LXRα phosphorylation in cardiometabolic disease: insight from mouse models

Maud Voisin[1*], Matthew Gage[2*], Natalia Becares[3], Elina Shrestha[1], Edward A. Fisher[1,4#], Ines Pineda-Torra[5#], Michael J. Garabedian[1#]

[1]Department of Microbiology, New York University School of Medicine, New York, NY 10016, USA.

[2]Department of Comparative Biomedical Sciences Royal Veterinary College, 4 Royal College Street, London NW1 0TU, UK

[3]Centre of Clinical Pharmacology, Division of Medicine, University College of London, London WC1 E6JF, UK.

[4]Department of Medicine, New York University School of Medicine, New York, NY 10016, USA.

[5]Centre of Cardiometabolic and Vascular Science, Division of Medicine, University College of London, London WC1 E6JF, UK.

*These authors contributed equally to this work.

#Corresponding authors;

Michael J. Garabedian, PhD (michael.garabedian@nyumc.org)

Ines Pineda-Torra, PhD (i.torra@ucl.ac.uk)

Edward A. Fisher, MD, PhD (edward.fisher@nyumc.org)

The authors have nothing to declare.

ABSTRACT

Post-translational modifications, such as phosphorylation, are a powerful means by which the activity and function of nuclear receptors such as LXRα can be altered. However, despite the established importance of nuclear receptors in maintaining metabolic homeostasis, our understanding of how phosphorylation affects metabolic diseases is limited. The physiological consequences of LXRα phosphorylation have, until recently, only been studied *in vitro* or non-specifically in animal models by pharmacologically or genetically altering the enzymes enhancing or inhibiting these modifications. Here we review recent reports on the physiological consequences of modifying LXRα phosphorylation at serine 196 (S196) in cardiometabolic disease including non-alcoholic fatty liver disease (NAFLD), atherosclerosis and obesity. A unifying theme from these studies is that LXRα S196 phosphorylation rewires the LXR-modulated transcriptome, which in turn alters physiological response to environmental signals, and that this is largely distinct from the LXR-ligand-dependent action.

.

INTRODUCTION

Liver X Receptors (LXRs) are ligand-activated transcription factors that control lipid and glucose homeostasis, as well as inflammation (1,2). LXRs consist of two subtypes, LXRα and LXRβ (NR1H3 and NR1H2, respectively), which exhibit 75% sequence homology (3). LXRα is expressed in tissues involved in lipid metabolism, such as adipose tissue and liver, as well as in bone marrow and immune cells while LXRβ is ubiquitously expressed (4). Like other nuclear receptors LXR is organized with an N-terminal activation function (AF), a central DNA binding domain (DBD) followed by a flexible the hinge region, and a C-terminal the ligand binding domain (LBD) (Fig. 1A). LXRs forms obligatory heterodimers with *9-cis* Retinoid X receptor (RXR) and are activated by desmosterol and oxysterols (2,5), which are oxidized-cholesterol derivatives. LXR can also homodimerize (6), however, the biological significance of this interaction has not been demonstrated. In the absence of ligand, the LXR/RXR heterodimer occupies LXR response elements (LXREs) on the regulatory regions of target genes (7). Unliganded LXR/RXR interacts with co-repressors, such as the Nuclear Receptor Co-Repressor (NCoR) and Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptor (SMRT), thereby silencing gene expression (8). This is also known as ligand-independent active repression. Once ligand binds to nuclear receptors, their ligand binding domain (LBD) undergoes a structural shift to a more stable position, which inhibits co-repressor interaction and creates a high-affinity site for co-activator proteins (9). These co-activator complexes are responsible for the modification of chromatin structure, thereby facilitating the assembly of the general transcriptional machinery to the gene promoter and inducing ligand-dependent transactivation of gene expression (10). LXRs can also repress gene expression through a mechanism known as transrepression (11,12), by which LXRs antagonize the activity of other signal-dependent transcription factors, such as the nuclear factor-κβ (NFκβ) and activator protein 1 (AP-1) (13,14). It appears this is partly the mechanism through which LXRs exert their anti-inflammatory functions (15,16). LXRs must be present in the nucleus to regulate target gene transcription, and the LXRα subtype has shown nuclear localization both in the absence and presence of its ligands (17,18).

Most proteins are subjected to post-translational modifications (PTMs), which are covalent modifications catalyzed by specific enzymes that result in changes in protein function (19,20). PTMs of nuclear receptors, such as phosphorylation, acetylation, sumoylation, methylation and O-GlcNAcylation, play important regulatory roles and allow rapid and reversible molecular changes in the nuclear receptor response (21). Phosphorylation (22-24), O-GlcNAcylation (25), and acetylation (26) of LXRα have been shown to influence LXRα-mediated transcriptional activation of target genes through changes in LXRα stability, transcriptional activity (either increasing or decreasing LXRα transcriptional potential), and/or recruitment of transcription factors at specific LXR target genes.

We have demonstrated that altering LXRα phosphorylation modifies its target gene repertoire *in vitro* in LXRα-overexpressing macrophage-like cell lines (22,27), and more recently *in vivo* in the context of cardiometabolic disease models of atherosclerosis progression (28), atherosclerosis regression and diabetes (29), obesity (30) and non-alcoholic fatty liver disease (NAFLD) (31). In this review, we discuss these recent findings and the potential of targeting LXRα phosphorylation for chronic inflammatory diseases.

LXRα PHOSPHORYLATION AT SERINE 198 AND ITS KINASES

LXRα is phosphorylated at Serine198 (S198) in human LXRα, and at the cognate serine196 (S196) site in mouse (22-24). Phosphorylation of this site is observed in mouse and human macrophages *in vitro* and in atherosclerotic plaque macrophages in Apolipoprotein E knock out (*Apoe KO)* mice (22,27), as well as in the liver (24,31). This site is located in the hinge region of LXRα, and is conserved between humans, rats, and mice while absent in LXRβ (Fig. 1A). Phosphorylation at this site is increased by both endogenous and synthetic LXR ligands (24(S),25-epoxycholesterol and T0901317 or GW3965, respectively), and has been reported to be a substrate of Casein Kinase 2 (CK2) (22), and Protein Kinase A (PKA) (24). Phosphorylation at S198 also affects LXRα transcriptional activity in a gene-selective manner. Molecular modeling of the DNA-bound LXRα-RXRα heterodimer (32) reveals alterations in the orientation of the

LXRα S198 residue upon its phosphorylation. Phosphorylated S198 is exposed on the surface of the complex, consistent with a site for protein interactions (Fig. 1B), whereas the unphosphorylated S198 is buried. However, a segment containing the unphosphorylated S198 adopts a short alpha helical stretch, suggesting an additional surface for protein interaction in this S198 non-phosphorylated state (27) (Fig. 1C). This is consistent with the phosphorylated and unphosphorylated LXRα imparting distinct structural characteristics to the hinge domain to influence cofactor recruitment, and modulating LXRα activity in a gene-specific manner. Given the structural impact and functions effects of LXRα phosphorylation at this site in cell based models, we examined the relevance of LXRα phosphorylation in mouse models of cardiometabolic disease, which is the focus of this review.

