JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008, 59, Suppl 7, 31–55
www.jpp.krakow.pl

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BIOLOGICAL ROLE OF LIVER X RECEPTORS

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Liver X receptors (LXRs) are ligand-activated transcription factors of the nuclear receptor superfamily. There are two LXR isoforms termed α and ß which upon activation form heterodimers with retinoid X receptor and bind to LXR response element found in the promoter region of the target genes. Their endogenous agonists include a variety of oxidized cholesterol derivatives referred to as oxysterols. In the recent years LXRs have been characterized as key transcriptional regulators of lipid and carbohydrate metabolism. LXRs were shown to function as sterol sensors protecting the cells from cholesterol overload by stimulating reverse cholesterol transport and activating its conversion to bile acids in the liver. This finding led to identification of LXR agonists as potent antiatherogenic agents in rodent models of atherosclerosis. However, first-generation LXR activators were also shown to stimulate lipogenesis *via* sterol regulatory element binding protein-1c leading to liver steatosis and hypertriglyceridemia. Despite their lipogenic action, LXR agonists possess antidiabetic properties. LXR activation normalizes glycemia and improves insulin sensitivity in rodent models of type 2 diabetes and insulin resistance. Antidiabetic action of LXR agonists is thought to result predominantly from suppression of hepatic gluconeogenesis. However, recent studies suggest that LXR activation may also enhance peripheral glucose uptake. The purpose of this review is to summarize the present state of knowledge on the physiological and pathophysiological implications of LXRs with the special consideration of their role in lipid and carbohydrate metabolism and associated diseases.

Key words: *liver X receptors, nuclear receptors, atherosclerosis, cholesterol, diabetes, lipids, lipogenesis*

INTRODUCTION

Liver X receptors (LXRs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily. They were first identified in 1994 by

screening a rat liver cDNA library (1, 2). LXRs were initially classified as orphan nuclear receptors because their natural ligands were unknown. In the following years identification of several physiological ligands has "adopted" these receptors. The LXR subfamily consists of two isoforms, LXRα (NR1H3) and LXRβ (NR1H2) that are highly related and share ~78% identity of their amino acid sequences in both DNA and ligand-binding domains (3). High expression of LXRα is restricted to spleen, liver, adipose tissue, intestine, kidney and lung whereas LXRβ is expressed in all tissues examined (1, 2, 4, 5). Upon ligand-induced activation both isoforms form obligate heterodimers with the retinoid X receptor (RXR) and regulate gene expression through binding to LXR response elements (LXREs) in the promoter regions of the target genes (*Fig. 1*). LXRE consists of two idealized hexanucleotide sequences (AGGTCA) separated by four bases (DR-4 element). LXR/RXR is a so called "permissive heterodimer" that may be activated by ligands for either partner in an independent manner (4). In the absence of ligands LXR recruits complexes of corepressors that are exchanged with coactivators upon receptor activation (6).

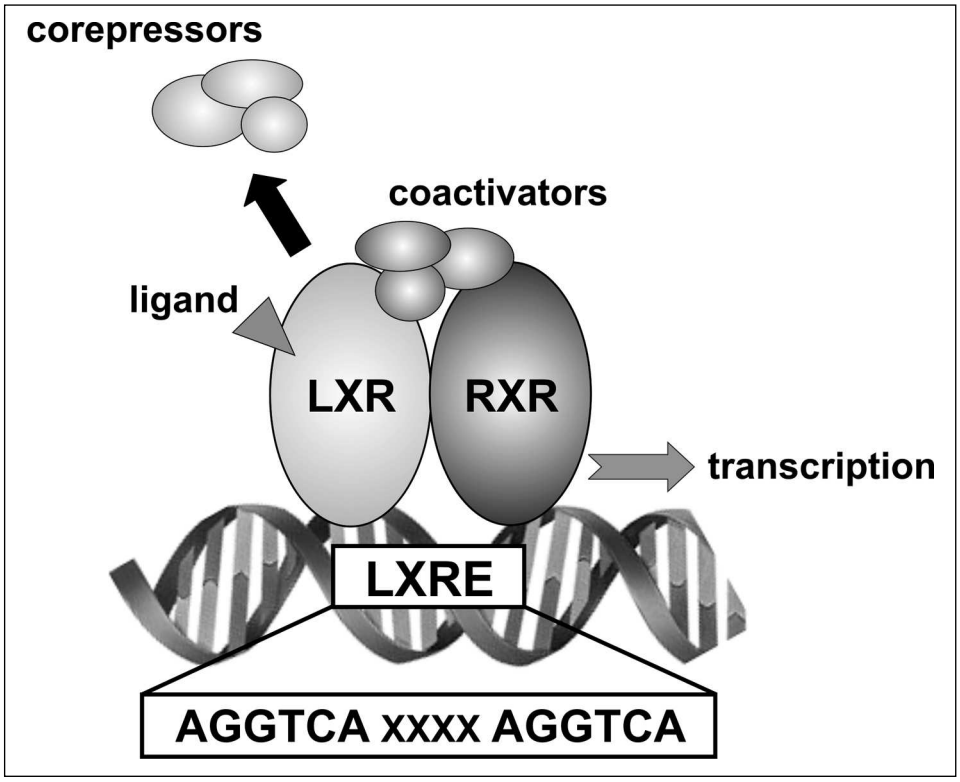

Fig. 1. Mechanism of transcriptional regulation mediated by LXRs. RXR - retinoid X receptor, LXRE - LXR response element.

It is widely accepted that endogenous LXR agonists are oxidized cholesterol derivatives referred to as oxysterols. The most potent activators of this group are 22(R)-hydroxycholesterol and 20(S)-hydroxycholesterol (intermediates in steroid hormone synthesis), 24(S)-hydroxycholesterol (produced in the brain, the major oxysterol of human plasma) and 24(S),25-epoxycholesterol (abundant in the liver) which were shown to bind to and stimulate transcriptional activity of LXRs at concentrations within the physiological range (7-10). Most oxysterols have similar affinity toward both LXR isoforms with the exception of 5,6-24(S),25-dieopxycholesterol and 6α-hydroxy bile acids which are somewhat selective for LXRα (11). In addition, Mitro *et al*. (12) demonstrated that D-glucose and D-glucose-6-phosphate are endogenous LXR agonists with efficacy comparable to that of oxysterols. However, this finding was recently questioned on the basis of inability of glucose and its metabolites to influence the interaction of cofactors with either LXRα or LXRβ and the lack of involvement of LXRs in regulation of glucose-sensitive genes in liver (13). In addition to natural ligands, a number of potent synthetic LXR agonists have been developed. The two most commonly used in experimental studies are T0901317 and GW3965 which show EC_{50} values for both LXRα and LXRβ in the low nanomolar range (14, 15). It should be noted, however, that T0901317 was reported to activate also pregnane X receptor (PXR) (16). The lack of widely available isoform-specific LXR agonists slows the progress of research on the function of individual LXR subtypes. To date only one selective LXRα activator has been described (17). In addition, Molteni *et al*. (18) have recently identified LXRβ-specific agonist which, however, was found to be inactive *in vivo*.

