Liver X Receptors at the Intersection of Lipid Metabolism and Atherogenesis

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Introduction

Cholesterol homeostasis is maintained through the coordinated regulation of the trafficking, biosynthesis, absorption, and efflux of cholesterol. From the discovery that atherosclerotic plaques contain >20-fold more cholesterol than healthy aorta, to the identification of mutations in the LDL receptor as the cause of Familial Hypercholesterolemia, to the discovery of the statin drugs, cholesterol metabolism has been a focus of intensive investigation for decades¹. Cholesterol is crucial for normal biological functions, including hormone synthesis, bile acid synthesis and maintaining cell membrane fluidity; however, excess circulating cholesterol is associated with CVD, especially when the cholesterol is carried by low-density lipoproteins (LDL). The evidence linking cholesterol to heart disease comes from population studies²⁻⁴, genetics, experimental models^{5, 6}, and therapeutic intervention⁷. Despite tremendous gains in our understanding of CVD, it remains the leading cause of mortality worldwide⁸. In this review, we focus on molecular mechanisms by which Liver X Receptors (LXRs) mediate physiological responses to cellular and systemic cholesterol overload and the relationship of these pathways to atherogenesis (Figure 1).

Transcriptional Regulation of Cholesterol Homeostasis

Intracellular cholesterol homeostasis is maintained primarily through the actions of opposing transcription factors: LXRs, which are activated when cells accumulate excess cholesterol, and Sterol Response Element Binding Protein 2 (SREBP-2), which is active when cholesterol levels are low. The intricate details of how the SREBP-2 senses and responds to membrane cholesterol content have been elucidated, and readers are directed to a recent excellent review of this topic⁹. LXRs are ligand-activated transcription factors belonging to the nuclear receptor superfamily. There are two highly homologous LXR proteins with distinct tissue expression patterns. LXR β (*NR1H2*) is ubiquitously expressed, while LXR α (*NR1H3*) is highly expressed in liver, intestine, kidney, adipose and macrophages¹⁰⁻¹².

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Conflict of Interest

The authors have no conflicts to disclose

Crystal structures of the two LXRs have revealed highly similar ligand binding domains¹³⁻¹⁶. Although they appear to respond to the same endogenous sterol ligands¹⁷, *in vivo* studies suggest that the two LXRs perform distinct physiological roles. This particularly true in the liver where the expression of LXR α dominates. LXR α is uniquely required for the efficient elimination of hepatic cholesterol in mice¹⁸.

LXRs form obligate heterodimers with Retinoid X Receptor (RXR) and modulate transcription of target genes via direct DNA binding. The optimal LXR response element is a direct repeat 4 (DR4) motif: repeats of the six base pair sequence AGTTCA separated by four base pairs^{10, 12}. Under basal conditions, corepressors bind the LXR/RXR heterodimer leading to transcriptional silencing. Ligand binding induces a conformational change in the heterodimer, releasing the corepressors and recruiting coactivators^{19, 20}. LXRs are activated physiologically by oxidized derivatives of cholesterol, oxysterols, which are synthesized when the cell accumulates excess cholesterol²¹⁻²³. Many LXR target genes have been identified in experiments screening for the gene expression consequences of cellular cholesterol loading or synthetic LXR agonist treatment²⁴.

The LXR Signaling Pathway in Atherogenesis

Atherosclerotic lesion formation begins with lipid deposition and the retention of ApoB-containing lipoproteins beneath the vascular endothelium. This process leads to the infiltration and accumulation of monocytes and macrophages in the subendothelial space. Macrophages take up native and modified lipoproteins via receptor-mediated endocytosis, macro pinocytosis, and scavenger receptor pathways, reviewed in²⁵. Cholesteryl esters taken up from lipoproteins are hydrolyzed to free cholesterol and fatty acids in the late endosome. Excess free cholesterol is then trafficked to the endoplasmic reticulum, where it is re-esterified for storage in lipid droplets²⁶. Lipid-laden macrophages within the artery wall are referred to as foam cells due to their characteristic microscopic appearance. Foam cells release inflammatory cytokines and chemokines, initiating an inflammatory state (reviewed extensively in^{25, 27, 28}).