LXRα S196 PHOSPHORYLATION DEFICIENT KNOCK-IN MICE

To determine the effect of mouse LXRα S196 phosphorylation (pS196) *in vivo*, we developed a global LXRα S196A knock-in mouse carrying a serine-to-alanine mutation (S196A) introduced by site-directed mutagenesis. This mutation prevents phosphorylation at this site but does not significantly affect the level of LXRα expression. The LXRα S196A mice do not present any apparent morphological phenotype, have similar developmental growth compared to matched wild type (WT) mice, and exhibit equivalent metabolic parameters (31). These mice have been used to determine the effect of LXRα phosphorylation *in vivo* on various diseases, such as NAFLD (31), atherosclerosis (28), diabetes (29), and obesity (30). Their phenotypes and mechanisms are discussed below and summarized graphically in Figure 2 and the models, sex of animals, diets used and phenotypes detailed in Table 1.

LXRα PHOSPHORYLATION IN NAFLD - LXRα S196A RETARDS NAFLD PROGRESSION

NAFLD is a progressive liver disease that ranges from simple fatty liver (steatosis) to non-alcoholic steatohepatitis (NASH), and can progress to fibrosis, cirrhosis and hepatocellular carcinoma (33). NAFLD is the major cause of chronic liver disease in the Western world, and is predicted to become the main cause for liver transplantation by 2030 (34). NAFLD may progress from benign steatosis to inflammatory and

fibrotic steatohepatitis. LXRs contribute to this pathology by promoting fatty acid and triglyceride accumulation (35). While deletion of LXRs in mouse models have shed light onto the LXR-mediated pathways controlling steatohepatitis (36,37), the impact of LXRα phosphorylation on NAFLD progression remained unclear, and we sought to address this gap.

To this end, Becares *et al*. (31) used a global knock-in mouse carrying an LXRα homozygous serine-to-alanine mutation at Ser196 (S196A) that impairs LXRα phosphorylation without affecting overall hepatic LXRα levels (LXRα S196A mouse). As cholesterol induces LXRα phosphorylation (22); it was hypothesized that LXRα S196A mice may respond differently to a high-fat-high-cholesterol (HFHC) diet when compared to chow.

Phenoytpe: LXRα S196A mice fed an HFHC diet showed higher levels of hepatic non-esterified fatty acids (NEFAs) and triglycerides compared to WT mice and exhibited enhanced hepatic steatosis. Livers of the LXRα S196A mice featured increased numbers of larger lipid droplets, associated with enhanced expression of lipid droplet genes. Since plasma NEFAs, triglycerides and insulin levels did not differ between genotypes, the increased hepatic fat accumulation in S196A mice was likely a result of enhanced *de novo* lipogenesis. In fact, S196A livers showed an increase in the total amount of saturated as well as unsaturated fatty acids, specifically ω9 and certain ω6 fatty acid species. Thus, LXRα S196A expressed globally induces hepatic steatosis and alters fatty acid profiles in response to a HFHC diet.

Despite the increased steatosis, HFHC-fed LXRα S196A mice displayed *less* liver inflammation and collagen deposition than their WT counterparts. This was associated with reduced expression of pro-inflammatory and pro-fibrotic mediators. Expression of factors involved in the activation of ER stress were reduced in LXRα S196A mice suggesting these animals are protected from lipotoxicity through a reduction in ER stress. In addition, HFHC-fed LXRα S196A mice were protected from plasma and hepatic cholesterol accumulation. This was accompanied by a 20% reduction in liver weight in LXRα S196A compared to controls and a decrease in the expression of the cholesterol efflux transporter ATP Binding Cassette Subfamily G Member 1 (*Abcg1*). The HFHC-fed LXRα S196A mice also showed a unique induction in the expression of the cholesterol transporter ATP Binding Cassette Subfamily G Member 5

(*Abcg5*). Thus, reduced cholesterol accumulation in S196A mice is likely due to increased hepatobiliary secretion of cholesterol.

Mechanism: RNA-seq was performed on livers from LXRα S196A and WT mice fed a HFHC diet to gain insight into the mechanism of steatosis managed by LXRα phosphorylation. This revealed increased expression of a subset of the hepatic enzymes involved in fatty acid elongation and fatty acid oxidation. These changes likely contribute to the distinct hepatic fatty acid profile of S196A mice.

LXRα S196A mice also showed a decrease in the levels of wound healing and fibrotic mediators, including collagen genes and enzymes responsible for collagen stabilization. Moreover, expression of a subset of genes involved in extracellular matrix remodeling and tissue regeneration that distinguish between low-risk and high-risk NAFLD among pre-symptomatic patients (38) were remarkably different between WT and S196A mice. Thus, LXRα phosphorylation could alter pre-clinical NAFLD progression by regulating tissue regenerative and remodeling pathways.

Variations in the hepatic transcriptome observed in the liver of LXRα S196A mice compared to WT coincided with changes in chromatin modifications: differences in H3K27 acetylation, which mark active genes, between LXRα WT and S196A mice strongly overlapped with changes in gene expression. For example, expression of the Carboxylase 1F (*Ces1f*) gene was strongly induced in livers from LXRα S196A on a HFHC diet while showing a significant increase in H3K27Ac compared to WT. *In silico* analysis of the *Ces1f* gene for putative LXR binding sites revealed a degenerate DR4 (direct repeat 4) sequence resembling the LXRE consensus binding site (7). In livers from the LXRα S196A HFHC-fed mice this site was preferentially bound by LXR compared to WT, and was associated with increased RNA Polymerase II (Pol II) and phosho-Ser2 Pol II (pSer-Pol II) occupancy, indicative of enhanced transcriptional initiation and elongation. This suggests that impaired LXRα S196 phosphorylation allows for the transcriptional activation of new target genes containing non-canonical DR4 sequences.

We have previously reported using a macrophage cell line that upon LXR ligand activation, phosphorylation affected the transcriptional activity of LXRα by modulating the binding of the NCoR corepressor to genes controlled by LXRα phosphorylation (22). However, differences in NCoR occupancy

in the livers of mice exposed to the HFHC diet were not detected, suggesting responses to cholesterol *in vivo* involve other transcriptional regulatory factors whose interaction with LXRα is sensitive to phosphorylation. One such factor is the Transducin Beta Like 1 X-Linked Receptor 1 (TBLR1), which participates in nuclear receptor cofactor exchange (39) and modulates LXR target gene expression in hepatic cells (40). TBLR1 was found to preferentially bind to LXRα S196A and, occupy the *Ces1f* LXRE in livers from LXRα S196A as compared to WT HFHC-fed mice. *Ces1f* is a carboxylesterase that regulates VLDL lipid packaging and assembly in the hepatocytes (41), with enhanced expression expected to result in increased hepatic lipid clearance. This suggests changes in LXRα phosphorylation at Ser196 in the context of a cholesterol rich diet affect its interactions with regulatory factors such as TBLR1 to drive new sets of target genes that influence diet-induced responses in liver.

LXRα PHOSPHORYLATION IN ATHEROSCLEROSIS

Atherosclerosis is a metabolic and inflammatory disease that begins when cholesterol-rich Low Density Lipoprotein (LDL) and other apolipoprotein B-containing lipoproteins in the circulation are deposited in the sub-endothelial space of large arteries, where they and their modified forms promote inflammatory responses (42). In macrophages, LXRα signaling is critical for the homeostatic response to cellular lipid loading (43). Macrophage uptake of normal and oxidized LDL leads to increased cellular concentrations of cholesterol and oxysterols. Activation of LXRs by oxysterols induces the expression of genes involved in cellular cholesterol trafficking and efflux. However, in the face of persistent high cholesterol (hyperlipidemia) the LXR-mediated cholesterol homeostatic mechanism in macrophages is overwhelmed. This results in their becoming foam cells retained in the subendothelial space and the growth of an atherosclerotic plaque (42). In addition, plaque macrophage content reflects additional parameters including monocyte recruitment, macrophage proliferation and apoptosis, and efferocytosis, the process where dead cells are removed by phagocytosis. We initially reported an increase in LXRα pS196 in plaque macrophages under inflammatory conditions associated with atherosclerosis progression, and a decrease in LXRα pS196 during the resolution of inflammation in regressing plaques. We also demonstrated *in vitro,*

that LXRα pS196 selectively regulates a subset of LXRα target genes in macrophages, including genes with anti-inflammatory and atheroprotective functions (22,27). However, the specific contribution of LXRα phosphorylation in macrophages and immune cells to atherosclerosis development remained unknown.

