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Chapter 2

Liver X receptors and heart failure

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Manuscript in preparation
ABSTRACT

Liver X receptors (LXRs) are master regulators of metabolism, and have been studied for their pharmacological potential in vascular and metabolic disease. Besides their established role in metabolic homeostasis and disease, there is mounting evidence to suggest that LXRs may exert direct beneficial effects in the heart. Here, we aim to provide a conceptual framework to explain the broad mode of action of LXRs and how LXR signaling may be an important local and systemic target for the treatment of heart failure. We discuss the potential role of LXRs in systemic conditions associated with heart failure, such as hypertension, diabetes, and renal and vascular disease. Further, we expound on recent data that implicate a direct role for LXR activation in the heart, for its impact on cardiomyocyte damage and loss due to ischemia, and effects on cardiac hypertrophy, fibrosis, and metabolism. Taken together, the accumulating evidence supports the notion that LXRs may represent a novel therapeutic target for the treatment of heart failure.
INTRODUCTION

Heart failure has increasing prevalence in our aging society and is associated with significant morbidity and mortality (1). It is a complex clinical syndrome and diagnosis is based on physical signs and symptoms of dyspnea, fluid retention, and fatigue upon exertion. Heart failure is attributed to a process of pathological cardiac remodeling that is initiated via molecular, cellular, and interstitial changes that structurally and functionally alter the myocardium. Cardiac remodeling is initially a compensatory response to injury such as myocardial infarction or hypertension, but is maladaptive when these factors continue unabated, perpetuating the progression toward cardiac dysfunction and heart failure. The time course and extent of remodeling are influenced by several factors such as the severity of the pathological insult, secondary events including recurrent ischemia or infarction, elevated hemodynamic load, and neurohormonal activation.

Heart failure is essentially a state of impaired pump function leading to inadequate cardiac output, and classification is based on left ventricular (LV) ejection fraction which is either reduced (HFrEF) or preserved (HFpEF) (2). The primary cause of HFrEF is coronary artery disease, and the underlying mechanisms that drive ventricular remodeling are a result of the ischemic injury caused by myocardial infarction. In the infarct zone, a fibrotic scar replaces loss of cardiomyocytes, whereas the remote myocardium undergoes eccentric remodeling that leads to LV dilatation, decreased contractility, and impaired systolic function. In contrast, HFpEF patients demonstrate evidence of diastolic dysfunction, including prolonged LV relaxation time, reduced LV filling, and increased myocardial stiffness (3), and often involves concentric remodeling of the myocardium.

Most pharmacological therapies such as β-blockade and inhibitors of the renin-angiotensin-aldosterone system (RAAS) target HFrEF and have been beneficial in reducing morbidity and mortality (4), however, this strategy has not been as successful for treating HFpEF (5), therefore diagnosing and treating HFpEF remains a challenge. In essence, the underlying pathophysiology of HFpEF is poorly understood largely due to the presence of multiple co-morbidities such as hypertension, diabetes, metabolic syndrome, atrial fibrillation, and renal and vascular disease, which impact the pathogenesis of this syndrome and contribute to the complexity of mechanisms governing cardiac remodeling (6) (Figure 1). Lack of a clear understanding of these mechanisms thus pose several challenges in targeting and treating heart failure. Identification of novel pleiotropic targets that are integrative in multi-organ systems may therefore be effective therapeutic strategies in preventing or reversing heart failure.

Liver X receptors (LXR) α and β belong to the nuclear receptor superfamily of ligand-activated transcription factors. LXRs have emerged as important regulators of cholesterol homeostasis, lipid and glucose metabolism, and inflammation, and so have been regarded as
promising therapeutic targets for intervention in atherosclerotic and metabolic disease (7). They were first discovered in the mid-nineties as “orphan receptors” because their natural ligands were unknown (8-10). Soon thereafter, endogenous oxysterols were identified as activators of LXR, thus LXRs were “deorphanized” (11). In the nucleus, LXRs form obligate heterodimers with the retinoid X receptor (RXR) and are bound to LXR response elements (LXREs) in regulatory regions of target genes. Binding of either their natural or synthetic ligands, such as T0901317 (T09) and GW3965, induces a conformational change in the LXR/
RXR complex to facilitate activation of target gene transcription (Figure 2). LXRβ is expressed ubiquitously, whereas LXRα is more abundant in metabolically active systems such as liver, adipose tissue, and macrophages, as well as in heart, skeletal muscle, kidney, and lung (12).