More than a decade of research has established the LXR pathway as a major determinant of atherosclerosis susceptibility, at least in rodent models²⁹⁻³¹. Loss of LXRs greatly accelerates atherosclerotic lesion deposition in both *Ldlr*^{-/-} and *ApoE*^{-/-} mice. Conversely, pharmacologic agonists of LXR protect against lesion development in multiple mouse models of atherosclerotic disease. LXRs exert several different anti-atherogenic effects: they increase peripheral cholesterol efflux, they promote reverse cholesterol transport to the liver and hepatic sterol secretion, repress inflammation, enhance macrophage survival, and they reduce the uptake of ApoB-containing lipoproteins. These pathways are summarized schematically in Figure 2.

LXRs and Inflammation

Inflammation in the atherosclerotic plaque is postulated to be a key determinant of the progression of atherosclerosis and other coronary artery diseases^{27, 28, 32}. Recent work has demonstrated that inflammatory signaling in plaque macrophages can be initiated by pattern recognition receptors that were traditionally associated with responses to infection^{25, 33-35}.

A considerable body of evidence supports the hypothesis that LXRs can exert anti-inflammatory effects in multiple cell type and contexts³⁶. Treatment of cells or animals with synthetic LXR ligands, or genetic manipulation to achieve a constitutively active LXR³⁷, attenuates inflammatory responses triggered by TLR4 and TNF α ^{38, 39}. Interestingly, LXR activity represses a distinct subset of LPS-sensitive genes and only minimally suppresses inflammation activated by TLR3³⁹. LXR agonists attenuate the production of inflammatory mediators by cells within atherosclerotic lesions³⁸. Mice lacking LXRs show increased inflammatory gene and protein expression in a number of different experimental models. Inflammatory signaling has also been shown to feedback on the LXR response. For example, TLR4 activation inhibits LXR activity in an interferon regulatory factor 3-dependent manner⁴⁰.

The precise mechanism(s) by which LXRs reduce inflammation have been the subject of intense investigation. The most accepted candidate explanation posits that SUMOylated LXRs bind as monomers to the promoters of inflammatory genes and promote the presence of corepressor proteins⁴¹. The production of anti-inflammatory high-density lipoprotein (HDL)^{42, 43} has also been proposed to contribute to LXRs anti-inflammatory effects. Recent studies have also suggested that some LXR's anti-inflammatory action may be secondary to alterations in lipid metabolism. Induction of the phospholipid-remodeling enzyme lysophosphatidylcholine Acyltransferase 3 (Lpcat3) by LXR has been shown to be an important contributor to hepatic inflammatory responses both *in vitro* and *in vivo*⁴⁴.

Regulation of Apoptosis by LXRs

Some of the anti-atherogenic properties of LXR have been attributed to the enhanced survival of macrophages within lesions. To date, three LXR target genes have been identified that inhibit apoptosis in macrophages: *API6/AIM*, *LBP* and *MAFB*. The Breslow group first demonstrated that a member of the scavenger receptor cysteine rich repeat family called apoptosis Inhibitor 6 (API6) responded to cholesterol feeding⁴⁵. API6 is also commonly referred to as or apoptosis inhibitor expressed by macrophages (AIM). Our group identified *Aim* as determinant of macrophage survival in mice infected with *Listeria monocytogenes*⁴⁶. Further work demonstrated that *Aim* is induced when macrophages are exposed to oxidized LDL and is highly expressed in atherosclerotic lesions. Analysis of *Aim*^{-/-} *Ldlr*^{-/-} mice revealed that loss of AIM was atheroprotective⁴⁷, likely due to increased macrophage apoptosis at the early stages of lesion development. Subsequent studies by Hamada *et al.* reported that the expression of *Aim* is at least in part dependent on the expression of another LXR target gene, *MAFB*⁴⁸. Loss of bone marrow expression of *MAFB* attenuated early atherosclerosis by reducing the expression of *Aim*⁴⁸.

LPS binding protein (LBP) is an other LXR target linked to the control of apoptosis⁴⁹. LBP is a member of a family of lipid transfer proteins, which includes PLTP and CETP. Hepatic *LBP* is a well-characterized acute phase response gene⁵⁰. Little is known about the role of macrophage-expressed *LBP* in atherosclerosis; however, LBP's ability to inhibit the release of TNF α in models of septic shock suggests an anti-inflammatory role⁵¹. Sallam *et al.* used a bone marrow transplant approach to demonstrate that deletion of *Lbp* in hematopoietic cells was protective against atherosclerosis in *Ldlr*^{-/-} recipient mice. The mice receiving

Lbp^{-/-} bone marrow had decreased apoptotic cells in their aortas, and macrophages isolated from the mice receiving *Lbp*^{-/-} bone marrow had altered gene expression suggestive of a pro-apoptotic state.