Altering LXRα phosphorylation in macrophages during atherosclerosis progression: impact on FoxM1 expression and macrophage proliferation.

Phenotype: To address the impact of LXRα phosphorylation in macrophages in atherosclerosis, Gage *et al*. (28) generated a mouse model expressing LXRα S196A specifically in myeloid cells on the LDL receptor (LDLR)-deficient (*Ldlr*-KO) atherosclerotic-prone background (M-S196A$^{Ldlr\text{-}KO}$). This was accomplished using a conditional LXRα S196A mouse whereby LysM-Cre-mediated recombination of lox sites results in the replacement of the wild type LXRα with the non-phosphorylatable S196A allele. The LysM-Cre promotes significant recombination in the monocyte/macrophage lineage, as well as promoting recombination in neutrophils (44).

M-S196A$^{Ldlr\text{-}KO}$ mice fed a western diet for 12 weeks developed accelerated plaque progression compared to WT^{LdlrKO} controls shown through increased lipid content measured by *en face* examination of aortas and plaque coverage in aortic roots. Despite no change in the metabolic characteristics such as systemic glucose or lipid homeostasis, atherosclerotic plaques were larger in M-S196A$^{Ldlr\text{-}KO}$ compared to control mice. This reflected increased proliferation of cells within the plaque (likely macrophages). Smaller necrotic cores and reduced fibrous caps of the M-S196A$^{Ldlr\text{-}KO}$ plaques were also observed.

Mechanism: To determine the mechanism of increased atherosclerosis in M-S196A$^{Ldlr\text{-}KO}$mice the transcriptomic profiles of bone marrow derived macrophages (BMDMs) from the western diet-fed mice were compared. This revealed significant genome-wide transcriptional changes and an enrichment in G2/M checkpoint and E2F targets, indicating cell cycle and cell proliferation pathways were induced in macrophages expressing LXRα S196A. This included a threefold increase in the proto-oncogene *FoxM1* and several of its target genes from western diet-fed LXRα S196A compared to WT LXRα BMDMs. Macrophages expressing the LXRα S196A also showed increased proliferation. Interrogation of ChIP-seq

data and *in silico* analysis identified specific LXRα occupancy at the *FoxM1* gene in macrophages. Furthermore, pharmacological inhibition of *FoxM1* using the specific inhibitor FDI-6 (45) reduced the LXRα S196A macrophage proliferation and *FoxM1* target gene expression confirming that *FoxM1* up-regulation mediates the increased macrophage proliferation observed in M-S196A$^{Ldlr\text{-}KO}$ mice. Interrogation of the LXRα S196A regulated transcriptome also revealed selective expression of several pro- and anti-phagocytic molecules predicted to increase efferocytosis, which may contribute to the decreased necrotic core size observed.

To further understand the magnitude of the transcriptional changes imposed by the LXRα phosphorylation disruption, response to an LXR ligand GW3965 was explored through RNA-seq analysis on western diet exposed M-196A$^{Ldlr\text{-}KO}$ and WT^{LdlrKO} BMDM. GW ligand activation promoted substantial changes in macrophage gene expression that was different and of a different magnitude in cells expressing the LXRα S196A mutant compared with WT macrophages highlighting the significance of LXRα S196 phosphorylation in rewiring the LXRα transcriptome.

Inhibiting LXRα phosphorylation in bone marrow cells during atherosclerosis progression promotes an anti-atherogenic phenotype

In addition to macrophages, other immune cells accumulate in the arterial wall and contribute to atherosclerosis (46-48). In a complementary approach to the macrophage specific knock-in described above, Voisin *et al.* (30) interrogated the requirement of LXRα phosphorylation on the entire cadre of immune cells expressing LXRα S196A compared to WT using a bone marrow transplantation model into the atherosclerotic-prone *Ldlr*-KO fed a western diet to promote plaque formation. This strategy can provide evidence for communication between immune cells types in atherosclerosis that respond to LXRα phosphorylation.

Phenotype: In contrast to myeloid-specific LXR S196A, plaques from LXRα S196A bone marrow transplant mice showed reduced lipid accumulation in the plaques and reduced macrophage proliferation. LXRα S196A decreased the recruitment of inflammation-prone Ly6C^{high} monocytes, which was opposed

by a decrease in macrophage apoptosis. Consequently, we observed a small reduction in plaque size and macrophage (CD68+ cells) accumulation. In this model, unlike the myeloid-specific knock in, lipoprotein profiles were affected with an increase in atheroprotective HDL-C and a decrease in atherogenic LDL-C in LXRα S196A compared to WT. This increase in HDL-C may occur through recruitment of LXRα S196A expressing resident macrophages (Kupffer cells) to the liver in the context of bone marrow transplantation after whole body irradiation to influence liver cells to either increase HDL-C particle secretion or decrease HDL-C uptake (49,50).

Mechanism: To characterize the mechanisms governing macrophage phenotype in atherosclerotic plaques when LXRα phosphorylation at S196 is inhibited in all bone marrow-derived cells, we examined by RNA-seq the gene expression profiles of CD68+ plaque macrophages collected by laser-capture microdissection from LXRα S196A and LXRα WT mice (51). Genes repressed by LXRα S196A aligned to pro-inflammatory pathways, while genes induced by LXRα S196A associated with mitochondrial function, oxidative phosphorylation pathways, but not fatty acid oxidation. This indicates that mitochondrial activity and energy metabolism are induced in LXRα S196A CD68+ cells relative to WT. These are characteristics of metabolic pathways upregulated in anti-inflammatory macrophages. In parallel, we found that LXRα S196A M1 BMDMs had increased basal respiration, ATP production, fatty acid metabolism and mitochondrial abundance. Since the metabolic dependence of macrophages on oxidative phosphorylation is a hallmark of the anti-inflammatory response by M2 macrophages (52), our findings indicate that inhibiting LXRα phosphorylation at S196 in CD68+ plaque macrophages promotes the acquisition of an anti-inflammatory metabolic phenotype.

The difference in phenotype between the myeloid-specific expression of S196A described above versus the bone marrow transplant model, in which the entire complement of hematopoietic cells express LXRα S196A, suggest that LXRα S196A expression within additional bone marrow-derived cells restrain macrophage proliferation in the plaque to deter atherosclerosis. Recent advances in single cell RNA-seq revealed different leukocyte clusters, including T cells, B cells, dendritic cells, macrophages, monocytes

and NK cells, are present in the plaques of *Ldlr*-KO mice fed a western diet (53). Elucidating which cell subset influences macrophage proliferation within the plaques of LXRα S196A mice remains an interesting and open question.

LXRα PHOSPHORYLATION IN CARDOMETABOLIC DISEASES

Disrupting LXRα phosphorylation in bone marrow during atherosclerosis progression protects against diet-induced obesity

Obesity shares pathological features with atherosclerosis, including chronic inflammation mediated by both innate and adaptive immune cells that accumulate in the visceral adipose tissue (VAT) and contribute to adipocyte dysfunction (54). Given the pivotal role of LXRα in cholesterol metabolism and inflammation and the influence of LXRα pS196 on gene expression, strategies that reduce LXRα pS196 could help diminish obesity. To determine the effect of LXRα pS196 on diet-induced obesity, we took advantage of the bone marrow transplant model of LXRα S196A in *Ldlr*-KO background (30).