Here, we review recent evidence regarding the systemic effects of LXR as it relates to co-morbidities that are relevant in heart failure pathogenesis. Although studies examining LXR specifically in the heart are limited, we also address the potential cardioprotective role for LXR signaling in cardiac remodeling and myocardial disease. Altogether, we provide a perspective on the potential of LXR as an integrative target for heart failure prevention.

I. SYSTEMIC EFFECTS OF LXR SIGNALING IN THE DEVELOPMENT AND PROGRESSION OF HEART FAILURE

Combined co-morbidities have a major impact on the pathogenesis of heart failure, which led to the supposition that myocardial dysfunction may not exclusively originate in the heart itself, but extrinsic factors that stem from these co-morbidities may perturb the heart (13). Here, we summarize current knowledge on the role of LXRs in the pathogenesis of several of these co-morbidities, including atherosclerosis and vascular disease, hypertension, diabetes, and chronic kidney disease (Figure 1). Given the widespread effects of LXRs, we postulate that systemic LXR activation may play an important role in conferring myocardial protection from these disorders that collectively contribute to the pathogenesis of HFrEF and HFpEF (Table).

LXR and atherosclerosis
Atherosclerosis contributes to multi-organ dysfunction involving the kidney, brain, gut, and skeletal muscle, and is a major cause of HFrEF following myocardial infarction (Figure 1). LXRs have been extensively studied for their putative atheroprotective functions. LXR agonist treatment significantly reduced atherosclerosis in both Ldlr<sup>-/-</sup> and apoE<sup>-/-</sup> mice (14), whereas selective loss of macrophage LXR activity through bone marrow transplantsations markedly increased lesion development in these models (15). The initial stages of atherosclerosis involve the formation of foam cells by the uptake of oxidized LDL in macrophages in the arterial wall. Mice deficient for both LXRα and LXRβ develop increased foam cell formation, implicating a basal role in cholesterol homeostasis (16). LXRs limit pathogenic accumulation of cholesterol in macrophages by enhancing the rate of cholesterol efflux (17) which is mediated through upregulation of genes involved in all aspects of the reverse cholesterol transport (RCT) pathway, including cellular cholesterol efflux, plasma lipid transport, intestinal absorption, and bile acid excretion (18). Interestingly, liver-specific deletion of LXRα in mice led to decreased RCT, cholesterol catabolism, and excretion while substantially increasing atherosclerosis, underscoring their importance as whole-body cholesterol sensors (19).
Additionally, the anti-inflammatory functions of LXRs strongly contribute to the mechanism underlying their atheroprotective effects. Selectively increasing LXRα in macrophages led to reduced atherosclerotic lesions in $Ldlr^{-/-}$ mice, as well as decreased plasma inflammatory cytokines, IL6 and TNFα (20). In the absence of the RCT pathway mediated by $Abca1/Abg1$-deficient macrophages, LXR agonism nonetheless reduced atherosclerotic lesion development and inflammation, including pro-atherogenic plasma MCP1 levels (21).

More recent evidence implicates additional atheroprotective properties of LXR agonists in the pathogenesis of atherosclerosis that extend beyond their capacity to promote cholesterol efflux and inhibit inflammation. Bories et al. recently identified a novel anti-atherosclerotic mechanism for LXRα through regulation of macrophage iron homeostasis. By increasing iron export, LXRα reduced iron loading which promotes formation of oxidized lipids, an inducer of cell death (22). Other functions for LXRs within the vasculature are emerging. Endothelial dysfunction is the underlying cause of all vascular disease and it is an early marker of atherosclerosis. LXRs are expressed in endothelial cells (23), and their distribution in mouse aortas reveals that LXRs and their target genes are more highly expressed in the atheroprotective thoracic region than in atheroprone areas such as the aortic arch, supporting an anti-atherosclerotic function (24). In atherosclerotic vessel walls, LXR activation decreased vascular expression of adhesion molecules such as E-selectin, ICAM-1, and CD44 (25), and improved vasomotor function in arteries of $apoE^{-/-}$ mice through enhanced endothelium-dependent vasorelaxation (26).
Also of relevance are the reparative effects of LXRs in vascular injury. Endothelial progenitor cells (EPCs) are important for re-endothelialization of damaged vessel walls as they are either incorporated into the vessel wall directly, or secrete angiogenic growth factors. LXR ligands repaired carotid artery injury in mice by increasing proliferation and migration of EPCs, enhancing their secretion of vascular endothelial growth factor (VEGF), and accelerating endothelial regeneration (27). Furthermore, in a rat model of carotid artery balloon injury, the LXR agonist T09 inhibited vascular smooth muscle cell proliferation and neointima formation (28), a major cause of postangioplasty restenosis and thrombosis in the arterial wall. Also of note is the identification of a novel role for LXRs in thrombosis and platelet function. Although platelets are anuclear, they reportedly express LXRβ, and GW3965 treatment inhibited platelet accumulation and thrombi formation (29).