In contrast to oxysterols that stimulate transcriptional activity of LXRs, geranylgeranylpyrophosphate, an intermediate of cholesterol biosynthesis pathway, inhibits both LXR isoforms by antagonizing their interaction with coactivators (19, 20). Transcriptional activity of LXRs was also shown to be inhibited by distinct oxidized cholesterol 3-sulfates normally found in human plasma (21). Moreover, polyunsaturated fatty acids (PUFA) were reported to be competitive LXR antagonists in various cell lines (22, 23). However, their antagonistic effect on LXRs was not confirmed in rodent liver and hepatocytes (24, 25). Although PUFA have been shown to suppress transcription of sterol regulatory element binding protein 1 (SREBP-1), one of the major LXR target genes, this action is independent of LXRα (25).

LXR activity is regulated not only by agonists and antagonists but also by changes in receptor expression. Several studies have demonstrated that expression of LXRα (but not LXRβ) is controlled by an autoregulatory mechanism. A functional LXRE activated by both LXR isoforms was identified in the human LXRα gene promoter (26). Synthetic as well as natural LXR agonists were shown to increase LXRα expression in human macrophages, adipocytes, hepatocytes, skin fibroblasts and myotubes (26-29). The LXRα autoregulatory loop is generally thought to be specific to human cells since it was not observed in murine macrophages and

preadipocytes (27, 28). However, Ulven *et al*. (30) identified functional LXRE in murine LXRα gene and demonstrated the existence of LXRα autoregulation in white adipose tissue but not in the liver and skeletal muscle of T0901317-treated mice. Human and rodent LXRα gene promoter contains also functional peroxisome proliferator response element (PPRE) (27, 31) and peroxisome proliferator-activated receptor (PPAR) α and γ agonists were shown to stimulate LXRα expression in rodent as well as human macrophages, adipocytes and hepatocytes (31-36). Another factor controlling LXRα expression is insulin which was shown to increase receptor mRNA in rat hepatocytes in a dose-dependent manner, primarily by stabilization of the transcripts (37). Transcriptional activity of LXRα is also regulated posttranslationally by protein kinase A that phosphorylates receptor protein at two sites thereby impairing its dimerization and DNA-binding (38).

The purpose of this review is to summarize the present state of knowledge on the physiological and patophysiological implications of LXRs with the special consideration of their role in lipid and carbohydrate metabolism and associated diseases.

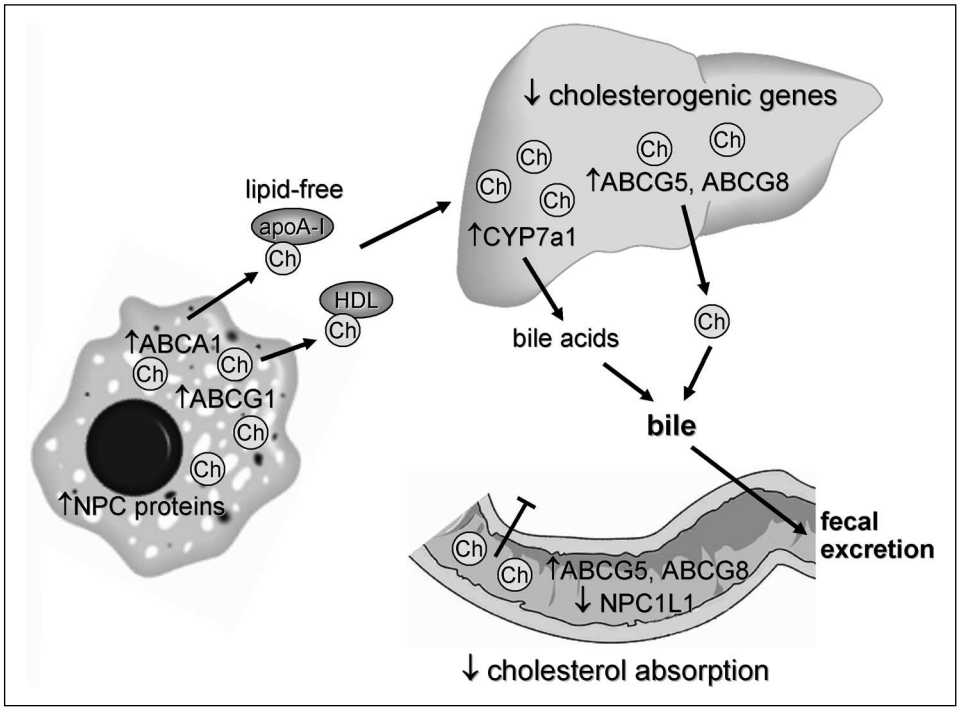

Fig. 2. Role of LXRs in cholesterol transport and metabolism. LXR-regulated genes are indicated. ABC - ATP-binding cassette transporters, apoA-I - apolipoprotein A-I, Ch - cholesterol, CYP7a1 - cholesterol 7-α-hydroxylase, HDL - high density lipoprotein, NPC proteins - Niemann-Pick C proteins, NPC1L1 - Niemann-Pick C1 like 1 protein.

Identification of oxysterols as endogenous LXR ligands pointed to a role for these receptors in regulating expression of genes involved in cholesterol homeostasis. Indeed, the first reported gene directly regulated by LXRs was Cyp7a1 encoding cholesterol 7-α-hydroxylase (CYP7a1), the rate-limiting enzyme in hepatic bile acid synthesis (7). Further studies demonstrated that LXRs regulate expression of an array of genes involved in virtually all aspects of cholesterol transport and metabolism. In the recent years LXRs have emerged as key sensors of intracellular sterol levels that trigger various adaptive mechanisms in response to cholesterol overload. These mechanisms include stimulation of reverse cholesterol transport and biliary cholesterol excretion, inhibition of intestinal absorption of dietary cholesterol and suppression of cholesterol synthesis *de novo* (*Fig. 2*).