Phenotype: *Ldlr*-KO mice reconstituted with LXRα WT or S196A bone marrow fed a western diet become obese. Interestingly, LXRα S196A mice gained less weight (15% less than WT mice) after 16 weeks of western diet. Despite equivalent food intake, bone mineral density, and lean body mass, LXRα S196A mice showed reduced fat mass, in particular perigonadal white adipose tissue mass (pWAT), compared to LXRα WT mice. Consistent with the reduced lipid accumulation in perigonadal adipose tissue, we also observed a decrease in adipocyte size when LXRα S196 phosphorylation was inhibited.

Mechanism: Associated with the reduction of adipose tissue, flow cytometry of the perigonadal adipose tissue revealed that LXRα S196A altered a unique type of immune cell population. Total adipose tissue macrophages (ATMs) and recruited pro-inflammatory FBC (triple positive F4/80+CD11b+CD11c+) macrophages (M1-like macrophages) were reduced, although the number of resident anti-inflammatory FB (double positive F4/80+CD11b+ CD11c-) macrophages (M2-like macrophages) did not change. This resulted in a higher ratio of inflammation-resolving FB to inflammation-promoting FBC macrophages in

LXRα S196A adipose tissue compared to LXRα WT (55). Dendritic cell (DC) and T cell numbers were not affected by LXRα S196A. RNA-seq analysis of ATMs revealed a large change in the LXRα S196A transcriptomes between ATM subtypes consistent with a less inflammatory phenotype suggestive of reduced inflammatory-mediated signaling to adipocytes.

Thus, expression of LXRα S196A in the bone marrow significantly decreased mouse weight, total fat, pWAT, and adipocyte size in pWAT compared to WT. This phenotype was not reported in myeloid-specific S196A expressing mice in the *Ldlr*-KO background, suggesting that other bone marrow-derived cells in combination with macrophages are promoting this phenotype (30).

Disrupting LXRα phosphorylation alters atherosclerosis regression associated with diabetes

Diabetic patients have earlier onset and more extensive atherosclerosis than non-diabetic patients (56,57). Clinical data shows that the diabetes significantly hampers the regression of human atherosclerosis, even when lipid levels are lowered (58). This effect has been recapitulated in mouse model of atherosclerosis where the favorable effects of lowering lipid levels on plaque development are impaired in diabetic mice (59). Plaque regression- the ability to resolve inflammation upon lipid lowering- is also significantly impaired in diabetic mice compared to non-diabetic mice, even when LDL cholesterol is similarly reduced in both (59). We have demonstrated that chronically high level of glucose alters LXR-dependent gene activation in BMDM, suggesting that LXR function is dysregulated by hyperglycemia in diabetes (60). Given that phosphorylation of LXRα at S196 was increased by diabetes-relevant high glucose levels in a macrophage cell line and also modulated LXRα- gene expression, we examined whether LXRα function in atherosclerosis regression is impaired in diabetic mice as a function of S196 phosphorylation. To determine whether LXRα phosphorylation affected macrophage function during atherosclerosis regression in diabetes, we used an inducible LXRα S196A phosphorylation deficient knock-in mouse so plaques could develop normally under LXRα WT expression and then be switched to LXRα S196A to isolate the effect of LXR phosphorylation on regression. Mice homozygous for LXRα S196A floxed allele ($S196A^{FL/FL}$) with or without a tamoxifen inducible CRE recombinase (*RosaCreER*^{T2};*Cre*+ or *Cre*-) were

used as donors for bone marrow (BM) transplantation into recipient Reversa mice (*Ldlr*$^{-/-}$; *ApoB*$^{100/100}$; *Mttp*$^{fl/fl}$; *Mx1-Cre*$^{+/+}$) (61). This is an *Ldlr*-KO model where the hyperlipidemia can be reversed after conditional inactivation of microsomal triglyceride transfer protein (*Mttp*). Mice were fed a western diet for 16 weeks to allow plaques to develop. Tamoxifen was administered at week 14.5 to all groups. This induced recombination of *LoxP* sites in the *Cre* expressing mice resulting in the replacement of LXRα WT with the LXRα S196A allele after the development of atherosclerosis. The regression/diabetic mice were administered an intraperitoneal injection of Streptozotocin (STZ) for 5 days to induce hyperglycemia (29).

Phenotype: Expression of LXRα S196A in hematopoietic cells had differential effects on atherosclerosis regression in normoglycemic versus hyperglycemic conditions. Under normoglycemia, LXRα S196A expression increased macrophage accumulation, macrophage apoptosis, and plaque necrotic area. In diabetes, LXRα S196A reduced macrophage retention in the plaque, which would be predicted to be anti-atherogenic and enhance plaque regression. However, this favorable effect on regression was negated by increased monocyte infiltration in the plaque, attributed to increased leukocytosis in LXRα S196A mice.

Mechanism: The increase in macrophage plaque accumulation under normoglycemia observed in LXRα S196A mice is due to an increase in the number of circulating white blood cells, especially monocytes. In fact, elevated white blood cell counts have been linked to an increased incidence of coronary heart disease in humans (62). Monocytosis occurs in hypercholesterolemic animal models as a result of western diet feeding, which promotes accumulation of macrophages in the plaque (63). Plaques from LXRα S196A mice also showed increased necrotic area and higher numbers of apoptotic macrophages compared to WT. This is likely the result of a defect in efferocytosis since plaque macrophages expressing LXRα S196A showed reduced expression of the efferocytosis promoting factor *Mertk*, a known LXR target gene (64).

While LXRα S196A reduced macrophage retention in plaques in diabetes, this favorable effect on regression is masked by increased monocyte infiltration in the plaque attributed to increased leukocyte production in LXRα S196A mice. Thus, in the absence of leukocytosis, the phosphorylation-deficient LXRα S196 has atheroprotective effects in diabetes by reducing the retention and accumulation of macrophages in atherosclerotic plaque (29).

LXRα S196 PHOSPHORYLATION AS A POTENTIAL DRUG TARGET

LXRα represents a promising target for the treatment of diseases involving dysregulation of metabolism and inflammation given that its activity can be controlled by both small molecule ligands and phosphorylation. This includes atherosclerosis (15), diabetes (65), obesity (36), and fatty liver disease (37,66), as well as cancer (67,68), and Alzheimer's disease (35). However, undesirable side effects of targeting LXR using ligands– such as elevated liver triglycerides via LXR-dependent activation of *SREBPs*- have hindered the clinical utility of compounds targeting LXRα (69-71). Therefore, finding ways to selectively activate (or repress) LXRα targets genes via changes in LXRα phosphorylation could represent a new therapeutic strategy, since alterations in LXRα phosphorylation elicit different patterns of gene expression. This is consistent with the finding that phosphorylation of PPARγ at S273 in the hinge region by the Cyclin dependent kinase 5 (CDK5), changes expression of specific genes involved in insulin sensitivity (72). In fact, PPARγ ligands that block CDK5-mediated phosphorylation of the receptor have potent anti-diabetic activity through selective changes in gene expression, suggesting the possibility of novel pharmacology achieved by modulating phosphorylation of nuclear receptors (72,73). We have reported that we can stimulate the non-phosphorylated form of LXRα pharmacologically by blocking the upstream kinase (e.g. CK2) or using a combination of LXRα ligands, such as T0901317 and 9cRA (22). To circumvent systemic side effects of LXR activation, site-specific delivery of LXRα ligands and/or kinase inhibitors that block phosphorylation could be accomplished using nanoparticles. Nanomedicine using LXRα ligands has been demonstrated success in treating atherosclerosis in a mouse model while minimizing systemic effects in the liver (74). However, a better understanding of the complex activities of LXRα pS196 in pathological contexts, especially in models that recapitulate human pathophysiology, is necessary to develop alternative targeted therapies.