Interestingly, loss of sterol transport protein ABCG1 in the background of *Ldlr* deficiency also resulted in reduced lesion development^{52, 53}. This effect was attributed to increased apoptosis of lesion macrophages, and is believed to be due to the accumulated of apoptosis-inducing oxysterols in the absence of ABCG1.

The findings that deletion of the LXR targets *Aim*, *Lbp* and *Abcg1* in mice reduces the severity of early atherosclerotic lesions appear to be contradictory to anti-atherosclerotic actions of LXRs themselves. There are several possible explanations to this apparent contradiction. LXRs control the expression of a battery of genes, many of which exert strong effects on lipid homeostasis. It is possible that the anti-atherogenic actions of other LXR target genes have a greater impact than *Aim*, *Abcg1*, and *Lbp*. It is also important to remember that LXR deletion reduces but generally does not abolish expression of LXR target genes. Readers are directed to an excellent literature review on the changing role of apoptosis in different phases of atherosclerosis progression⁵⁴.

Regulation of Lipid Uptake

As foam cells become overloaded with lipid, LXRs activate a negative feedback mechanism to limit the uptake of more cholesterol. Treatment of macrophages with LXR ligands reduces protein levels of LDLR. We identified the E3 ubiquitin ligase IDOL as the mediator of this LXR-dependent reduction in LDLR⁵⁵. IDOL limits the uptake of additional lipid by targeting members of the LDL receptor family for degradation. IDOL dimers interacted with members of the UBE2D family of E2 ubiquitin ligases to transfer ubiquitin to a conserved region on the cytoplasmic tail of three receptors: LDLR, the VLDL receptor, and the ApoE Receptor 2⁵⁶⁻⁵⁸. Lipoprotein receptors are poly-ubiquitinated by IDOL at both K48 and K63 residues, and thereby targeted for degradation in the lysosome⁵⁹. Macrophages and ES cells from *Idol*^{-/-} mice have more LDLR protein on their plasma membranes compared with wild-type mice^{60, 61}. Polymorphisms and loss of function mutations in human *IDOL* have been linked to circulating cholesterol levels⁶²⁻⁶⁴. However, further studies are required to determine the effect of IDOL expression in macrophages and other tissues on the development of atherosclerosis.

LXRs and Macrophage Cholesterol Efflux

Considerable effort has been directed towards understanding the critical role of LXRs in cholesterol efflux from macrophages and foam cells^{29, 65, 66}. The ability of LXRs to drive removal of cholesterol from foam cells is a key step in the prevention and perhaps even regression of atherosclerosis^{65, 67}. Mutations in the LXR-target gene *ABCA1* cause Tangier disease; a rare autosomal disease characterized by the absence of HDL-cholesterol accompanied by accumulation of cholesteryl ester-rich foam cells throughout the body⁶⁸⁻⁷⁰. Studies using fibroblasts from Tangier disease patients demonstrated that while flux of cholesterol to mature HDL is only diminished by 30-50%, the cells are completely unable to efflux cholesterol to lipid poor ApoA1^{71, 72}, suggesting that ABCA1 is responsible for the

flux of cholesterol to nascent HDL. Several groups subsequently identified LXR as the primary trans-activating factor mediating *ABCA1* transcription in both mouse and human macrophages^{73, 74}.

LXR also regulates the expression of *ABCG1*, which encodes a half transporter that may account for much of the *ABCA1*-independent flux of cholesterol to HDL⁷⁵. *Abcg1* knockout mice accumulate cholesterol and phospholipids in their macrophages, consistent with a role for *ABCG1* in cholesterol efflux⁷⁶. Overexpression of *ABCG1* in cell culture models promotes the efflux of cholesterol to a range of acceptors, including phosphatidylcholine vesicles and reconstituted HDL particles⁷⁷⁻⁸⁰. *ABCG1* is an intracellular protein, localized in the endosome compartment⁸¹, and appears likely to promote efflux from cells indirectly through the regulation of cholesterol homeostasis in intracellular membranes and perhaps trafficking to the plasma membrane.