Hepatic cholesterol excretion

Physiological significance of individual LXR subtypes in the regulation of liver cholesterol metabolism was clearly shown in experiments where mice lacking either LXRα or LXRβ were challenged with high-cholesterol diet. In contrast to wild type animals, which are highly resistant to cholesterol feeding, LXRα-deficient mice develop massive hepatic accumulation of cholesterol esters, hepatomegaly and hypercholesterolemia. This phenotype results from the inability to upregulate Cyp7a1 expression, and in consequence bile acid synthesis and fecal excretion, in response to cholesterol-rich diet (39). Although intact LXRβ is present in the liver of these animals it cannot compensate for the absence of LXRα in regulating Cyp7a1 expression likely due to the fact that the LXRE found in this gene is a substantially stronger response element for LXRα than it is for LXRβ (39). Consistently, LXRβ-deficient mice retain normal hepatic cholesterol metabolism and are able to adapt to cholesterol-rich diet which underscores the key role of LXRα in this process (40). In contrast to rodents, human Cyp7a1 gene promoter is not induced by LXRα which may be responsible for greater susceptibility of humans to diet-induced hypercholesterolemia (41, 42). In addition to stimulation of bile acid synthesis, LXR activation further potentiates cholesterol excretion by increasing transcription of the ATP-binding cassette transporters (ABC) G5 and G8 in the liver (43, 44). These transporters are expressed at the canalicular membrane of hepatocytes where they drive cholesterol transport into the bile (45). Consistently, T0901317 fails to increase biliary cholesterol excretion in ABCG5/ABCG8 double knockout mice (43).

Intestinal cholesterol absorption

ABCG5 and ABCG8 also play a key role in the intestinal absorption of dietary cholesterol. These transporters are localized on the apical membrane of enterocytes

and their primary function is to transport absorbed cholesterol back to the lumen of the intestines (46). Expression of both ABCG5 and ABCG8 is substantially increased upon LXR activation in murine intestine and in human enterocyte CaCo-2 cell line (44, 47, 48). In consequence administration of LXR agonists markedly decreases intestinal net cholesterol absorption in mice (44, 49). Initially this effect was associated with increased ABCA1 expression in enterocytes (49). However, subsequent experiments on mice lacking either ABCA1 or ABCG5 and ABCG8 revealed that only the latter two transporters are involved in the LXR-induced inhibition of dietary cholesterol absorption (43, 50). In addition, it was reported recently that expression of Niemann-Pick C1 like 1 (NPC1L1), a protein critical for intestinal cholesterol absorption, is decreased by synthetic LXR agonists in murine intestine as well as in cultured CaCo-2 cells (47).

De novo cholesterol synthesis

SREBPs are a family of transcription factors synthesized as inactive precursor molecules that are bound to the endoplasmic reticulum. Upon activation these precursors are proteolytically cleaved to release a mature fragment that migrates to the nucleus and stimulates expression of the target genes. There are three SREBP isoforms: SREBP-1a that regulates expression of genes involved in both fatty acid and cholesterol biosynthesis, SREBP-1c stimulating lipogenic genes and SREBP-2 which stimulates cholesterol-synthesizing enzymes (51). LXRα-deficient mice exhibit higher hepatic expression of SREBP-2 and several of its target genes including hydroxymethyl glutaryl-coenzyme A synthase and reductase, farnesyl diphosphate synthase and squalene synthase (39). A similar, although milder, upregulation of cholesterogenic genes was observed in the liver of LXRβ-null mice (40). Concordantly, administration of synthetic LXR agonist reduces hepatic expression of squalene synthase and hydroxymethyl glutaryl-coenzyme A synthase in wild-type mice (14). However, it should be noted that downregulation of hepatic cholesterogenic genes in response to cholesterol-rich diet is not impaired in either LXRα- or LXRβ-deficient mice (39, 40) which indicates that sterol-induced inhibition of SREBP-2 cleavage (51) rather than LXR activation plays the major role in this phenomenon. Although the above data suggest that LXRs inhibit cholesterol biosynthesis their role in the regulation of this pathway is unclear and requires further investigation. For instance, Peet *et al*. (39) reported that elevated expression of cholesterogenic enzymes in the liver of LXRα-deficient mice does not result in the predicted increase in hepatic cholesterol synthesis. In addition, surprisingly, T0901317 and GW3965 were shown to enhance cholesterol synthesis in human hepatoma HepG2 cells (52).

Reverse cholesterol transport

Elimination of cholesterol from the organism occurs almost exclusively in the liver. Therefore, excess cholesterol from most other tissues must be transported *via* HDL particles or lipid-free apolipoproteins to the liver for excretion in the

bile. This process is termed "reverse cholesterol transport" (RCT). The initial finding that LXRα is crucial for maintaining hepatic cholesterol homeostasis suggested that LXRs may regulate RCT as well. Indeed, subsequent studies have revealed that LXRs stimulate almost every aspect of this process. Cholesterol efflux from the cells is the first step in RCT and is primarily mediated by ABCA1 and ABCG1 transporters. ABCA1 transfers both cholesterol and phospholipids from plasma membrane to lipid-free apolipoprotein A-I (apoA-I). This transporter is also crucial for the formation of nascent HDL particles in the liver. On the other hand, the function of ABCG1 is to transfer cholesterol to HDLs (48). It was demonstrated that LXRα and LXRβ upregulate expression of rodent as well as human ABCA1 and ABCG1 *via* functional LXREs found in their genes (49, 53, 54). Numerous studies showed that synthetic and natural LXR agonists increase ABCA1 and ABCG1 expression as well as cholesterol efflux from various cell types including macrophages, primary fibroblasts, CaCo-2 cells, C2C12 myotubes, primary hepatocytes, 3T3-L1 adipocytes and HepG2 cells (12, 55-59). Moreover, Naik *et al*. (60) demonstrated that administration of GW3965 to mice preinjected with ^{3}H-cholesterol-labeled macrophages markedly increases fecal excretion of the tracer, thus providing direct evidence that LXR activation enhances RCT *in vivo*. Similar results were recently reported by other groups for T0901317 (61, 62). In primary human macrophages, LXR agonists were also shown to increase cholesterol availability for extracellular acceptors *via* upregulating expression of Niemann-Pick C proteins that control cholesterol trafficking from the intracellular pools to the plasma membrane (63).

Another gene involved in cholesterol homeostasis that is directly regulated by LXRα and LXRβ is apoE. This apolipoprotein is present on the surface of plasma lipoproteins and is a high-affinity ligand of the LDL receptor. ApoE is required for hepatic uptake of chylomicron remnants, very low density lipoproteins and some subtypes of HDLs. It can also serve as extracellular acceptor for cholesterol effluxed *via* ABCA1 (64). Laffitte *et al*. (65) demonstrated that LXRs mediate lipid-inducible expression of apoE selectively in adipose tissue and macrophages, through interaction with LXREs present in two enhancers that direct macrophage- and adipose-specific expression of the apoE gene. Thus, LXRs stimulate RCT not only by enhancing expression of ABC transporters but also by increasing availability of extracellular cholesterol acceptors such as apoE. Consistent with multilevel stimulatory effect of LXRs on reverse cholesterol transport, synthetic LXR agonists were repeatedly shown to increase plasma HDL concentration in rodents (14, 15, 66-70).