CONCLUSIONS AND FUTURE DIRECTIONS

We have shown that LXRα phosphorylation at S196 plays a key role in the control of LXRα transcriptional activity in cardiometabolic disease. One overarching theme from these studies is that S196

phosphorylation reprograms the LXR-modulated transcriptome, and this appears distinct from the LXR transcriptome in response to ligand (28,31). For example, Gage *et al.* found that the majority of the genes in the macrophage transcriptional response that were sensitive to the LXRα phosphomutant were observed in the absence of LXR ligand stimulation (28). This suggest that environmental cues such as diet are perceived by LXR phosphorylation. These findings are also supported by work from Ramon-Vazquez and colleagues in immortalized BMDMs from LXR-deficient mice reconstituted with either LXRα or LXRβ that found most of the gene expression differences were seen when receptors were expressed rather than with pharmacological activation of LXR (75). In addition, comparison of gene expression profiles between macrophages from the plaque and macrophages from the visceral adipose tissue from the same mouse were non-overlapping (30), highlighting the role of the local tissue microenvironment in determining gene expression managed by LXRα.

While our studies have established the physiological relevance of LXRα phosphorylation in cardiometabolic diseases, several questions remain. For example, which kinase(s), CK2 or PKA, phosphorylate LXRα *in vivo*, and is this dependent on the diet or cell type? To address these questions, we could use genetics and pharmacological manipulation via kinase conditional knock out mice, and kinase inhibitors. Another remaining question is how does LXRα S196A induce such a dramatic change in transcription? LXRα S196A can selectively occupy certain genes via preferential interaction with co-regulators (22,31). This reinforces work on LXRβ phosphorylation at S426 by Calcium/calmodulin-dependent protein kinase II γ (CaMKIIγ) leading to the de-repression of inflammatory genes via the release of Coronin 2 (CORO2A), a component of the NCoR repression complex (76). But are these mechanisms the exception or the rule? To gain insight into this question, ChIP-seq studies of LXRα WT and LXRα S196A upon dietary changes, as well as characterization of the LXRα S196 phospho-cistrome using LXRα phospho-specific antibody in macrophages and liver could inform LXRα phosphorylation-dependent gene regulatory mechanisms by identifying potential collaborating transcription factors.

We are also interested in applying the LXRα S196A mouse model to other diseases linked to LXRα signaling including neurodegenerative diseases such as Alzheimer (77). Additionally, an atlas of LXRα

phosphorylation in normal and diseased human tissues would help inform homeostatic or pathological states where LXRα phosphorylation could be relevant. Together, such studies would reveal mechanisms underlying LXRα phosphorylation-dependent gene expression, and inform physiological settings where LXRα phosphorylation is important.

ACKNOWLEDGEMENTS

This work was supported by grants from the NIH (R01HL117226, MJG, EAF), and an American Heart Association postdoctoral fellowship (19POST34410010, MV), Jan Vilcek/David Goldfarb Fellowship (MV), British Heart Foundation Project Grants (PG/13/10/30000, IPT and PG/16/87/32492, MG & IPT), Medical Research Council New Investigator Grant (G0801278 to IPT) and University College of London Grand Challenges PhD Studentship (IPT, NB).

REFERENCES

1. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nature reviews Molecular cell biology*. 2012;13:213-224.
2. Spann NJ, Garmire LX, McDonald JG, Myers DS, Milne SB, Shibata N, Reichart D, Fox JN, Shaked I, Heudobler D, Raetz CR, Wang EW, Kelly SL, Sullards MC, Murphy RC, Merrill AH, Jr., Brown HA, Dennis EA, Li AC, Ley K, Tsimikas S, Fahy E, Subramaniam S, Quehenberger O, Russell DW, Glass CK. Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. *Cell*. 2012;151(1):138-152.
3. Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G, Pfahl M. A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. *Mol Cell Biol*. 1994;14(10):7025-7035.
4. Zelcer N, Tontonoz P. Liver X receptors as integrators of metabolic and inflammatory signaling. *J Clin Invest*. 2006;116(3):607-614.
5. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature*. 1996;383(6602):728-731.
6. Fradera X, Vu D, Nimz O, Skene R, Hosfield D, Wynands R, Cooke AJ, Haunso A, King A, Bennett DJ, McGuire R, Uitdehaag JC. X-ray structures of the LXRalpha LBD in its homodimeric form and implications for heterodimer signaling. *J Mol Biol*. 2010;399(1):120-132.
7. Boergesen M, Pedersen TA, Gross B, van Heeringen SJ, Hagenbeek D, Bindesboll C, Caron S, Lalloyer F, Steffensen KR, Nebb HI, Gustafsson JA, Stunnenberg HG, Staels B, Mandrup S. Genome-wide profiling of liver X receptor, retinoid X receptor, and peroxisome proliferator-activated receptor alpha in mouse liver reveals extensive sharing of binding sites. *Mol Cell Biol*. 2012;32(4):852-867.
8. Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, Jepsen K, Baek SH, Heyman RA, Rosenfeld MG, Schulman IG, Glass CK. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Mol Cell Biol*. 2003;23(16):5780-5789.
9. Sever R, Glass CK. Signaling by Nuclear Receptors. *Csh Perspect Biol*. 2013;5(3).
10. Kouzarides T. Chromatin modifications and their function. 2007;128:693-705.
11. Glass CK, Saijo K. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nature Reviews Immunology*. 2010;10(5):365-376.
12. Chinenov Y, Gupte R, Rogatsky I. Nuclear receptors in inflammation control: repression by GR and beyond. *Mol Cell Endocrinol*. 2013;380(1-2):55-64.
13. Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, Rosenfeld MG, Glass CK. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. *Mol Cell*. 2007;25(1):57-70.
14. Lee SK, Kim JH, Lee YC, Cheong J, Lee JW. Silencing mediator of retinoic acid and thyroid hormone receptors, as a novel transcriptional corepressor molecule of activating protein-1, nuclear factor-kappaB, and serum response factor. *J Biol Chem.* 2000;275(17):12470-12474.
15. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat Med.* 2003;9(2):213-219.
16. Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, Westin S, Hoffmann A, Subramaniam S, David M, Rosenfeld MG, Glass CK. Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. *Cell*. 2005;122(5):707-721.
17. Prufer K, Boudreaux J. Nuclear localization of liver X receptor alpha and beta is differentially regulated. *J Cell Biochem*. 2007;100(1):69-85.
18. Watanabe Y, Tanaka T, Uchiyama Y, Takeno T, Izumi A, Yamashita H, Kumakura J, Iwanari H, Shu-Ying J, Naito M, Mangelsdorf DJ, Hamakubo T, Kodama T. Establishment of a monoclonal antibody for human LXRalpha: Detection of LXRalpha protein expression in human macrophages. *Nucl Recept*. 2003;1(1):1.