Genetically modified mouse models have been used to demonstrate the importance of LXR target genes in cholesterol efflux from hematopoietic cells. Whole body deletion of *Abca1* did not affect the development of atherosclerosis, but this observation is complicated by the fact that the mice showed a 50% decrease in Apolipoprotein B and LDL cholesterol levels when fed a western diet^{82, 83}. However, bone marrow transplantation studies demonstrated that expression of both *ABCA1* and *ABCG1* in bone marrow cells is atheroprotective in mouse models^{82, 84-87}. The results of recently published studies in myeloid-specific conditional *Abca1* knockout mice suggest that cells other than macrophages may be contributors to these atheroprotective effects^{83, 88}.

LXRs and Reverse Cholesterol Transport

The movement of cholesterol from a lipid-laden macrophage in the periphery to the liver for excretion requires transportation by lipoproteins through the circulatory system. LXRs regulate the expression of several lipoprotein-metabolizing enzymes and improve the flux of cholesterol from the periphery to the liver. This process is believed to principally involve cholesterol transport by high-density lipoproteins (HDL). The initial step in RCT is the transfer of unesterified cholesterol from a lipid-laden foam cell in the atherosclerotic lesion to HDL, facilitated by *ABCA1* or *ABCG1*, as discussed above. LXR activation in the macrophage also induces the expression of Apolipoprotein E (ApoE)^{89, 90}, which both promotes efflux by acting intracellularly and also serves as an acceptor for newly effluxed cholesterol.

Interestingly, recent data suggests that the enhanced RCT observed with LXR agonists might be driven by LXR activity in multiple tissues. Breevort *et al.* used tissue specific LXR knockout mice and synthetic LXR agonist to demonstrate that macrophage to feces RCT is largely independent of macrophage LXR activity⁹¹. The authors found that on a standard chow diet, intestinal activation of LXR was the primary driver for RCT, confirming the results of other groups that used transgenic⁹² or tissue-specific LXR agonists^{93, 94}. These groups and others postulate that intestinal *ABCA1* is a significant driver of systemic HDL levels, thereby driving RCT^{95, 96}.

Nascent HDL particles mature through the acceptance of cellular cholesterol and lipid remodeling. Several circulating enzymes catalyze lipoprotein remodeling. Lecithin CoA Acyltransferase (LCAT) is activated by apolipoprotein A1 on HDL to transfer the 2-acyl group from phosphatidylcholine or phosphatidylethanolamine to unesterified cholesterol⁹⁷. Cholesteryl esters carried by HDL are exchanged for triacylglycerol from ApoB-containing lipoproteins through the actions of Cholesteryl Ester Transfer Protein (CETP). Human *CETP* expression is driven by LXR binding to a response element in its promoter, which was conserved in CETP transgenic mice⁹⁸. It is possible that effective RCT involves CETP-mediated transport of cholesterol to LDL for clearance by hepatic LDLR. Rader's group tested this hypothesis in mice using an adeno-associated virus (AAV) to express human CETP. They demonstrated that CETP improved the fecal excretion of macrophage-derived cholesterol in an LDLR-dependent manner⁹⁹. The data published using CETP transgenic mice is less clear. Breevoort *et al.* reported that CETP transgenic mice had reduced LXR-mediated RCT from macrophages to HDL, paradoxically accompanied by increased loss of macrophage-derived cholesterol from the body, due to synthesis of bile acids⁹¹.

Activation of LXR in the liver or in macrophages induces expression of *PLTP*, the gene encoding the Phospholipid Transfer Protein (PLTP)¹⁰⁰⁻¹⁰³, a secreted protein that facilitates the transfer of phospholipids between ApoB-containing lipoproteins and HDL¹⁰⁴. PLTP may also contribute to the anti-inflammatory activities of LXRs by transporting α -tocopherol, which reduces oxidation in lipoproteins¹⁰⁵. The relationship between PLTP and atherosclerosis is complicated. Mouse models of atherosclerosis suggests that local expression of *PLTP* by macrophages may be protective¹⁰⁶, while prolonged increased systemic levels worsen the progression of the disease^{107, 108}.