LXRS AS ANTIATHEROSCLEROTIC TARGETS

Identification of the role of LXRs in cholesterol efflux from macrophages and reverse cholesterol transport pointed to a possible antiatherosclerotic effect of LXR activation. This notion was confirmed by subsequent experiments on

murine models of atherosclerosis. Joseph *et al*. (71) showed that administration of GW3965 reduces the formation of aortic lesions by about 50% in both LDL receptor-deficient and apoE-deficient mice, the two most commonly used animal models of atherosclerosis. T0901317 was reported to exert even stronger antiatherogenic effect (up to 71% reduction in lesion formation) in LDL receptor-deficient mice (72). Importantly, in this model T0901317 is able not only to delay progression of atherosclerosis but also to induce regression of preexisting lesions (73). It should be noted that antiatherosclerotic action of synthetic LXR agonists in murine models is to a large extent independent from changes in plasma lipid profile which indicates that this effect is predominantly a consequence of direct action of LXR activators on the vascular wall. Consistent with this notion synthetic LXR agonists were shown to stimulate ABCA1 and ABCG1 expression in the atherosclerotic lesions of both LDL receptor- and apoE-deficient mice (71-73). Subsequent experiments using bone marrow transplantation approaches provided direct evidence for protective role of macrophage LXRs in atherosclerosis development. Tangirala (74) demonstrated that hematopoietic stem cells-specific LXRα/β deficiency aggravates atherosclerosis in both apoE- and LDL receptor-null mice. Furthermore, T0901317 treatment has no inhibitory effect on atherosclerosis in LDL-deficient mice with macrophages devoid of LXRs (73). These animals have intact LXR signaling in other tissues which underscores the key role of macrophage LXRs in the antiatherosclerotic effect of LXR agonists.

Another mechanism that could potentially contribute to the antiatherosclerotic action of LXR activators is their suppressing effect on macrophage inflammatory mediators production. Joseph *et al*. (75) demonstrated that GW3965 and T0901317 inhibit expression of inducible nitric oxide synthase, cyclooxygenase-2 and interleukin-6 in macrophages subjected to bacterial infection or lipopolysaccharide stimulation. This inhibition depends on both LXRα and LXRβ and is mediated through suppression of the nuclear factor-κB signaling. Anti-inflammatory action of LXR agonists was confirmed *in vivo* in a model of contact dermatitis and in the aortas of the atherosclerotic mice (75). LXR activation was also shown to inhibit vascular smooth muscle cell proliferation (76) and to reduce macrophage production of matrix metalloproteinase-9 (77), thereby decreasing growth of the atherosclerotic plaque and increasing its stability.

Although LXR agonists were proved to posses potent antiatherosclerotic properties in mice, the results of these studies cannot be directly extrapolated to humans due to species differences in lipoprotein metabolism and LXR targets. For instance, in contrast to rodents, human Cyp7a1 is not under transcriptional control of LXRs (41, 42). Another significant difference between mice and humans is the absence in mice of cholesteryl ester transfer protein (CETP), a well known LXR target gene (78). CETP is involved in the exchange of triglycerides in apoB-containing lipoproteins for HDL-cholesterol, thereby decreasing HDL-

cholesterol concentration and promoting a more atherogenic plasma lipoprotein profile (79). Interestingly, T0901317 increases HDL-cholesterol concentration in wild type but not in "humanized" mice expressing CETP (80). In addition, Groot *et al*. (81) reported that synthetic LXR agonists failed to increase HDL-cholesterol level in hamsters and cynomolgus monkeys, two CETP expressing species. Importantly, LDL-cholesterol concentration was simultaneously elevated. The results of these studies strongly indicate that the biological response to LXR activation is to a large extent modulated by the presence of CETP, thus raising the need for reevaluation of antiatherosclerotic potential of LXR agonists in CETP expressing species.

FATTY ACID METABOLISM

Liver

In addition to cholesterol metabolism LXRs have also been shown to regulate hepatic fatty acid biosynthesis. This process is governed by SREBP-1c that regulates all the genes involved in this pathway, namely acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD) (51). Peet *et al*. (39) were the first to report that LXRα-deficient mice are characterized by markedly lower hepatic expression of SREBP-1c and several of its target genes including FAS and SCD-1. These findings were confirmed in LXRα/β double knockout mice that in addition to reduced expression of lipogenic genes exhibit decreased level of hepatic and plasma triglycerides (14, 82, 83). On the other hand, LXRβ-deficient mice retain normal expression of SREBP-1c and its target genes in the liver (40, 84), indicating that LXRα is the subtype responsible for controlling hepatic lipogenesis. Consistently, LXRα-deficient mice are characterized by markedly reduced hepatic fatty acid synthesis and incorporation of palmitoleic and oleic acid into phospholipids and triglycerides (84).

In agreement with the data from knockout animals, administration of T0901317 increases hepatic expression of SREBP-1c, ACC, FAS and SCD-1 in wild type but not in LXRα/β- or LXRα-deficient mice (14, 85). T0901317-induced activation of lipogenesis leads to massive hepatic accumulation of triglycerides (and ultimately to liver steatosis and dysfunction), and hypertriglyceridemia in mice, rats and hamsters (14, 86 and Baranowski *et al*., unpublished observation). Similar phenotype was recently observed in mice with liver-specific LXRα overexpression (87). The key role of LXRα in the control of hepatic lipogenesis was confirmed by Lund *et al*. (17) in experiments with specific LXRα activator. In wild type mice this compound was reported to be as potent as LXR pan-agonist in inducing hypertriglyceridemia, stimulating expression of hepatic lipogenic genes and accumulation of triglycerides, whereas in the LXRα-deficient animals it was completely ineffective. On the other hand, LXR pan-agonist exerted similar effects in wild type and LXRβ-deficient mice.

Chu and Miyazaki (86) demonstrated that SCD-1 is crucial for the lipogenic effect of LXRs. They reported that SCD-1 deficiency completely protects against hypertriglyceridemia and attenuates hepatic accumulation of triglycerides in T0901317-treated mice.