19. Lalevee S, Ferry C, Rochette-Egly C. Phosphorylation control of nuclear receptors. *Methods Mol Biol*. 2010;647:251-266.
20. Berrabah W, Aumercier P, Lefebvre P, Staels B. Control of nuclear receptor activities in metabolism by post-translational modifications. *FEBS Lett*. 2011;585(11):1640-1650.
21. Becares N, Gage MC, Pineda-Torra I. Posttranslational Modifications of Lipid-Activated Nuclear Receptors: Focus on Metabolism. *Endocrinology*. 2017;158(2):213-225.
22. Torra IP, Ismaili N, Feig JE, Xu CF, Cavasotto C, Pancratov R, Rogatsky I, Neubert TA, Fisher EA, Garabedian MJ. Phosphorylation of liver X receptor alpha selectively regulates target gene expression in macrophages. *Mol Cell Biol*. 2008;28(8):2626-2636.
23. Chen M, Bradley MN, Beaven SW, Tontonoz P. Phosphorylation of the liver X receptors. *FEBS Lett*. 2006;580(20):4835-4841.
24. Yamamoto T, Shimano H, Inoue N, Nakagawa Y, Matsuzaka T, Takahashi A, Yahagi N, Sone H, Suzuki H, Toyoshima H, Yamada N. Protein kinase A suppresses sterol regulatory element-binding protein-1C expression via phosphorylation of liver X receptor in the liver. *J Biol Chem*. 2007;282(16):11687-11695.
25. Anthonisen EH, Berven L, Holm S, Nygard M, Nebb HI, Gronning-Wang LM. Nuclear receptor liver X receptor is O-GlcNAc-modified in response to glucose. *J Biol Chem*. 2010;285(3):1607-1615.
26. Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol Cell*. 2007;28(1):91-106.
27. Wu C, Hussein MA, Shrestha E, Leone S, Aiyegbo MS, Lambert WM, Pourcet B, Cardozo T, Gustafson JA, Fisher EA, Pineda-Torra I, Garabedian MJ. Modulation of Macrophage Gene Expression via Liver X Receptor alpha Serine 198 Phosphorylation. *Mol Cell Biol*. 2015;35(11):2024-2034.
28. Gage MC, Becares N, Louie R, Waddington KE, Zhang Y, Tittanegro TH, Rodriguez-Lorenzo S, Jathanna A, Pourcet B, Pello OM, De la Rosa JV, Castrillo A, Pineda-Torra I. Disrupting LXRalpha phosphorylation promotes FoxM1 expression and modulates atherosclerosis by inducing macrophage proliferation. *Proc Natl Acad Sci U S A*. 2018;115(28):E6556-E6565.
29. Shrestha E, Voisin M, Barrett TJ, Nishi H, Cantor DJ, Hussein MA, David G, Pineda-Torra I, Fisher EA, Garabedian MJ. Phosphorylation of LXRα impacts atherosclerosis regression by modulating monocyte/macrophage trafficking. *bioRxiv*. 2018:363366.
30. Voisin M, Shrestha E, Rollet C, Josefs T, Barrett T, J., Chang HR, Ruoff R, Nikain CA, Garabedian M, L., Brown E, J., Goldberg I, J., Pineda-Torra, Inés, Fisher E, A., Garabedian MJ. Inhibiting LXR alpha Phosphorylation in Hematopoietic Cells Reduces Inflammation and Attenuates Atherosclerosis and Obesity. *bioRxiv*. 2020:939090.
31. Becares N, Gage MC, Voisin M, Shrestha E, Martin-Gutierrez L, Liang N, Louie R, Pourcet B, Pello OM, Luong TV, Goni S, Pichardo-Almarza C, Roberg-Larsen H, Diaz-Zuccarini V, Steffensen KR, O'Brien A, Garabedian MJ, Rombouts K, Treuter E, Pineda-Torra I. Impaired LXRalpha Phosphorylation Attenuates Progression of Fatty Liver Disease. *Cell Rep*. 2019;26(4):984-995 e986.
32. Lou X, Toresson G, Benod C, Suh JH, Philips KJ, Webb P, Gustafsson JA. Structure of the retinoid X receptor alpha-liver X receptor beta (RXRalpha-LXRbeta) heterodimer on DNA. *Nat Struct Mol Biol*. 2014;21(3):277-281.
33. Sanyal AJ. Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat Rev Gastro Hepat*. 2019;16(6):377-386.
34. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018;67(1):123-133.
35. Hong C, Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. *Nat Rev Drug Discov*. 2014;13(6):433-444.

36. Beaven SW, Matveyenko A, Wroblewski K, Chao L, Wilpitz D, Hsu TW, Lentz J, Drew B, Hevener AL, Tontonoz P. Reciprocal regulation of hepatic and adipose lipogenesis by liver X receptors in obesity and insulin resistance. *Cell Metab*. 2013;18(1):106-117.
37. Wouters K, van Bilsen M, van Gorp PJ, Bieghs V, Lutjohann D, Kerksiek A, Staels B, Hofker MH, Shiri-Sverdlov R. Intrahepatic cholesterol influences progression, inhibition and reversal of non-alcoholic steatohepatitis in hyperlipidemic mice. *FEBS Lett*. 2010;584(5):1001-1005.
38. Moylan CA, Pang H, Dellinger A, Suzuki A, Garrett ME, Guy CD, Murphy SK, Ashley-Koch AE, Choi SS, Michelotti GA, Hampton DD, Chen Y, Tillmann HL, Hauser MA, Abdelmalek MF, Diehl AM. Hepatic gene expression profiles differentiate presymptomatic patients with mild versus severe nonalcoholic fatty liver disease. *Hepatology*. 2014;59(2):471-482.
39. Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell*. 2004;116(4):511-526.
40. Jakobsson T, Venteclef N, Toresson G, Damdimopoulos AE, Ehrlund A, Lou X, Sanyal S, Steffensen KR, Gustafsson JA, Treuter E. GPS2 is required for cholesterol efflux by triggering histone demethylation, LXR recruitment, and coregulator assembly at the ABCG1 locus. *Mol Cell*. 2009;34(4):510-518.
41. Lian J, Nelson R, Lehner R. Carboxylesterases in lipid metabolism: from mouse to human. *Protein Cell*. 2018;9(2):178-195.
42. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol*. 2013;13(10):709-721.
43. Lee SD, Tontonoz P. Liver X receptors at the intersection of lipid metabolism and atherogenesis. *Atherosclerosis*. 2015;242(1):29-36.
44. Abram CL, Roberge GL, Hu Y, Lowell CA. Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice. *J Immunol Methods*. 2014;408:89-100.
45. Gormally MV, Dexheimer TS, Marsico G, Sanders DA, Lowe C, Matak-Vinkovic D, Michael S, Jadhav A, Rai G, Maloney DJ, Simeonov A, Balasubramanian S. Suppression of the FOXM1 transcriptional programme via novel small molecule inhibition. *Nat Commun*. 2014;5:5165.
46. Cochain C, Vafadarnejad E, Arampatzi P, Pelisek J, Winkels H, Ley K, Wolf D, Saliba AE, Zernecke A. Single-Cell RNA-Seq Reveals the Transcriptional Landscape and Heterogeneity of Aortic Macrophages in Murine Atherosclerosis. *Circ Res*. 2018;122(12):1661-1674.
47. Fernandez DM, Rahman AH, Fernandez NF, Chudnovskiy A, Amir ED, Amadori L, Khan NS, Wong CK, Shamailova R, Hill CA, Wang Z, Remark R, Li JR, Pina C, Faries C, Awad AJ, Moss N, Bjorkegren JLM, Kim-Schulze S, Gnjatic S, Ma'ayan A, Mocco J, Faries P, Merad M, Giannarelli C. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med*. 2019;25(10):1576-1588.
48. Lin JD, Nishi H, Poles J, Niu X, McCauley C, Rahman K, Brown EJ, Yeung ST, Vozhilla N, Weinstock A, Ramsey SA, Fisher EA, Loke P. Single-cell analysis of fate-mapped macrophages reveals heterogeneity, including stem-like properties, during atherosclerosis progression and regression. *JCI Insight*. 2019;4(4).
49. Beattie L, Sawtell A, Mann J, Frame TCM, Teal B, de Labastida Rivera F, Brown N, Walwyn-Brown K, Moore JWJ, MacDonald S, Lim EK, Dalton JE, Engwerda CR, MacDonald KP, Kaye PM. Bone marrow-derived and resident liver macrophages display unique transcriptomic signatures but similar biological functions. *J Hepatol*. 2016;65(4):758-768.
50. Takezawa R, Watanabe Y, Akaike T. Direct evidence of macrophage differentiation from bone marrow cells in the liver: a possible origin of Kupffer cells. *J Biochem*. 1995;118(6):1175-1183.
51. Trogan E, Choudhury RP, Dansky HM, Rong JX, Breslow JL, Fisher EA. Laser capture microdissection analysis of gene expression in macrophages from atherosclerotic lesions of apolipoprotein E-deficient mice. *Proc Natl Acad Sci U S A*. 2002;99(4):2234-2239.

