1 and ABCG1 expression by LXR agonist treatment appears critical in this respect. Inactivation of ABCA1 specifically in macrophages promotes atherosclerosis, whereas ABCA1 overexpression reduces lesion formation [79, 134]. ABCA1 expression is induced in macrophages in response to oxidized LDL, oxysterols and synthetic LXR agonists, but not in the absence of LXRs [65]. Similarly, LXR activation also increased ABCG1 mRNA levels in macrophages [64, 133], and furthermore in liver and adipose tissue [135, 136]. Via activation of the ABCA1, ABCG1 and other genes, the LXR pathway is critical to the regulation of cholesterol homeostasis, as well as the prevention of cholesterol accumulation in macrophages leading to atherosclerosis [28, 133, 137]. Evidence for the potential use of LXR activators in atherosclerosis has recently been provided by intervention studies in murine models. LXR agonist treatment reduced the lesion development in both apoE$^{-/-}$ and LDLR$^{-/-}$ mice to 50% [132]. Interestingly, chronic ligand administration only moderately affected the lipoprotein profile of these mice, suggesting that a direct effect of ligand on cells of the artery wall may also be involved in the antiatherogenic effects. Consistent with this idea, LXR agonist was shown to induce expression of ABCA1 and ABCG1 in the atherosclerotic aortas of apoE mice [132]. Considering all these data, increased ABCA1 and ABCG1 expression by LXR activation might play an important role in reduction of atherosclerosis.

In addition to enhancing cholesterol efflux, LXR agonist treatment provides for cholesterol-accepting lipoproteins to be used in reverse cholesterol efflux. ApoE is involved in macrophage cholesterol efflux, and apoE overexpression in mice results in reduced atherogenesis, whereas apoE deletion increased the susceptibility to the disease [86]. ApoE is part of a gene cluster including apoE/apoCI/apoCII/apoCIV, and the entire cluster is induced by LXR agonists in both human and murine macrophages [138]. Other LXR target genes expressed in macrophages or adipose tissues and involved in reverse cholesterol are PLTP and CETP [87–89, 139]. Immunohistochemistry studies revealed that PLTP protein is highly expressed by macrophages within human atherosclerotic lesions, suggesting a potential role for this enzyme in lipid-loaden macrophages [88]. One possible mechanism for its antiatherosclerotic action is that PLTP remodels lipoproteins within the arterial wall to generate particles that can better serve as cholesterol acceptors. Recently, bone marrow expression of PLTP was shown to be antiatherogenic, which indicates a benefical role for this enzyme within macrophages [140]. CETP, another HDL remodeling enzyme, is suggested to be antiatherogenic by facilitating reverse cholesterol transport [141, 142], but the impact of CETP on atherogenesis is not clear at present since overexpression could also prevent lesion formation [143]. Despite the plethora of antiatherogenic effects of LXR activation, the induction of large triglyceride-rich VLDL particles acts in a proatherogenic manner but fails to neutralize the overall antiatherogenic action of LXRs according to various animal models [101].

Together, these studies suggest that LXRs promote macrophage cholesterol efflux not only by inducing cholesterol transporter proteins (ABCA1 and ABCG1), but also through increased production of cholesterol acceptors (apoE and apoCs) and lipoprotein remodeling proteins (PLTP, CETP) contributing to anti-atherosclerotic effects of LXRs.

Antiatherogenic effects of LXR mediated by the immune system

Atherosclerosis is now recognized not only as a metabolic but also as a chronic inflammatory disease. Hence, the antiatherosclerotic action of LXR agonists could also be based on their ability to inhibit the expression of inflammatory genes in macrophages [119, 126].

The anti-inflammatory action of LXR agonists appears to depend on suppression of NF-κB, though the precise mechanism how LXRs block NF-κB signaling is yet unknown [119]. Inhibition of MMP9 expression by LXR agonists that also involves the NF-κB pathway could contribute to their antiatherogenic action [121]. Many proinflammatory genes contributing to atherosclerosis are target genes of NF-κB. In 'free' cholesterol-loaden macrophages, NF-κB is required for induction of TNF-α and IL-6, which contributes to atherogenesis [144]. Accordingly, inhibition of IκB kinase activation, which is necessary for NF-κB activation, reduces TNF-α and IL-1β cytokine production in macrophages [145].

Thus, the LXR pathway and particularly its interaction with NF-κB signaling could critically regulate inflammatory processes involved in atherogenesis.