Taken together, evidence of an important role for LXR in atherosclerosis continues to broaden as studies reveal new functions in cholesterol efflux, macrophage activity, and vascular protection. Preventing atherosclerotic development offsets the potential for myocardial infarction, the major cause of systolic dysfunction leading to HFrEF, thus LXRs represent a potential target in the underlying etiology of HFrEF and preventing morbidity.

**LXR and hypertension**

In HFpEF, hypertension is the most prevalent co-morbidity (30) and precedes heart failure in 90% of all cases (31). Besides being a risk factor for atherosclerosis, hypertension affects the cardiac muscle through increased hemodynamic afterload, which contributes to concentric hypertrophic remodeling (Figure 1).

The RAAS is a predominant hormonal signaling pathway in the regulation of blood pressure and fluid balance, and LXRs have been implicated in blood pressure regulation through modulation of the RAAS. Initial observations identified LXRα as a regulator of renin transcription (32,33). Acute administration of LXR agonists directly increased renin transcription in vivo, whereas LXR-null mice lost their capacity to upregulate renin under β-adrenergic stress (33), suggesting a crosstalk between LXR signaling and the RAAS. In subsequent studies, chronic LXR activation inhibited isoproterenol-induced components of the RAAS, including renin, but also ACE and angiotensin type I receptor (AT1R) expression in kidneys and heart (34), as well as reduced the cellular response to angiotensin (Ang) II in aortic smooth muscle cells via downregulation of AT1R (35). In vivo investigation into the functional effects of LXRs on RAAS activation revealed that LXR agonism abolished Ang II-induced increases in blood pressure in rats (36). Although improved vasoreactivity was not unequivocally linked to the level of RAAS activation, these latter findings suggest that LXRs decrease peripheral vascular resistance and potentially lower blood pressure. In line with this, the LXR agonist T09 was found to reduce the elevation in blood pressure due to chronic pressure overload in mice (37). Overall, existing evidence suggests that LXRs play a role in antagonizing the effects of RAAS activation, as well as alleviating the hemodynamic burden imposed on the heart.
LXR and diabetes

Disturbances in energy balance lead to impaired peripheral glucose utilization and the development of insulin resistance and type II diabetes, both of which increase the risk for cardiovascular disease. Diabetes accelerates atherosclerosis, but also directly causes myocardial hypertrophy and diastolic dysfunction in the absence of hypertension or coronary artery disease (Figure 1).

LXR agonists have been recognized as a potential pharmacological strategy for the treatment of diabetes and associated metabolic disorders (38). Multiple studies have established the importance of LXRs in glucose metabolism and in the adaptation to metabolic stress that triggers diabetes. In rodent models of type II diabetes and insulin resistance, LXR agonists reduced plasma glucose (39,40) and improved glucose tolerance and insulin sensitivity (39-43). Mechanisms underlying the beneficial effects of LXRs on glucose homeostasis span several organ systems including liver, adipose tissue, skeletal muscle, as well as the pancreas. In the liver, LXR agonists suppress gluconeogenesis and promote hepatic glucose utilization (39,41,44). In adipose tissue and skeletal muscle, LXRs enhance peripheral glucose uptake largely through direct transcriptional regulation of the glucose transporter, Glut4, to promote both basal and insulin-stimulated glucose uptake (41,45), as well as in diabetes (46,47). In pancreatic islet cells, an important homeostatic role for LXRβ has been elucidated as LXRβ−/− mice are intolerant to glucose due to impaired glucose-stimulated insulin secretion (48), and furthermore, LXR ligands promote β-cell insulin secretion (42,48,49).