52. Viola A, Munari F, Sanchez-Rodriguez R, Scolaro T, Castegna A. The Metabolic Signature of Macrophage Responses. *Front Immunol*. 2019;10:1462.
53. Winkels H, Ehinger E, Ghosheh Y, Wolf D, Ley K. Atherosclerosis in the single-cell era. *Curr Opin Lipidol*. 2018;29(5):389-396.
54. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112(12):1796-1808.
55. Hill AA, Reid Bolus W, Hasty AH. A decade of progress in adipose tissue macrophage biology. *Immunol Rev*. 2014;262(1):134-152.
56. Chait A, Bornfeldt KE. Diabetes and atherosclerosis: is there a role for hyperglycemia? *J Lipid Res*. 2009;50 Suppl:S335-339.
57. Katakami N. Mechanism of Development of Atherosclerosis and Cardiovascular Disease in Diabetes Mellitus. *J Atheroscler Thromb*. 2018;25(1):27-39.
58. Nicholls SJ, Ballantyne CM, Barter PJ, Chapman MJ, Erbel RM, Libby P, Raichlen JS, Uno K, Borgman M, Wolski K, Nissen SE. Effect of two intensive statin regimens on progression of coronary disease. *N Engl J Med*. 2011;365(22):2078-2087.
59. Parathath S, Grauer L, Huang LS, Sanson M, Distel E, Goldberg IJ, Fisher EA. Diabetes adversely affects macrophages during atherosclerotic plaque regression in mice. *Diabetes*. 2011;60(6):1759-1769.
60. Hussein MA, Shrestha E, Ouimet M, Barrett TJ, Leone S, Moore KJ, Herault Y, Fisher EA, Garabedian MJ. LXR-Mediated ABCA1 Expression and Function Are Modulated by High Glucose and PRMT2. *PLoS One*. 2015;10(8):e0135218.
61. Lieu HD, Withycombe SK, Walker Q, Rong JX, Walzem RL, Wong JS, Hamilton RL, Fisher EA, Young SG. Eliminating atherogenesis in mice by switching off hepatic lipoprotein secretion. *Circulation*. 2003;107(9):1315-1321.
62. Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. White blood cell count and incidence of coronary heart disease and ischemic stroke and mortality from cardiovascular disease in African-American and White men and women: atherosclerosis risk in communities study. *Am J Epidemiol*. 2001;154(8):758-764.
63. Murphy AJ, Akhtari M, Tolani S, Pagler T, Bijl N, Kuo CL, Wang M, Sanson M, Abramowicz S, Welch C, Bochem AE, Kuivenhoven JA, Yvan-Charvet L, Tall AR. ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. *J Clin Invest*. 2011;121(10):4138-4149.
64. N AG, Bensinger SJ, Hong C, Beceiro S, Bradley MN, Zelcer N, Deniz J, Ramirez C, Diaz M, Gallardo G, de Galarreta CR, Salazar J, Lopez F, Edwards P, Parks J, Andujar M, Tontonoz P, Castrillo A. Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. *Immunity*. 2009;31(2):245-258.
65. Cao G, Liang Y, Broderick CL, Oldham BA, Beyer TP, Schmidt RJ, Zhang Y, Stayrook KR, Suen C, Otto KA, Miller AR, Dai J, Foxworthy P, Gao H, Ryan TP, Jiang XC, Burris TP, Eacho PI, Etgen GJ. Antidiabetic action of a liver x receptor agonist mediated by inhibition of hepatic gluconeogenesis. *J Biol Chem*. 2003;278(2):1131-1136.
66. Zhou J, Febbraio M, Wada T, Zhai Y, Kuruba R, He J, Lee JH, Khadem S, Ren S, Li S, Silverstein RL, Xie W. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology*. 2008;134(2):556-567.
67. Lin CY, Vedin LL, Steffensen KR. The emerging roles of liver X receptors and their ligands in cancer. *Expert Opin Ther Targets*. 2016;20(1):61-71.
68. Lin CY, Gustafsson JA. Targeting liver X receptors in cancer therapeutics. *Nat Rev Cancer*. 2015;15(4):216-224.
69. Groot PH, Pearce NJ, Yates JW, Stocker C, Sauermelch C, Doe CP, Willette RN, Olzinski A, Peters T, d'Epagnier D, Morasco KO, Krawiec JA, Webb CL, Aravindhan K, Jucker B, Burgert M, Ma C,

Marino JP, Collins JL, Macphee CH, Thompson SK, Jaye M. Synthetic LXR agonists increase LDL in CETP species. *J Lipid Res*. 2005;46(10):2182-2191.
70. Joseph SB, Laffitte BA, Patel PH, Watson MA, Matsukuma KE, Walczak R, Collins JL, Osborne TF, Tontonoz P. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. *J Biol Chem*. 2002;277(13):11019-11025.
71. Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B. Role of LXRs in control of lipogenesis. *Genes Dev*. 2000;14(22):2831-2838.
72. Choi JH, Banks AS, Estall JL, Kajimura S, Bostrom P, Laznik D, Ruas JL, Chalmers MJ, Kamenecka TM, Bluher M, Griffin PR, Spiegelman BM. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5. *Nature*. 2010;466(7305):451-456.
73. Choi JH, Banks AS, Kamenecka TM, Busby SA, Chalmers MJ, Kumar N, Kuruvilla DS, Shin Y, He Y, Bruning JB, Marciano DP, Cameron MD, Laznik D, Jurczak MJ, Schurer SC, Vidovic D, Shulman GI, Spiegelman BM, Griffin PR. Antidiabetic actions of a non-agonist PPARgamma ligand blocking Cdk5-mediated phosphorylation. *Nature*. 2011;477(7365):477-481.
74. Zhang XQ, Even-Or O, Xu X, van Rosmalen M, Lim L, Gadde S, Farokhzad OC, Fisher EA. Nanoparticles containing a liver X receptor agonist inhibit inflammation and atherosclerosis. *Adv Healthc Mater*. 2015;4(2):228-236.
75. Ramon-Vazquez A, de la Rosa JV, Tabraue C, Lopez F, Diaz-Chico BN, Bosca L, Tontonoz P, Alemany S, Castrillo A. Common and Differential Transcriptional Actions of Nuclear Receptors Liver X Receptors alpha and beta in Macrophages. *Mol Cell Biol*. 2019;39(5).
76. Huang W, Ghisletti S, Saijo K, Gandhi M, Aouadi M, Tesz GJ, Zhang DX, Yao J, Czech MP, Goode BL, Rosenfeld MG, Glass CK. Coronin 2A mediates actin-dependent de-repression of inflammatory response genes. *Nature*. 2011;470(7334):414-418.
77. Zelcer N, Khanlou N, Clare R, Jiang Q, Reed-Geaghan EG, Landreth GE, Vinters HV, Tontonoz P. Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. *Proc Natl Acad Sci U S A*. 2007;104(25):10601-10606.