Initially, activation of hepatic lipogenesis upon LXR stimulation has been presumed to be mediated solely *via* induction of SREBP-1c. Functional LXRE activated by both LXR subtypes was identified in the promoter region of the SREBP-1c gene and stimulation of LXR/RXR heterodimer was reported to increase the content of precursor as well as mature, nuclear SREBP-1c protein in HepG2 cells (84, 88). The importance of SREBP-1c in LXR-induced stimulation of hepatic lipogenesis was confirmed in SREBP-1c knockout mice treated with T0901317. In the liver of these animals transcriptional response of the majority

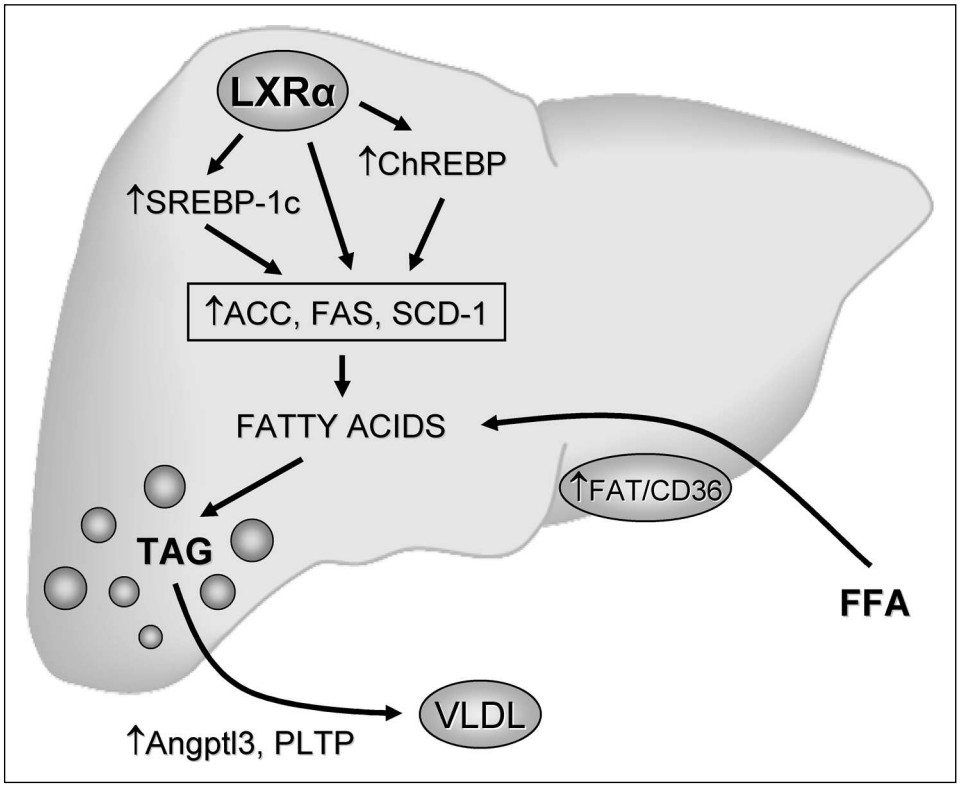

Fig. 3. LXRα-mediated stimulation of lipogenesis in the liver leads to hepatic steatosis and hypertriglyceridemia. LXR-regulated genes are indicated. ACC - acetyl-CoA carboxylase, Angptl3 - angiopoietin-like protein 3, ChREBP - carbohydrate response element binding protein, FAS - fatty acid synthase, FAT/CD36 - fatty acid translocase/CD36, FFA - free fatty acids, PLTP - phospholipid transfer protein, SCD-1 - stearoyl-CoA desaturase 1, SREBP-1c - sterol regulatory element binding protein 1c, TAG - triacylglycerol, VLDL - very low density lipoprotein.

of lipogenic genes to LXR activation is absent and hepatic accumulation of triglycerides as well as hypertriglyceridemia are substantially reduced (86, 89). However, the increase in expression of ACC, FAS and SCD-1, albeit blunted, is still present indicating contribution of the SREBP-1c-independent mechanism. Subsequent studies revealed that these three genes contain functional LXREs and, therefore, are directly regulated by LXRs (86, 90, 91).

Carbohydrate response element binding protein (ChREBP) is a glucose-sensitive transcription factor stimulating expression of lipogenic genes, thereby promoting hepatic conversion of excess carbohydrate to lipids. Cha and Repa (92) reported that administration of T0901317 increases mRNA level and activity of ChREBP as well as expression of ChREBP-regulated genes in the liver of wild type but not LXRα/β-deficient mice. They also identified two LXREs, activated by both LXRα and LXRβ, in the ChREBP gene promoter region. Furthermore, in ChREBP knockout mice stimulatory effect of T0901317 on the hepatic expression of lipogenic genes including ACC, FAS and SCD-1 was attenuated. T0901317-induced activation of ChREBP seems to involve AMP-stimulated protein kinase (AMPK). ChREBP transcriptional activity is inhibited by the active phosphorylated form of AMPK and T0901317 was reported to decrease AMPK phosphorylation in murine liver in LXR-dependent manner, thereby, removing its inhibitory effect on ChREBP (92). However, experiments on fasted and fed mice and on primary hepatocytes incubated with either high or low glucose concentration revealed that increased intracellular glucose flux is required for T0901317-induced increase in ChREBP transcriptional activity (13). Although LXR agonist is able to stimulate ChREBP expression under both conditions, its activation and translocation to the nucleus is induced only in fed mice and in hepatocytes incubated with high glucose concentration. Taking together, LXRs appear to regulate hepatic expression of lipogenic genes by multiple mechanisms, including both direct (*via* LXREs) and indirect (*via* SREBP-1c and ChREBP) effects (*Fig. 3*).

Grefhorst *et al*. (93) reported that hypertriglyceridemia induced by LXRs results from augmented hepatic VLDL-triglyceride secretion. Interestingly, the number of VLDL particles formed does not change, instead, their diameter increases due to higher amount of triglycerides per particle. Hypertriglyceridemic action of LXR agonists may be partially mediated by phospholipid transfer protein (PLTP). This protein not only mediates phospholipid transport from apoB-containing lipoproteins to HDLs but also promotes VLDL secretion from the liver (94) and T090137 was shown to upregulate hepatic expression and plasma activity of PLTP in mice (95). In addition, angiopoietin-like protein 3 (Angptl3) was demonstrated to play a critical role in LXR-induced hypertriglyceridemia. Angptl3 is a secretory protein, expressed exclusively in the liver, that was shown to play an important role in lipid metabolism (96). Synthetic and natural LXR agonists increase Angptl3 expression in human hepatoma cells and murine liver through binding to a functional LXRE identified in its gene (97).

Inaba *et al*. (98) reported that Angptl3-deficient mice treated with T0901317 are completely protected from hypertriglyceridemia but not from hepatic triglyceride accumulation. The authors suggested that Angptl3 promotes hypertriglyceridemia through inhibition of lipoprotein lipase (LPL) activity.