LXR agonists are thus promising anti-diabetic agents given their insulin-sensitizing effects. However, for these compounds to be of potential clinical use, their beneficial effects on glucose metabolism need to be dissociated from their lipogenic effects (Table). LXR agonists enhance hepatic and skeletal muscle lipid accumulation and increase circulating triglycerides (46,50), which worsens the lipogenic pathology in diabetes (51). Chronic LXR activation may also impair insulin secretion by contributing to lipotoxicity-induced pancreatic β-cell apoptosis (52). Alternative approaches include the development of partial LXR ligands or LXRβ-specific agonists since lipogenesis is primarily mediated via LXRα (53). Interestingly, a recent study reported that administration of the LXR agonist T09 in combination with metformin, an established oral anti-diabetic drug, ameliorated the development of hepatic steatosis induced by LXR agonism in diabetic rats (54), suggesting that combinatorial therapies may be viable.

Apart from metabolic dysregulation, diabetes is also characterized by low-grade inflammation that stems from macrophage infiltration in adipose tissue and secretion of pro-inflammatory cytokines (55). The anti-inflammatory functions of LXRs have been established – LXRs inhibit the induction of pro-inflammatory iNOS, COX2, IL6, and MCP1 by antagonizing NFκB signaling in the nucleus (56). However, data decisively demonstrating the link between the anti-inflammatory effects of LXRs on diabetic pathophysiology are
lacking. Since chronic systemic inflammation predisposes toward myocardial dysfunction and resultant HFpEF (57), LXRs may arguably protect the heart, as well as other susceptible organs, from diabetes- and obesity-induced inflammation.

In summary, LXRs are implicated in the protection from diabetes through modulation of glucose metabolism, β-cell insulin secretion, and inflammatory signaling, including recent developments indicating beneficial effects in hyperglycemia-induced endothelial dysfunction (58,59). Further, the potential of LXRs in targeting metabolic, inflammatory, and vascular components of diabetes has several implications regarding other co-morbidities affecting heart failure pathogenesis, such as atherosclerosis, which is further aggravated by the presence of diabetes and is the major cause of HFrEF, as well as nephropathy leading to chronic kidney disease (discussed below), which influences HFpEF.

LXR and chronic kidney disease

Nephropathy is a microvascular complication of diabetes mellitus and uncontrolled hypertension, leading to chronic kidney disease (60). The disease itself is a major contributor to cardiac damage and is associated with an increased risk for cardiovascular disease (61,62). LXRs have been implicated as a renoprotective target, preserving intrinsic renal structure and function both basally and in diabetic nephropathy.

A homeostatic role for LXRs in kidney function has been postulated. LXRβ−/− mice exhibit polyuria and polydipsia, features of diabetes insipidus (63), and mice deficient for both LXRs display a renal phenotype analogous to diabetic nephropathy with elevations in albumin-to-creatinine ratio and glomerular lipid accumulation (64). When challenged with diabetes, these mice demonstrated accelerated mesangial matrix expansion, glomerular lipid accumulation, and upregulation of inflammatory and oxidative stress markers (64).

In the kidney, expression levels of both LXRs are significantly decreased in animal models of type I diabetes (65) and in patients with diabetic nephropathy (66). Studies conducted in several diabetic rodent models demonstrated that LXR activation with T09 and GW3965, as well as a new generation agonist, N,N-dimethyl-3β-hydroxycholenamide (DMHCA), prevented renal damage and dysfunction by reducing urinary albumin excretion and inhibiting macrophage infiltration, inflammation, and lipid accumulation (64,67,68). Besides local renal effects, macrophage-derived LXR signaling is also pertinent in renal pathophysiology as transgenic LXRα overexpression in macrophages protected from hyperlipidemic-hyperglycemic nephropathy (68). These findings suggest that LXRs play an important role in hyperglycemic-induced kidney disease. Whether LXRs affect hypertension associated renal impairment remains to be established.
**Table.** Systemic Effects of Liver X Receptor Activation in Relation to Co-Morbidities Relevant in Heart Failure Pathogenesis