Therapeutic aspects of LXR agonists for atherosclerosis

The identification of LXRs as regulators of the ABCA1 together with their anti-inflammatory effects has raised

the possibility that LXR agonists could be effective drugs for the prevention and treatment of atherosclerosis. These exciting possibilities for LXR agonists as new therapeutics, however, could be limited by significant adverse and potential proatherogenic effects. These include the induction of lipogenesis and triglyceride accumulation, the induction of proinflammatory cytokines such as TNF-α and the potential proatherogeneic effect of LPL in macrophages.

The deleterious lipogenic side effects predominantly originate in the liver. One potential solution to dissociate LXR activation from hepatic side effects could be LXRβ-selective agonists. In contrast to LXR$\beta^{-/-}$ mice, LXR$\alpha^{-/-}$ mice showed reduced plasma triglyceride levels as well as reduced hepatic mRNA levels for multiple enzymes of fatty acid synthesis, indicating that LXRα is the subtype controlling hepatic SREBP-1c transcription [57, 58]. Moreover, LXRβ is expressed in liver only to a minor extent [7]. In contrast, peritoneal macrophages from LXR$\beta^{-/-}$ mice, but not LXR$\alpha^{-/-}$ mice, show increased basal expression of ABCA1 mRNA, suggesting that LXRβ is the subtype responsible for controlling basal ABCA1 transcription in macrophages [62]. On the other hand, LXR agonist treatment of peritoneal macrophages from both LXR$\alpha^{-/-}$ and LXR$\beta^{-/-}$ mice showed a comparable increase of ABCA1 mRNA expression similar to wild-type mice [28], indicating that both LXRα and LXRβ are equally important for inducing ABCA1 transcription. Based on these data in mice, agonists specific for LXRβ could perhaps combine induction of cholesterol export from macrophages, while preventing induction of hepatic lipogenesis.

LXR target genes differ in their recruitment of coactivators and corepressors. E.g., basal ABCA1 gene expression and HDL cholesterol serum concentration are increased in LXR$\alpha^{-/-}\beta^{-/-}$ mice due to release of corepressors and recruitment of coactivators by other transcription factors [28, 38, 101]. This 'derepression', however, does not occur on the SREBP-1c promoter. ChIP experiments indicate that NCoR is recruited to the ABCA1 promotor in an LXR-dependent manner, and that either addition of an LXR agonist or loss of LXR expression similarly results in decreased corepressor recruitment to the ABCA1 promoter. The importance of ABCA1 in reverse cholesterol transport is also exemplified in ABCA1 knockout mice. These results suggest that LXR is capable of both activation and repressing target genes that control cholesterol efflux and HDL biogenesis. Thus, LXR ligands that release corepressors without recruiting coactivators could mimic the differential transcriptional effects of ABCA1 and SREBP-1c seen in LXR$\alpha^{-/-}\beta^{-/-}$ mice. Such components would promote reverse cholesterol transport without inducing lipogenesis. Synthetic ligands with these properties may provide an improved therapeutic index over first-generation LXR agonists such as T0901317. Ligands that mediate corepressor release without recruiting coactivators have been identified for retinoic acid receptors, which are closely related to LXRs, suggesting that similar compounds could be found for LXRs [146]. The development of selective estrogen receptor modulators (SERMs) indicates the feasibility to design nuclear receptor ligands that function as agonists in one cell type but as antagonists in others depending on the coregulator levels present [147]. In analogy to SERMs, selective LXR modulators could exploit the intracellular levels of coactivators and corepressors within different cells to result in LXR activation restricted to certain tissues [148]. The LXR agonist GW3965 appears to have such properties of an LXR modulator. This compound increases ABCA1 expression in the intestine and HDL cholesterol concentration, but only minimally induces hepatic gene expression including SREBP-1c and FAS. These results point to the feasibility of selective LXR modulators to induce LXR target gene expression selectively in non-hepatic tissue, thereby avoiding hepatic lipogenesis and perhaps hepatotoxicity. Another approach for dissociating HDL elevating from the lipogenic effects of LXR agonists is to modulate cholesterol loading in parallel. Compounds to be developed, perhaps steroid derivatives that not only activate LXRs but also suppress SREBP processing, could act in this manner.

Taken together, the development of potent antiatherogenic substances involved in LXR activation appears feasible, though compounds for several pharmacological mechanisms have to be tested initially to identify the most effective approach.