It should be noted that hypertriglyceridemic effect of LXR agonists is usually transient and limited to the first few days of the treatment (17, 90), likely due to enhanced VLDL-triglyceride hydrolysis resulting from increased expression of hepatic LPL (17, 93). Functional LXRE activated by LXRα and, to a lesser extent, by LXRβ was found in the LPL gene and T0901317 was reported to induce LPL expression in murine liver and macrophages but not in skeletal muscle or adipose tissue (99).

Recently Zhou *et al*. (87) identified fatty acid translocase/CD36 (FAT/CD36) as a novel transcription target of LXRα. They found functional LXRE in the promoter region of FAT/CD36 gene and demonstrated that its expression is induced by synthetic and oxysterol LXR ligands in a liver-specific manner in mice and humans. Furthermore, hepatic steatosis and stimulation of lipogenic genes were attenuated and hypertriglyceridemia was completely prevented in FAT/CD36-deficient mice treated with LXR agonists. The above data suggests that in addition to stimulation of fatty acid synthesis increased hepatic free fatty acid uptake may also contribute to liver steatosis induced by LXR activation.

Since the major function of LXRs is to maintain cholesterol homeostasis it may be difficult to understand why LXR activation should increase fatty acid synthesis. One reason may be to provide oleoyl-CoA (synthesized by SCD-1) that is the preferred substrate for cholesterol esterification. In addition, lipogenesis supplies lipids essential for cholesterol removal from the cell, including triglycerides needed for VLDL production in the liver and phospholipids required for HDL formation and maintenance of the appropriate ratio of cholesterol to other lipids in the plasma membrane (6, 100). Nevertheless, the paradoxical hyperstimulation of hepatic lipogenesis by synthetic LXR agonists (particularly T0901317) is still difficult to understand in the context of the role of these receptors in cholesterol homeostasis. It should be emphasized, however, that effects of pharmacologic and nonpharmacologic LXR activation differ markedly. In a physiological setting the underlying cause for LXR stimulation is cholesterol excess which results not only in generation of oxysterol ligands but also in suppression of SREBP-1c activation through inhibition of its proteolytic cleavage (51). Consistently, cholesterol-rich diet has very modest effect on hepatic expression of lipogenic genes and triglyceride content (39). In contrast, synthetic LXR agonists induce SREBP-1c expression without suppressing its activation which results in dramatic increase in fatty acid biosynthesis. It should be noted, however, that various synthetic LXR agonists seem to differ in their potency to stimulate lipogenesis. Several studies comparing effects of T0901317 and GW3965 revealed that the latter agonist has only limited ability to increase expression of lipogenic genes and in consequence does not induce hepatic

steatosis and hypertriglyceridemia in mice (16, 67, 85). There are, however, reports showing modest increase in liver and/or plasma triglycerides upon GW3965 administration (69, 81, 87).

The fact that the effect of insulin on expression of lipogenic genes is mediated by SREBP-1c (51) pointed to a possible role for LXRs as mediators of insulin action in the liver. This notion was confirmed by Tobin *et al*. (37) who showed that insulin stimulates LXRα expression in rat hepatocytes both *in vitro* and *in vivo*. Furthermore, insulin-induced increase in hepatic SREBP-1c and other lipogenic genes was completely abolished in LXRα/β-deficient mice. It was also shown that intact LXREs in the SREBP-1c gene promoter are required for induction of SREBP-1c expression by insulin (101), which further underscores the critical role of LXRs in the lipogenic action of this hormone.

LXRs were shown to regulate also other aspects of hepatic fatty acid metabolism. For instance, Hu *et al*. (102) demonstrated that T0901317 increases hepatic peroxisomal fatty acid β-oxidation rate and expression of the related enzymes in LXRα-dependent and PPARα-independent manner. In addition, LXR activation increases expression and activity of Δ6 and Δ5 desaturase in murine liver (103).

Undoubtedly, induction of lipogenesis by synthetic LXR agonists resulting in severe hepatic steatosis and hypertriglyceridemia represents the major barrier to their clinical use. However, it is currently unclear to what extent these adverse side effects observed in experiments on rodents will occur in higher species, including humans. Recently Kotokorpi *et al*. (56) showed significant differences between human and rat hepatocytes in response to GW3965, raising the question as to how well rodent models reflect the human situation. For instance, LXR activation reduced output of VLDL-triglycerides in human but not rat hepatocytes. Noteworthy, although GW3965 markedly stimulated lipogenic gene expression in human hepatocytes, the increase in the triglyceride content was very modest. In addition, two synthetic LXR pan agonists were shown to induce hypertriglyceridemia in hamsters whereas in cynomolgus monkeys such effect was not observed (81).

Skeletal muscle

Although both LXR subtypes have been shown to be expressed in human and murine skeletal muscle, to date only few studies have addressed the role of LXRs in this tissue. LXRβ was shown to be the dominant subtype in skeletal muscle of mice and humans (30, 100, 104, 105). However, Muscat *et al*. (59) reported similar level of LXRα and LXRβ mRNA in murine quadriceps and we observed predominance of LXRα protein in rat soleus and gastrocnemius (Baranowski *et al*., unpublished data). Both LXR isoforms are expressed early during differentiation of human myotubes and slightly increased in mature myotubes (106). On the other hand, in C2C12 cells only LXRα expression is induced during differentiation to myotubes and LXRβ is constitutively

expressed (59). Noteworthy, LXRα expression is markedly increased upon LXR activation in human differentiated myotubes (29, 105, 107) but not in murine skeletal muscle (30, 59, 69).

Available studies suggest marked differences in LXR signaling between liver and skeletal muscle. In contrast to liver, skeletal muscle fatty acid synthesis rate and expression of lipogenic genes are not reduced in LXRα/β-deficient mice (59, 83). In addition, although T0901317 and GW3965 strongly induce SREBP-1c expression in murine skeletal muscle, mRNA levels of lipogenic genes including FAS, ACC and SCD-1 are not upregulated (59, 69). Noteworthy, expression of other LXR target genes like ABCA1, ABCG1 and apoE is strongly induced upon T0901317 administration (59) indicating normal response to LXR stimulation. Studies by Kase *et al*. (29, 106) demonstrated that in contrast to murine skeletal muscle, human differentiated myotubes respond to T0901317 with marked upregulation of both SREBP-1c and its downstream targets, including ACC, FAS and SCD-1. Noteworthy, although in the same experiments 22(R)-hydroxycholesterol increased SREBP-1c expression, other lipogenic genes were not induced by this oxysterol. Consistently, T0901317 but not 22(R)-hydroxycholesterol was found to stimulate lipogenesis in human myotubes (29). It should be noted, however, that in contrast to Kase *et al*. (29, 106), Cozzone *et al*. (105) reported very modest increase in the expression of lipogenic genes in human differentiated myotubes treated with T0901317, likely due to the fact that LXR activation upregulated only precursor but not mature SREBP-1c protein. In human myotubes T0901317 was also shown to induce expression of FAT/CD36 and acyl-CoA synthetase long chain family member-1 which results in increased palmitate uptake and incorporation into cellular lipids (29, 106, 107). Although palmitate oxidation rate was simultaneously elevated, the increase was not sufficient to compensate for higher fatty acid uptake. In consequence LXR activation resulted in shunting of palmitate toward formation of complex lipids (107).