<table>
<thead>
<tr>
<th>Positive Effects of LXR Activation</th>
<th>Negative Effects of LXR Activation</th>
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<tr>
<td>Atherosclerosis</td>
<td>Liver steatosis</td>
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<tr>
<td>↑ Cholesterol efflux</td>
<td>↑ Liver steatosis</td>
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<td>↓ Inflammation</td>
<td>↑ Hypertriglyceridemia</td>
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<td>↑ Vascular protection</td>
<td>Neurological side effects</td>
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<td>Hypertension</td>
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<td>↑ RAAS modulation</td>
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<td>Chronic kidney disease</td>
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<td>↓ Diabetic nephropathy</td>
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II. LOCAL CARDIAC EFFECTS FOR LXR

Relatively few studies have evaluated LXR signaling in the heart itself. Both LXRα and LXRβ are expressed in the heart where, in comparison to the cardiomyocyte fraction, their expression levels are 10- to 15-fold higher in the non-myocytic fraction consisting of fibroblasts and endothelial cells (69). In the heart, LXRs are induced and activated by myocardial infarction (69,70), chronic pressure overload (71,72), and in diabetes (73,74), indicating that LXRs are regulated in cardiac (patho)physiology. We review current evidence regarding local cardiac effects for LXRs and speculate on the potential role of LXR signaling in the fundamental cellular processes governing cardiac remodeling, including cardiomyocyte hypertrophy and death, fibrosis, and metabolic and vascular remodeling (Figure 3).

**Cardiomyocyte hypertrophy**

Cardiomyocytes are non-proliferative, therefore they respond to pathophysiological stimuli through hypertrophic growth in order to reduce ventricular wall stress and augment contractile function. Increases in mechanical load as well as various neurohumoral signals such as catecholamines, growth factors, cytokines, and vasoactive peptides, activate a cascade of signal transduction pathways within the myocyte that orchestrates transcriptional reprogramming and posttranslational modification of protein synthesis and growth, as well as reactivation of fetal contractile and metabolic gene expression (fetal gene program). Initially, these adaptations are compensatory and considered protective, but with unremitted stress become maladaptive and yield to pathological concentric or eccentric growth.
Several lines of evidence suggest an important role for LXRα in regulating hypertrophic cardiac remodeling. First, from *in vitro* experiments, LXR agonists decreased cellular hypertrophy induced by diverse hypertrophic stimuli such as Ang II and LPS (71), endothelin-1 (37), and phenylephrine (72), whereas knockdown of LXRα in cardiomyocytes led to increased cellular growth (72). Second, LXRα protein abundance is markedly upregulated in the pressure overloaded heart (71,72). Third, murine hearts deficient for LXRα demonstrated an exacerbated hypertrophic response to chronic pressure overload induced via transverse aortic constriction (TAC) (71), whereas LXR agonists T09 and AZ876 attenuated hypertrophy in wild-type mice (37,72), but not in LXRα-null mice (37). Finally, the effect of LXRs on cardiac hypertrophy is heart-specific and independent of confounding systemic effects attributed to either T09 activation, which are lipogenic (12), anti-inflammatory (75), and blood pressure-lowering (37) effects, or residual effects from whole-body LXRα deletion (76). Cardiac-specific LXRα overexpression in transgenic mice protected the heart from TAC-induced pressure overload and Ang II stimulation by attenuating LV hypertrophy and preventing cardiac dysfunction (Cannon et al., unpublished data). Taken together, these findings suggest a cell-specific role for LXRs in cardiomyocyte hypertrophy, and are supported by pharmacological and genetic murine studies (Figure 3).

The mechanism(s) in which LXRα antagonizes pathological hypertrophic growth remains to be elucidated. In cardiomyocytes, Wu et al. identified suppressed pro-inflammatory NFκB signaling to be operative in T09-mediated decreases in cellular growth (71). It is also likely that the protective effects conferred by LXRα may involve modulation of myocardial metabolism. Mice with cardiac LXRα overexpression display an endogenous protective

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**Figure 3**

Cardiac effects of liver X receptor signaling. In the ischemic or hypertrophic myocardium, LXRs decrease cardiomyocyte hypertrophy and loss (death), as well as attenuate fibrotic remodeling. LXRs also modulate myocardial metabolism, and have regulatory functions in angiogenesis and neovascularization. ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.
phenotype evidenced by enhanced myocardial glucose uptake alongside increased natriuretic peptide signaling that resulted in protection from hypertrophic perturbations (Cannon et al., unpublished data).