Induction of IDOL by LXRs affects lipoprotein metabolism in a species-specific manner. LXR activation in mice induces IDOL in macrophages and other peripheral tissues, but not in the liver. However, treatment of non-human primates with a synthetic LXR agonist induced *IDOL* in liver, which led to increased plasma LDL-cholesterol and APOB levels⁶⁰. These effects were at least in part attributable to degradation of LDLR, as antisense oligonucleotides against IDOL inhibit the ability of LXR agonist to raise LDL levels in non-human primates.

In order to distinguish between whole body and hepatic clearance of LDL, we created transgenic mice that overexpress a dominant active form of human *IDOL* hepatocytes. These mice had near complete degradation of liver LDLR resulting in a human-like lipoprotein profile. The mice developed early stage atherosclerotic lesions in a diet-dependent manner with a more mild lipoprotein phenotype than existing mouse models of atherosclerosis¹⁰⁹. Both of these studies underscore the importance of the LXR-IDOL axis to the regulation of LDLR *in vivo*.

Regulation of entero-hepatic cholesterol flux by LXRs

The liver and intestine are important regulators of whole body cholesterol homeostasis. LXR activity in the cells of these organs regulates multiple aspects of cholesterol metabolism, from uptake to trafficking and elimination. Several receptor-dependent pathways clear circulating cholesterol. LDL receptors bind APOB- and APOE-containing apolipoproteins

and internalize them via receptor-mediated endocytosis^{1, 110}. Cholesterol is also cleared by through selective uptake from HDL particles by SCARB1¹¹¹ and by the internalization of HDL particles¹¹². LXR reduces LDLR-mediated uptake through the induction of *IDOL*, as described above⁶⁰; however no clear role of LXR in the regulation of *SCARB1* expression has been described. LXR target genes have been reported to increase *SCARB1*-mediated cholesteryl ester uptake in human macrophages but there have not been any studies demonstrating LXR-mediated effects on SCARB1-mediated uptake in the liver¹¹³.

Cholesterol carried by LDL is taken up by receptor-mediated endocytosis and enters the endocytic trafficking pathway. The receptor dissociates from the complex in early endosomes before the lipoprotein particle is degraded in late endosomes. Fusion with lysosomes introduces hydrolytic enzymes that produce unesterified cholesterol¹¹⁴. There is a significant gap in knowledge explaining how cholesterol traffics from the late endosome to other organelles. Genetic studies of the cholesterol storage disease Niemann Pick C identified two causal genes: *NPC1* and *NPC2*^{115, 116}. Both of these genes are induced by synthetic LXR ligands in human macrophages¹¹⁷, but there have been no studies demonstrating that they are targets in liver. The membranes of the endocytic compartment comprise a pool of cholesterol important to the LXR activated ABCA1-mediated formation of nascent HDL²⁶. Lipoprotein-derived cholesterol is also trafficked to the endoplasmic reticulum where it is re-esterified by Acetyl Co Acyltransferase (ACAT) either for storage in lipid droplets or incorporation into nascent VLDL.

Recent work has identified a novel LXR-regulated phospholipid remodeling process that controls the assembly and secretion of VLDL. LXRs induce the expression of the gene encoding Lysophosphatidylcholine Acyltransferase 3 (*Lpcat3*)^{44, 118}. LPCAT3 transfers polyunsaturated fatty acyl groups to the *sn*-2 position of lysophospholipids^{119, 120}. We generated conditional knockout mice lacking *Lpcat3* and hypothesized that loss of this enzyme would increase the saturated fatty acid content of the PC pool, affecting the fluidity of membranes. Liver-specific *Lpcat3*^{-/-} mice had a pronounced neutral lipid storage phenotype accompanied by a reduction in the secretion of triglycerides in nascent ApoB-containing lipoproteins. Secretion of APOB was not affected by the loss of *Lpcat3*, but bulk lipidation of nascent lipoproteins was severely impaired. This defect was linked to a marked decrease in cellular membrane fluidity in live primary *Lpcat3*^{-/-} hepatocytes¹²¹. The enhanced membrane fluidity associated with *Lpcat3* activity is a mechanism by which LXR could potentially exert effects on multiple aspects of cellular lipid processing. The impact of this pathway on the progression of atherosclerosis has not yet been studied.