ANTIDIABETIC ACTION OF LXR AGONISTS

The close interdependence of lipid and carbohydrate metabolism as well as identification of LXRs as mediators of insulin action in the liver pointed to a possible role of these receptors in glucose homeostasis. This notion was confirmed by several studies demonstrating potent glucose-lowering and insulin-sensitizing effects of synthetic LXR agonists in various rodent models of diabetes and insulin resistance. Administration of T0901317 was reported to normalize plasma glucose level in db/db mice and Zucker diabetic fatty (ZDF) rats (108, 109). Noteworthy, LXR activators do not affect glycemia in nondiabetic animals (108, 110). In addition, GW3965 and T0901317 were shown to enhance insulin sensitivity in ob/ob mice and Zucker fatty rats as well as in high-fat fed rodents (69, 108-111). Interestingly, LXRα- as well as LXRβ-deficient mice retain

normal insulin sensitivity (112). Although still a matter of debate, several potential mechanisms for the antidiabetic action of LXR agonists have been proposed (*Fig. 4*).

Hepatic gluconeogenesis

Stulnig *et al*. (113) were the first to demonstrate that LXR activation results in striking downregulation of the key genes of gluconeogenesis (phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase) in the liver of wild type but not LXRα/β-deficient mice. This observation was subsequently confirmed by other groups in db/db mice and high-fat fed rats treated with synthetic LXR agonists (108, 110, 111). Consistent with the above findings, T0901317 and GW3965 markedly reduced hepatic glucose output in ZDF and high-fat fed rats, respectively (108, 111). It should be noted, however, that Grefhorst *et al*. (69) reported no effect of GW3965 on hepatic insulin sensitivity and glucose production in the liver of lean and ob/ob mice. Experiments

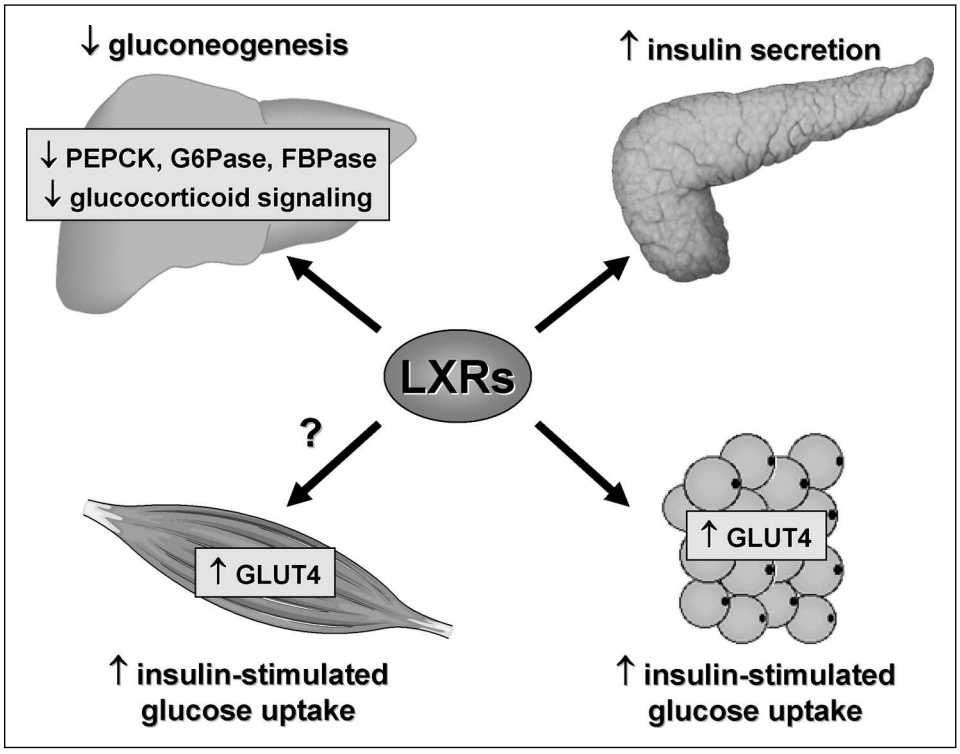

Fig. 4. Mechanisms underlying antidiabetic action of LXR agonists. FBPase - fructose-1,6-bisphosphatase, G6Pase - glucose-6-phosphatase, GLUT4 - glucose transporter 4, PEPCK - phosphoenolpyruvate carboxykinase.

on rat hepatoma cells and human primary hepatocytes demonstrated that inhibition of gluconeogenic gene expression by synthetic LXR agonists results from their direct action on hepatocytes (108, 111) and that both LXR subtypes are involved in this phenomenon (114). However, *in vivo*, the ability of GW3965 to suppress expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in murine liver depends on the presence of LXRα but not LXRβ (111).

Even though the exact mechanism underlying suppression of gluconeogenic genes remains obscure, it was shown that LXRs do not regulate glucose-6-phosphatase transcription directly, but rather by regulation of expression of another protein (114). Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and SREBP-1c are potential candidates for this protein. PGC-1α is the key regulator of hepatic glucose production (115) and its mRNA level is reduced upon LXR activation in rodent liver (110, 111). In addition, SREBP-1c overexpression was shown to downregulate expression of phosphoenolpyruvate carboxykinase (116) which points to a role of this transcription factor in LXR-induced inhibition of gluconeogenesis. Another potential mechanism involves suppression of glucocorticoid signaling. T0901317 was shown to reduce hepatic expression of glucocorticoid receptor and 11beta-hydroxysteroid dehydrogenase type 1 (the enzyme mediating synthesis of active corticosterone from inactive 11-dehydrocorticosterone) in wild type and db/db mice, but not in LXRα/β-deficient animals (109, 113, 117). However, Commerford *et al*. (111) did not confirm this observation in high-fat fed rats treated with GW3965.