**Cardiomyocyte death**

Loss of cardiomyocytes is crucial in the pathogenesis of myocardial infarction, ischemia/reperfusion (I/R), and heart failure. Since cardiomyocytes are terminally differentiated and generally incapable of replicating in the adult heart, their survival is critical for maintaining myocardial viability. Cell death arises when blood supply to the myocardium is disrupted by coronary occlusion. Ensuing hypoxic and ischemic stimuli increase ROS production which triggers necrosis and apoptosis.

Recently, a role for LXR in apoptosis has been evaluated in the infarcted heart. LXRα, but not LXRβ, protected against acute and chronic I/R injury where decreased magnitude of cardiac injury was associated with significant reductions in post-ischemic myocardial apoptosis. This effect occurred via inhibition of endoplasmic reticulum stress- and mitochondria-mediated apoptotic pathways through targeting of caspases 12 and 9, respectively (70). Similarly, GW3965 treatment in mice decreased infarct size and improved LV contractile function in response to global I/R injury, as well as prevented hypoxia-reoxygenation-induced apoptosis in HL-1 cells by attenuating caspase 3 (69). In an alternative therapeutic approach for cardiac repair following myocardial infarction, the combined therapy of LXR agonist T09 and adipose-derived mesenchymal stem cells (AD-MSCs) transplanted into infarcted hearts inhibited host cardiomyocyte apoptosis and improved cardiac function, while T09 further improved the survival of AD-MSCs under hypoxic conditions (77). These findings are consistent with other studies demonstrating protective effects for LXRs in ischemic models of intestinal and brain injury, although the beneficial effects conferred by LXR agonism in these models were linked to suppressed pro-inflammatory NFκB signaling (78-80).

LXRs have also been shown to regulate cell survival through inhibition of ROS production and oxidative stress (23,81). LXR agonists prevented hyperglycemia-induced apoptosis in cardiomyoblastic H9C2 cells (82) and in the diabetic db/db murine heart (74). Interestingly, the anti-apoptotic factor Spα, also known as apoptosis inhibitor 6 or AIM, is a direct target gene for regulation by specifically LXRα (83), and LXR signaling is also important for apoptotic cell clearance and enhanced macrophage phagocytosis (84,85), suggesting that LXRs may modulate various facets of cellular death (Figure 3).

**Fibrosis**

Cardiac fibrosis is central in the pathogenesis of heart failure and is a major constituent in LV remodeling resulting from myocardial infarction and chronic pressure overload. With myocardial infarction, cardiomyocyte loss is resolved by cardiac fibroblasts which initiate wound healing through replacement fibrosis to form a scar resilient in preventing ventricular rupture. Fibrosis that develops from pressure overload and in remote regions after
myocardial infarction is termed reactive fibrosis, and interferes not only with transduction of electrical impulses and contractile force in myocytes, but impedes diastolic relaxation through increased myocardial stiffness. In response to pathological stimuli, fibroblasts proliferate and differentiate into myofibroblasts which contract and secrete collagens. Increased collagen deposition as well as changes in the balance of proteins regulating extracellular matrix turnover consequentially disrupt normal matricellular architecture. To date, there are no therapeutic strategies that specifically target fibrogenesis in the heart.

LXR agonists exhibit anti-proliferative properties as demonstrated in pancreatic β-cells (86), smooth muscle cells (28,87), lymphocytes (88), and cancer cells (89), and thus, in the heart, they may potentially reduce expansion of the fibroblast population in LV remodeling. Both LXRs are expressed in cardiac fibroblasts, and treatment with the LXR agonist AZ876 suppressed fibroblast-to-myofibroblast transition and prevented Ang II- and transforming growth factor β (TGFβ)-induced collagen synthesis in vitro (72). In mice subjected to both chronic pressure overload and diabetes, LXR agonism reduced myocardial fibrosis and pro-fibrotic gene expression in conjunction with improved diastolic function (72,74). These observations are in accordance with other studies demonstrating anti-fibrotic effects of LXRs in liver injury (90), diabetic nephropathy (67,68), and experimental skin fibrosis (91). The latter two findings are associated with the interference of LXR ligands in macrophage infiltration and release of cytokines. In macrophages, LXRs downregulated MMP9 via repressed NFκB signaling (92), suggesting a link between LXRs and regulation of extracellular matrix turnover. The effect on macrophage activity is of clinical relevance considering that multiple co-morbidities contribute to a sustained pro-inflammatory state, particularly evident in HFpEF (93).