LXR and type 2 diabetes

Type 2 diabetes mellitus has become an epidemic in industrialized countries. The devastating complications of diabetes mellitus are mostly due to microvascular disease and accelerated atherogenesis. Cardiovascular morbidity in patients with type 2 diabetes is two to four times greater than that of non-diabetic subjects [149]. Type 2 diabetes is caused by insulin resistance and defective insulin secretion. Important target tissues of insulin action are liver, muscle and adipose tissues, where insulin stimulates the uptake of glucose from the blood, inhibits hepatic glucose production and regulates lipogenesis. Expression of LXRα is induced by insulin in hepatocytes [55], suggesting LXRα to be a mediator of insulin action. Hence, LXRs not only play a key role in metabolic regulation but could also be directly involved in metabolic alterations leading to type 2 diabetes. In addition, agonists for PPARs have been established in the treatment of diabetes and hyperlipidemia. In particular, agonists of PPARγ, the thiazolidinediones, significantly enhance insulin sensitivity and improve glycemic control in diabetes

patients, whereas PPARα agonists, the fibrates, exert considerable hypolipidemic actions. PPARγ agonists induce LXRα expression [50], suggesting that some of their beneficial effects could be mediated by LXRs and giving hope that LXR agonists could provide similar benefits as thiazolidinediones with fewer side effects. However, these initial ideas appear not to become true according to currently available data.

LXR and carbohydrate metabolism in type 2 diabetes

LXR agonist treatment significantly downregulates expression of gluconeogenic enzymes such as PEPCK and glucose-6-phosphatase in mouse liver (fig. 3) [114, 150]. Accordingly, LXR agonist treatment dramatically reduced plasma glucose in diabetic rodents [35, 115]. LXR agonists increased basal uptake of glucose in adipocytes and induced the expression of the predominant glucose transporters for basal and insulin-stimulated glucose uptake, GLUT1 and GLUT4, respectively [114, 151]. In addition, LXR agonists also increase GLUT1 and GLUT4 mRNA expression in muscle [116, 152]. Furthermore, activation of ectopically expressed LXRα in adipocytes resulted in increased synthesis of glycogen and cholesterol in adipose tissue and elevated circulating levels of glycerol and non-esterified fatty acids, indicating an regulatory role of LXR in lipolysis as well as in carbohydrate metabolism by stimulating glucose uptake and glycogen synthesis [151]. LXR agonist treatment also increased insulin sensitivity in insulin-resistant zucker rats and repressed gluconeogenic genes [115]. However, as detailed above, LXR agonists only slightly improved insulin resistance in diabetic animals in other studies and profoundly elevated insulin secretion and insulin serum concentrations [115, 117]. However, the stimulatory effects on insulin secretion from pancreatic β-cells *in vitro* appear to occur secondary to enhanced lipogenesis [118]. Thus, so-called β-cell lipotoxicity could abolish the beneficial effects of LXR agonists on carbohydrate metabolism and promote diabetes development when applied for longer treatment periods.

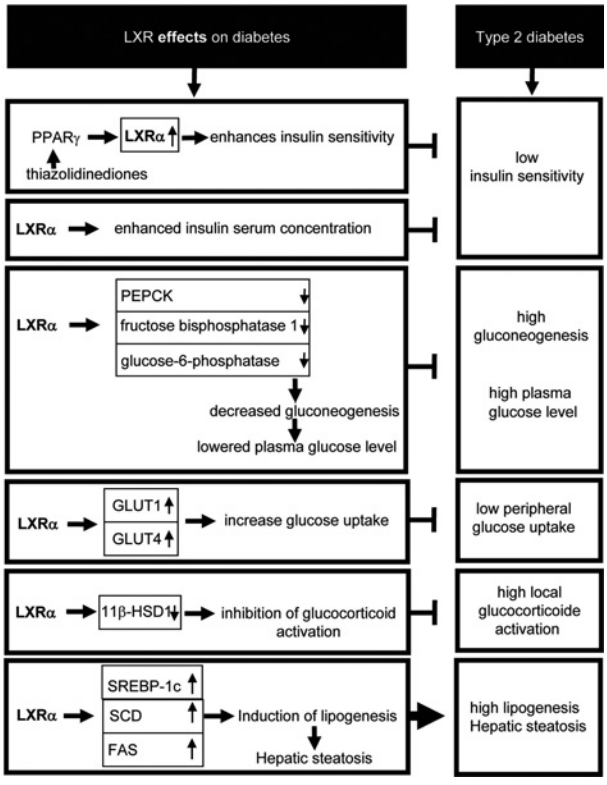

Figure 3. Effects of LXRs in type 2 diabetes. Alterations in type 2 diabetes are given on the right. Effects of LXRs on these derangements are depicted on the left. Positive LXR effects include enhancement of insulin sensitivity and insulin serum concentration, suppression of genes involved in gluconeogenesis (PEPCK, G6P and Fbp1), contributing to lower plasma glucose levels, induction of glucose uptake by induced expression of glucose transporters (GLUT1 and GLUT 4), inhibition of glucocorticoid activation by inhibited expression of 11β-HSD1 and induction of insulin serum concentration. Negative effects of LXR activation could be the induction of genes involved in lipogenesis (SREBP-1c and SCD), leading to hepatic steatosis. Arrowheads indicate up- or downregulation of genes. See text for details.