Peripheral glucose uptake

Functional LXRE was identified in the promoter region of glucose transporter 4 (GLUT4) gene in mice and humans (110, 118) and synthetic LXR activators were repeatedly shown to increase GLUT4 expression in white adipose tissue (WAT) of mice and rats as well as in cultured murine and human adipocytes (30, 69, 110, 111, 118, 119). This effect seems to be mediated by both LXR subtypes since T0901317 upregulates adipose tissue GLUT4 expression in either LXRα- or LXRβ-deficient mice (but not in LXRα/β double knockout animals) (118). It should be noted, however, that only LXRα-deficient mice are characterized by decreased GLUT4 mRNA level in WAT (118). Consistent with the above data, T0901317 was found to improve insulin-stimulated glucose uptake in 3T3-L1 adipocytes (110). This observation was, however, not confirmed by other group (120). Interestingly, in the study by Fernandez-Veledo *et al*. (119) LXR activation ameliorated tumor necrosis factor α-induced insulin resistance in rat brown adipocytes by completely restoring insulin-stimulated glucose uptake and GLUT4 translocation to the plasma membrane and correcting abnormalities in the insulin signaling cascade. In addition, Commerford *et al*. (111) reported marked (albeit statistically insignificant) increase in subcutaneous fat glucose uptake during euglycemic-hyperinsulinemic clamp in high-fat fed rats treated with

GW3965. There are also studies showing elevated basal glucose uptake and GLUT1 expression in rodent adipocytes upon LXR activation (110, 119, 120).

In contrast to adipose tissue, skeletal muscle GLUT4 expression is not stimulated upon LXR activation in rodents (30, 69, 110, 111) which suggests tissue-specific regulation of this gene by LXRs. Consistently, GW3965 did not affect skeletal muscle glucose uptake during euglycemic-hyperinsulinemic clamp in high-fat fed rats (111). It should be noted, however, that Dalen *et al*. (118) found increased GLUT4 mRNA level in skeletal muscle of mice treated with synthetic LXR agonist. In contrast to studies on rodents, GLUT4 expression was markedly upregulated upon T0901317 treatment in differentiated myotubes of both control and type 2 diabetic subjects (29, 107), a similar, albeit weaker effect was observed also for GLUT1. In support of this observation, increase in insulin-mediated glucose uptake and oxidation (but not glycogen synthesis) has been described in human differentiated myotubes treated with synthetic LXR agonist (107). Interestingly, although T0901317 promotes lipid accumulation in differentiated human myotubes in the presence of a high glucose concentration, it does not impair insulin signaling in these cells (105).

β-cell function

LXRβ and, to a lesser extent, LXRα are expressed in rodent as well as human pancreatic islets (121, 122). However, β-cells and insulin-secreting cell lines exclusively express LXRβ (122). It was shown that prolonged exposure of rat pancreatic islets and insulinoma cell lines to T0901317 increases insulin secretion induced by glucose and glucagon-like peptide 1 (121, 122). Consistently, LXRβ-deficient (but not LXRα-null) mice exhibit markedly decreased glucose tolerance due to impaired insulin secretion (112) and pancreatic islets isolated from these animals are characterized by significantly reduced basal and glucose-stimulated insulin secretion (122). However, the ability of LXR agonists to stimulate insulin secretion *in vivo* is controversial. There are reports demonstrating increased plasma insulin concentration upon LXR activation in wild type and db/db mice (69, 123), but other studies have shown no effect (108, 109, 111, 124).

The stimulatory effect of LXR agonists on insulin secretion seems to be mediated by regulation of both glucose and lipid metabolism in pancreatic β-cells. T0901317 was shown to stimulate expression of GLUT2 and glucokinase and to increase pyruvate carboxylase activity in rodent insulinoma cell lines (121, 122). Together these effects would be expected to elevate intracellular ATP level *via* promotion of glucose flux through the citric acid cycle and stimulation of anaplerosis (restoration of the citric acid cycle intermediates pool). In addition, T0901317 and GW3965 increase expression of the "classic" LXR target genes including SREBP-1c, ACC and FAS (121, 122, 125) which accelerates production of malonyl-CoA and fatty acids (stimulators of insulin biosynthesis and secretion). Consistently, suppression of the lipogenic response by either

siRNA targeting SREBP-1c or ACC inhibitor substantially reduces the stimulatory effect of LXR activation on insulin secretion (122).

Although in the short run LXR-induced stimulation of lipogenesis in pancreatic β-cells promotes insulin secretion, chronic activation of this pathway can lead to lipid overload and apoptosis resulting from lipotoxicity. This notion was confirmed by a recent study showing induction of apoptosis accompanied by accumulation of triglycerides and free fatty acids in isolated rat pancreatic islets and insulinoma cells upon chronic LXR activation with T0901317 (125). Synthetic as well as natural LXR agonists were also shown to increase the susceptibility of insulinoma cells to free fatty acid- and cytokine-induced apoptosis (126). In addition, it was recently suggested that increased expression of LXRs may contribute to the dysfunction of pancreatic β-cells observed in type 2 diabetes (125).

Potent glucose-lowering properties of T0901317 and GW3965 demonstrated in rodent studies suggest a potential clinical use of LXR agonists as antidiabetic drugs. However, enthusiasm over their favourable effects on carbohydrate metabolism is tempered by several adverse effects predominantly related to enhanced lipogenesis. Firstly, administration of T0901317 to db/db mice results in a more severe hypertriglyceridemia and hepatic lipid accumulation than observed in nondiabetic mice (124). Secondly, as discussed above, extended stimulation of lipogenesis in pancreatic β-cells may induce their apoptosis and in consequence impair insulin secretion. In addition, there are reports showing reduced expression of glycolytic enzymes in human hepatocytes and murine adipose tissue upon LXR activation (56, 113), a clearly undesirable effect under diabetic conditions.

CONCLUSIONS

Studies conducted over the last decade substantially extended our knowledge of the pathophysiological role of LXRs. It is now evident that these receptors function not only as sterol sensors but also as key regulators of fatty acid and carbohydrate metabolism. Experiments on rodents provided strong evidence for potential therapeutic application of LXR ligands for the treatment of various human diseases including atherosclerosis and type 2 diabetes. However, due to extensive side effects, particularly related to their lipogenic action, LXR-activating drugs must be specially designed in order to be applicable in clinical practice. Several potential strategies for dissociating antiatherosclerotic and antidiabetic effects of LXR agonists from their lipogenic effects have been proposed (127). These strategies include development of LXRβ-selective agonists, tissue or gene-specific LXR activators and sterol mimicking LXR agonists capable of suppressing SREBP processing. Clearly, development of such

compounds presents an exciting but challenging task for scientists in the pharmaceutical industry.

Acknowledgements: This work was supported by the Polish Ministry of Science and Higher Education grant no. N401 134 31/2926.

Conflicts of interest statement: None declared.

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Received: October 1, 2008
Accepted: December 3, 2008

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