Additionally, LXRs may counteract pro-fibrotic signaling in the heart by affecting key intracellular pathways. For example, Ang II signaling promotes fibroblast proliferation and extracellular matrix formation through AT1 receptor-dependent activation, which is downregulated by LXR agonism (34-36) through dephosphorylation of the transcription factor, Sp1 (35). TGFβ is another central mediator of multiple inflammatory and fibrotic cellular responses in cardiac remodeling (94). TGFβ and downstream Smad2/3 expression are attenuated in hypertrophied murine hearts (72), as well as in other diseases including diabetes-induced renal fibrosis (67) and chronic asthma-induced airway remodeling (95). These findings altogether suggest that LXRs play a role in fibrogenesis, and may serve as a protective target in preventing the development of fibrosis in cardiac remodeling (Figure 3).

**Metabolic remodeling**

The heart is a highly metabolic organ requiring continuous replenishment of its cellular ATP stores to support sarcomeric contraction and relaxation as well as functioning of membrane transport systems. The demand for energy is supplied primarily through oxidation of fatty acids, but also glucose and to a lesser extent lactate, ketones, and amino acids. However, in
pathological states myocardial substrate utilization is altered to maximize energy efficiency through enhanced glucose utilization and reciprocal downregulation of fatty acid oxidation. As heart failure progresses, such metabolic adaptation is insufficient as oxygen and substrate supply are decreased and energy transfer is impaired, resulting in mitochondrial dysfunction and inefficient energy utilization. In essence, the failing heart becomes “an engine out of fuel” (96) with energy production inadequate to support cardiac output.

Promoting the shift toward increased glucose reliance has been postulated to improve myocardial efficiency in heart failure, preventing energy depletion (97,98). LXRα has been implicated in the transcriptional regulation of cardiac glucose metabolism. Constitutive LXRα overexpression in isolated cardiomyocytes and murine hearts induced Glut1 and Glut4 expression which functionally enhanced glucose uptake and utilization. Furthermore, under conditions of hypertrophic stress, these mice exhibited substantially greater increases in glucose uptake levels compared to wild-type mice in the protection against LV hypertrophy and cardiac dysfunction, whereas this adaptive response was diminished in LXRα-null hearts (Cannon et al., unpublished data). Interestingly, LXRα was also shown to improve long-term cardiac performance following chronic I/R injury, evidenced in part by preserved glucose uptake which is indicative of myocardial viability in this setting (70). Whether preservation of glucose uptake was a direct effect of LXRα on glucose metabolic pathways, or secondary to reduced infarct size, was not established herein. Lei et al. observed increased lipid droplet accumulation with LXR agonism in association with improved tolerance to acute myocardial ischemia (69). At the onset of I/R there is increased availability and usage of fatty acids (99). However, the role of lipids in the heart remains controversial as it may lead to lipotoxicity and dysfunction (100). Glucose pathways were not assessed in this study and thus cannot be precluded as a protective mechanism against acute myocardial infarction.

Targeting LXRα to promote cellular glucose uptake may indeed be advantageous under conditions where glucose uptake is compromised. For example, insulin resistance is highly prevalent in the heart failure population and can impair the shift to glucose reliance (101,102). Strategies that sensitize the heart to glucose are therefore of clinical relevance. Inasmuch, insulin resistance induced by obesity did not adversely affect the capacity for enhanced glucose uptake in cardiac LXRα transgenic mice (Cannon et al., unpublished data), indicating that sustained increases in myocardial glucose uptake by LXRα is undeterred by a metabolic challenge. Further, LXR agonism was recently shown to protect against diabetic cardiomyopathy by attenuating cardiac dysfunction in db/db mice (74). Thus, overall, LXRs represent a potential metabolic modulator for optimization of myocardial substrate utilization in cardiac pathophysiology (Figure 3).