LXR activation in the liver drives the removal of cholesterol from the body. The original defect observed in the LXR α knockout mouse was an inability to process excess dietary cholesterol, leading to hepatomegaly and non-alcoholic hepatosteatosis¹⁸. The enzyme that catalyzes the rate-limiting step in bile acid synthesis, CYP7A1, is regulated transcriptionally by LXR α in mice. Synthesis of bile acids is a primary mechanism for the removal of cholesterol; however, direct secretion into bile also occurs. Hobbs' group demonstrated that LXRs regulate the expression of the half transporters ABCG5 and ABCG8¹²². These proteins form an obligate heterodimer on the canalicular membrane where they function to secrete cholesterol into the bile¹²³. Recent studies have implied that ABCG5/G8-

independent cholesterol efflux also occurs^{124, 125}, especially in response to cholesterol feeding¹²⁶, suggesting that additional efflux transporters have yet to be discovered. To date, all LXR-regulated processes that improve the secretion of cholesterol or bile acids from the liver into bile have been found to be atheroprotective, underscoring the importance of identifying new targets that further enhance the removal of excess cholesterol.

The intestine is the final organ in which LXRs contribute to RCT and help to promote negative whole body cholesterol balance. LXRs both reduce cholesterol absorption and promote a non-biliary route for cholesterol secretion¹²⁷⁻¹²⁹. Cholesterol, both dietary and biliary, is taken up from the intestinal lumen by enterocytes in the distal duodenum and proximal jejunum¹³⁰. Cholesterol in the apical membrane of enterocytes is subject to competing forces: it can be internalized for incorporation into nascent lipoproteins in an NPC1L1-dependent manner¹³¹ or it can be effluxed back into the lumen by the LXR targets ABCG5 and ABCG8¹³².

In addition to limiting cholesterol absorption, LXR activation has recently been shown to drive the secretion of non-biliary cholesterol through a process termed trans-intestinal cholesterol excretion (TICE)¹³³⁻¹³⁵. Under normal physiologic conditions this pathway only contributes a small percentage of the total neutral fecal loss; however, the pathway is highly inducible by LXR agonists or under pathophysiological conditions. For example, when biliary secretion is interrupted TICE can maintain fecal cholesterol excretion, reviewed in¹³⁴. The ability of TICE to compensate for lack of biliary secretion was recently demonstrated in mice with tissue-specific deletions of both *Abcg5* and *Abcg8*. Loss of the ABCG5/G8 transporter in either liver or intestine reduced the fecal excretion of infused cholesterol similarly, without affecting the clearance of cholesterol from the circulation¹²³. The molecular mechanisms involved in TICE are still being elucidated. Deciphering these mechanisms may uncover new cholesterol transport pathways^{136, 137}.

Pharmacology and Future Directions

The ability of LXRs to protect against atherosclerosis and even induce regression of lesions makes them potentially attractive drug targets. Despite this promise, however, only three LXR agonists have entered clinical trials and none progressed past phase I trials^{138, 139}. Although this review has focused on beneficial LXR-mediated effects on cholesterol metabolism, LXRs also induce the expression of lipogenic genes. A recent meta-analysis of genome wide association study datasets identified a subset of genes with pleiotropic effects on circulating lipids, cardiovascular disease, and metabolic disease. LXR was the most enriched pathway among these pleiotropic genes, underscoring the importance of LXRs in regulating a range of cardio-metabolic phenotypes¹⁴⁰. Unfortunately, this also illustrates the challenges associated with LXR pharmacology. First generation “pan LXR” agonists induced the expression of *Srebp1* as well as several downstream targets of SREBP-1C, including Fatty Acid Synthase and Stearoyl CoA Desaturase^{141, 142}. The up-regulation of lipid synthesis associated with these agonists lead to increased secretion of triglyceride-rich lipoproteins, transient hypertriglyceridemia, and ultimately hepatic steatosis¹⁴³. Studies in knockout mice demonstrated that these effects are primarily a result of LXRα.

activity^{144, 145} and, consequently, there has been a concerted effort to design either LXR β -specific or tissue specific pan-LXR agonists¹³⁸.