LXR and lipid metabolism in type 2 diabetes

Diabetes mellitus is accompanied by significant derangements of lipid homeostasis, particularly hyperlipidemia and low HDL concentrations that contribute to the cardiovascular complications of the disease. Insulin elevates expression of key genes involved in lipogenesis such as SREBP-1c and SCD-1 [24, 102, 103], and insulin action on hepatic lipogenesis appears to be mediated by LXRs [55]. However, increased SCD activity in liver leads to synthesis of triglycerides and esterification of cholesterol for transport out of the liver. LXRs by themselves induce hepatic lipogenesis, and LXR agonist treatment provokes excessive hepatic lipid accumulation in rodents. In type 2 diabetes, SCD activity is increased probably in response to increased levels of insulin [153]. Hence LXR-mediated upregulation of lipogenesis could considerably worsen lipid disorders associated with type 2 diabetes [154] (fig. 3). On the other hand, the potential role of LXRs in the pathogenesis of the diabetes-associated dyslipidemia has still to be elucidated.

In addition to effects in liver and adipose tissue, LXRs also regulate lipid and carbohydrate metabolism in muscle [155, 156]. In particular, LXR agonist treatment results in increased fatty acid uptake and lipid accumulation in myotubes [156]. Hence, activation of LXRs could be responsible for the accumulation of intracellular lipids in skeletal muscle, particularly in those from type 2 diabetes patients, that is associated with impaired insulin

sensitivity [152]. Thus, LXRs could be critically involved in the pathogenesis of type 2 diabetes by inducing myocellular lipogenesis.

LXR and local glucocorticoid activation

Recently, interference with insulin action by local activation of glucocorticoids mediated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in adipose tissue and liver has evolved as a novel principle of how obesity associates with insulin resistance and type 2 diabetes [150]. E.g., transgenic 11β-HSD1 overexpression in adipocytes provokes a metabolic syndrome in mice strongly resembling the human disease [157]. Moreover, inhibition of 11β-HSD1 enzymatic activity provided promising data on reducing blood glucose levels in diabetic mice [158, 159]. LXR also interferes with local glucocorticoid activation by inhibiting 11β-HSD1 expression. Hence, downregulation of 11β-HSD1 expression could contribute to the anti-diabetic action of LXR agonists [150].

Therapeutic aspects of LXR agonists for type 2 diabetes

Inhibition of hepatic gluconeogenesis and lowering serum glucose concentrations shown *in vitro* and in rodent models of type 2 diabetes suggests a potential clinical use of LXR agonists as antidiabetic drugs. However, enhanced hepatic and muscular lipid content along with increased circulating triglyceride concentrations strongly argue against LXR agonists to be candidates for therapeutic compounds against diabetes. Similar to their potential application for atherogenesis, LXR-activating compounds that reveal anti-diabetic action would require highly selective inhibitory actions on gluconeogenic genes while leaving lipogenesis unaffected or repressed. In liver, gluconeogenesis mainly occurs in periportal hepatocytes, whereas lipogenesis predominates in perivenous hepatocytes [160, 161]. A zonation of gene activation in the liver could be caused by differences in substrate availability, hormones, mediators, neural signals, and in cell-to cell or cell-to-matrix interactions [160]. This heterogeneity of hepatocytes leaves scope for development of LXR-activating compounds that act in a different manner in hepatocytes with somehow different gene regulation, thereby avoiding negative side effects. Generation of agonists and antagonists specific for LXRα and LXRβ, respectively, will help to elucidate isoform-specific functions of LXR.

Obesity has recently been found to be associated with a chronic subclinical inflammation that resides within the adipose tissue and appears to predispose towards metabolic and cardiovascular complications [162, 163]. Hence, the anti-inflammatory action of LXRs could theoretically exert beneficial effects on adipose tissue inflammation. However, it is currently not known whether LXR agonists affect obesity-associated adipose tissue inflammation or whether direct metabolic effects predominate.

Conclusions

Over the past several years, LXRs have arisen as important regulators of metabolism and the immune system. LXRs are particularly important for the control of cholesterol, lipid and carbohydrate metabolism in insulin target tissues. In addition, LXR activity plays important roles in macrophage biology, thereby inhibiting inflammatory responses. Due to their diverse actions, isoform-selective LXR agonists or selective LXR modulators need to be developed that could become potent drugs particularly for prevention and treatment of atherosclerosis. However, due to extensive side effects, particularly owing to their lipogenic action, these specific LXR-activating drugs need to be specially designed to allow potential clinical application.

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