**Angiogenesis**
Endothelial dysfunction involves a range of endothelial cell functions that become dysregulated, including impaired angiogenic responses that are crucial in salvaging the
infarcted and hypertrophic myocardium (103). In HFrEF, new microvascular networks are necessary for improving regional perfusion after ischemic injury, and in HFrEF, muscle-to-capillary ratio is reduced in pathologically hypertrophied hearts, which causes myocardial hypoxia and contractile dysfunction by compromising oxygen and nutrient delivery needed for growth. The aim of cardiovascular therapies is to stimulate angiogenesis within the myocardium to prevent or reverse heart failure.

The angiogenic potential of LXRs has been established in the ischemic rat brain (79) and following stroke (104). LXRs promote angiogenesis by directly regulating VEGF (105). Murine and human Vegfa genes harbor a functional LXRE in the promoter region, and regulation occurs independent of the hypoxia response element for HIF-1 (105). However, several studies provide evidence for an anti-angiogenic (yet protective) role for LXRs in settings of uncontrolled angiogenesis such as in tumor growth (106) and cancer (107,108). Whether LXRs stimulate angiogenesis in either the ischemic or hypertrophic myocardium remains to be determined.

LXRs may also regulate angiogenesis through modulation of cholesterol. Cholesterol promotes lipid raft formation in the plasma membrane from where cell surface receptors initiate signaling events that lead to angiogenesis (109). Since excess cholesterol or its removal determines the balance between either promoting or inhibiting angiogenesis, this balance may arguably be regulated by LXRs given their antagonistic actions of increasing HDL cholesterol (110) and stimulating cholesterol efflux via the RCT (17). These data therefore implicate a role for LXR in angiogenesis and suggest LXRs may prevent tissue hypoxia either through directly targeting VEGF or through modulation of cholesterol (Figure 3).

CONCLUSIONS

Heart failure is a clinical syndrome, and co-morbidities such as hypertension, diabetes, and kidney and vascular disease, including atherosclerosis, are increasingly recognized for their provocation of this disease. Left untreated, these co-morbidities accelerate the progression of adverse myocardial remodeling which results in impaired cardiac function, eventually leading to heart failure and death (Figure 1). The optimal window for therapeutic modulation likely occurs in the early phases when cardiac remodeling is largely reversible, and since systemic LXR activation confers protective effects in the pathogenesis of the aforementioned co-morbidities, targeting of LXR during this phase may represent a useful addendum in preventing remodeling progression. Within the myocardium, recent evidence implicates LXR as a cardioprotective molecule in pathological pathways involving cardiomyocyte hypertrophy and loss, fibrosis, and metabolism (Figure 3), and are therefore amenable to treatment with LXR ligands. However, translation of basic findings to clinical settings requires additional studies to further elucidate LXR signaling in the heart, and the impact of the integrative functions of LXRs in multi-organ systems on myocardial protection warrants further address.
Thus far, few data exist on the role of LXR signaling in humans. In a human genetic study, analysis of the LXR gene sequence in patients afflicted with coronary artery disease revealed mutations in the ligand-binding domain of LXRα that altered their conformation, rendering LXRα inept in binding its ligands (111), suggesting that humans having a mutated LXRα gene may develop a predisposition towards coronary artery disease. Results from GWAS studies provides evidence for a positive association between LXR and longevity (112), and genetic polymorphisms in the LXRα gene locus are associated with electrocardiographic LVH (Van der Harst et al., unpublished data), implicating anti-hypertrophic effects in the heart.

Undoubtedly, high affinity pharmacological agonists specific for LXRα are needed to further evaluate the potential of LXR activation in human disease. Testing of new generation LXR agonists with less lipogenic profiles in clinical trials should provide further insight into their therapeutic prospective. Thus far, only one study has been published regarding the LXR agonist, LXR-623, having activated LXR without causing hepatic lipogenesis. However, this clinical trial was prematurely halted due to adverse neurological side effects in subjects tested (113). Nevertheless, several other LXR agonists are in development, including natural modulators of LXR activity which are also being considered for their potential as nutraceuticals for therapy (114), and recently, a novel partial LXR agonist, BMS-779788, with decreased lipogenic potential and proven efficacy in primates (115). Given the global salutary effects of LXR activation in cardiovascular disease and its precursors, atherosclerosis, hypertension, diabetes, and inflammation, we postulate that effective and successful targeting of LXRs holds promise for future therapies.
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