FIGURE LEGENDS

Figure 1 LXRα S196 phosphorylation of the hinge region. A) Schematic of human LXRα showing the N-terminal activation function (AF), DNA binding domain (DBD), the hinge region, the ligand binding domain (LBD), and conservation of the LXRα phosphorylation site in mouse on the S196. Phosphorylated serine residues are in red. This site is not conserved in LXRβ. B-C) Molecular modeling of LXRα S198 phosphorylation on the DNA-bound LXRα-RXRα heterodimer reveals changes in LXRα S198 residue orientation upon phosphorylation: conformations of the human LXRα-RXRα heterodimer bound to DNA (multicolored cylinder with central plates) (B) in the S198 phosphorylated form (red arrow) and (C) in the S198 non-phosphorylated form (green arrow). Residues 181 to 195 loop orientation in the LXRα S198 non-phosphorylated state is altered in the phosphorylated state (panel C: black arrow). RXRα is shown in blue, and LXRα is depicted in gray. Adapted from (27).

Figure 2: LXRα S196A mouse models in cardiometabolic diseases. ***Top, Left,*** The full body knock-in of LXRα S196A showed reduced progression from steatosis to steatohepatitis in a model of diet-induced non-alcoholic fatty liver disease (NAFLD) (31). ***Top, Right,*** A myeloid specific knock-in of LXRα S196A in an atherosclerotic background demonstrated that LXRα S196A increased atherosclerosis via enhanced macrophage proliferation. ***Bottom, Left,*** and ***Bottom, Right,*** bone marrow transplant models where all hematopoietic cells express LXRα S196A, revealed that LXRα S196A decreased atherosclerosis progression and obesity (30) and reduced atherosclerosis during regression in a diabetic context (29).

Figure 1

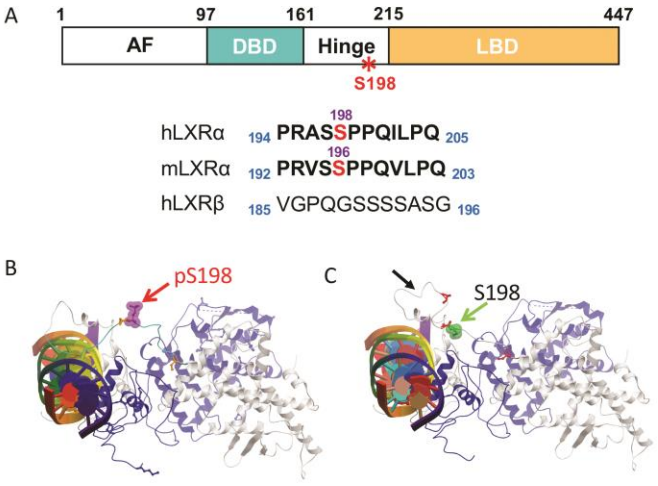

Figure 2

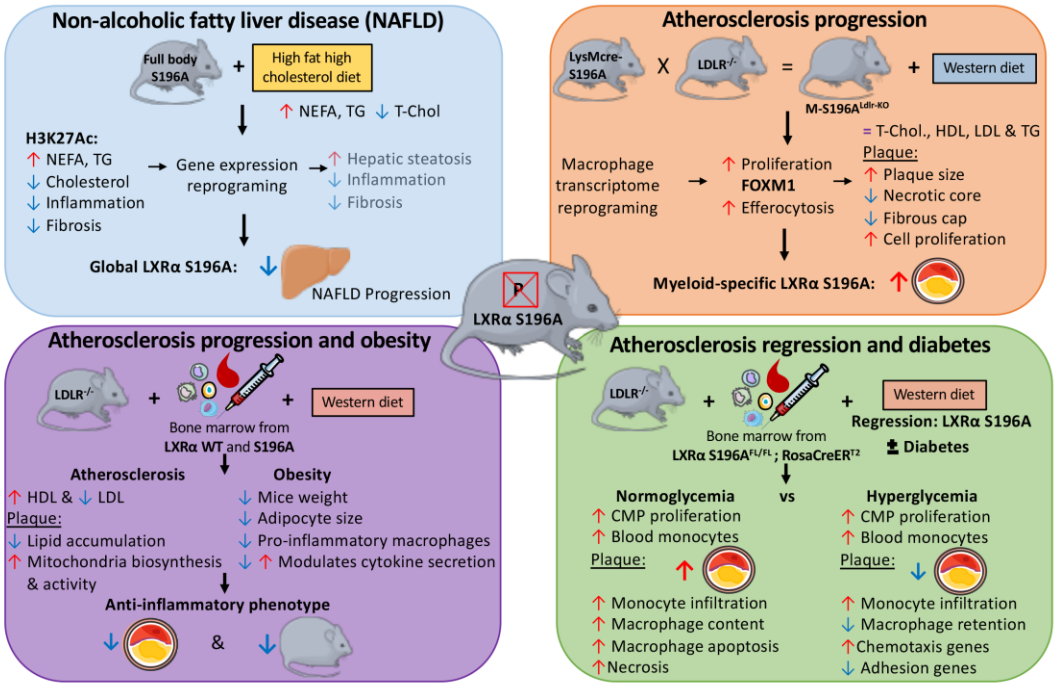

Table 1: Models used in assessing LXRα S196 phosphorylation in cardiometabolic diseases

Disease	Model	CRE	S196A Expression	Genetic Background	Diet	Sex	Phenotype	Reference
Atherosclerosis	LXRαS196A$^{FL/FL}$	LYSM-CRE	Monocyte/ Macrophage	C57Bl6; *Ldlr*$^{-/-}$	WD#	Male	Increased atherosclerosis & macrophages proliferation	Gage *et al*, PNAS 2018 (ref. 28)
Nonalcoholic fatty liver disease (NAFLD)	LXRαS196A$^{FL/FL}$	PGK1-CRE	Whole body	C57Bl6	HFHC*	Female	Retards NAFLD progression	Becares *et al*, Cell Reports 2019 (ref. 31)
Atherosclerosis and diabetes	LXRαS196A$^{FL/FL}$	ROSA-CRE-ERT2: BMT	All hematopoietic cells	C57Bl6; *Ldlr*$^{-/-}$	WD^	Male	Decreased macrophage adhesion in diabetic plaques	Shrestha *et al*, BioRxiv 2018 (ref. 29)
Atherosclerosis and obesity	LXRαS196A$^{FL/FL}$	ROSA-CRE-ERT2: BMT	All hematopoietic cells	C57Bl6; *Ldlr*$^{-/-}$	WD^	Male	Decreased atherosclerosis and obesity	Voisin *et al*, BioRxiv 2020 (ref. 30)

 # WD; Western Diet = 20% [wt/wt] fat, 0.15% cholesterol
** HFHC; High Fat High Cholesterol diet = 17.2% Cocoa Butter, 2.8% Soybean Oil, 1.25% Cholesterol, 0.5% Sodium Cholate*
^ Western Diet (WD) = 21% [wt/wt] fat, 0.3% cholesterol
BMT= bone marrow transplant