New studies are needed to fully understand how LXR α and LXR β achieve different patterns of gene expression. One recent study suggested that the hepatic LXR lipogenic program might be separated from cholesterol efflux. Silencing the LXR co-activator TRAP80 was reported to interfere with LXR-mediated induction of *Srebp1* but not *Abca1*¹⁴⁶. However, the robust expression of *Abca1* by Küpffer cells complicates the interpretation of these results. Nevertheless the studies set the stage for additional studies of selective LXR/co-activator interactions that could be important determinants of LXR responses in various tissues. Such interactions could also represent potential pharmacological targets to selectively agonize specific LXR programs.

Conclusions

In summary, LXRs are sterol-activated transcription factors that coordinate whole body lipid homeostasis. Studies using knockout mice and first generation synthetic LXR agonists demonstrated that LXR activity reduces atherosclerosis by several mechanisms and through actions in a range of cell types. Key biological effects of LXRs include promotion of cellular cholesterol efflux, inhibition of inflammation, prevention of cell death, and induction of macrophage to feces reverse cholesterol transport. Unfortunately, LXRs have proven to be difficult pharmacological targets due to their pleiotropic effects on a range of processes that impact cardio-metabolic diseases. Nevertheless, the drive to understand how LXRs regulate cellular and systemic metabolism has led to the discovery of new mechanisms governing lipid synthesis, metabolism and flux. These fundamental gains in understanding LXR biology will undoubtedly ultimately contribute to improved strategies for the treatment of cardiovascular disease.

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Highlights

- LXRs are sterol-responsive transcription factors that protect against atherosclerosis
- Activation of LXRs improves reverse cholesterol transport and reduces inflammation
- This review includes newly discovered atheroprotective gene targets of LXRs

Figure 1.

LXRs exert distinct effects on cholesterol and lipid metabolism in discrete cell types.

Globally, LXR activation induces reverse cholesterol transport and reduces atherosclerotic plaque burden. Macrophage LXRs respond to oxidized sterol by increasing cholesterol efflux to lipoprotein acceptors, while decreasing uptake through the targeted degradation of the LDL receptor. In hepatocytes, LXRs increase the removal of cholesterol either by conversion to bile acids or direct secretion into the bile by ABC transporters. LXRs also induce a lipogenic program in hepatocytes, leading to increased triglyceride production and secretion. LXR activation in the small intestine decreases cholesterol absorption by increasing the expression of apical efflux transporters. Intestinal LXRs raise circulating HDL levels by inducing the expression of *ABCA1*. Portions of this figure were modified with permission from Macmillan Publishers Ltd: *Nature Reviews Molecular Cell Biology* (reference ¹⁴⁷).

Early Atherosclerosis
(Fatty Streak / Foam Cell Lesion)

Time, genetic,
and environmental factors

Advanced Atherosclerosis
(Complex Lesion)

Feature	Effect of LXR agonist	Putative Gene(s) Responsible	References
Cholesterol-rich "foam cells"	↑ Cholesterol efflux	ABCA1, ABCG1	73-78, 80
Macrophage Recruitment / Activation	↓ Inflammatory gene expression	NF-κB target genes	36-40

Feature	Effect of LXR agonist	Putative Gene(s) Responsible	References
Necrotic core	↓ Apoptosis	AIM, LBP, MAFB, ABCG1	46-49, 52, 53
	↑ Efferocytosis	TGM2, MERTK	148, 149
Inflammatory cytokine release	↓ Inflammatory gene expression	NF-κB target genes	36-40
Foam cell accumulation	↑ Cholesterol efflux	ABCA1, ABCG1	73-78, 80
	↑ Macrophage egress	CCR7	67
Thinning fibrous cap	↓ Matrix degradation	MMP9	150

Figure 2. Activation of LXRs is protective at different stages of atherosclerosis. In early lesions, cholesterol accumulates in macrophages, which adopt “foam cell” morphology. LXR activation in these cells reduces inflammation and improves cholesterol efflux. As atherosclerosis progresses the lesions become more complex, characterized by acellular lipid accumulation, a necrotic core, and thinning fibrous cap. In advanced lesions LXR activation induces the expression of genes that increase cholesterol efflux, reduce inflammation, stabilize the fibrous cap, induce macrophage egress from the lesion, repress apoptosis, and improve the clearance of apoptotic debris.^{36-40, 46-49, 52-54, 67, 73-78, 80, 148-150}