Cardiac LXRα protects against pathological cardiac hypertrophy and dysfunction by enhancing glucose uptake and utilization

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Abstract

Pathological cardiac hypertrophy is characterized by a shift in metabolic substrate utilization from fatty acids to glucose, but the molecular events underlying the metabolic remodeling remain poorly understood. Here, we investigated the role of liver X receptors (LXRs), which are key regulators of glucose and lipid metabolism, in cardiac hypertrophic pathogenesis. Using a transgenic approach in mice, we show that overexpression of LXRα acts to protect the heart against hypertrophy, fibrosis, and dysfunction. Gene expression profiling studies revealed that genes regulating metabolic pathways were differentially expressed in hearts with elevated LXRα. Functionally, LXRα overexpression in isolated cardiomyocytes and murine hearts markedly enhanced the capacity for myocardial glucose uptake following hypertrophic stress. Conversely, this adaptive response was diminished in LXRα-deficient mice. Transcriptional changes induced by LXRα overexpression promoted energy-independent utilization of glucose via the hexosamine biosynthesis pathway, resulting in O-GlcNAc modification of GATA4 and Mef2c and the induction of cytoprotective natriuretic peptide expression. Our results identify LXRα as a key cardiac transcriptional regulator that helps orchestrate an adaptive metabolic response to chronic cardiac stress, and suggest that modulating LXRα may provide a unique opportunity for intervening in myocyte metabolism.

Keywords glucose metabolism; left ventricular hypertrophy; liver X receptor; nuclear receptor; O-GlcNAcylation

Subject Categories Cardiovascular System; Metabolism

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Introduction

Pathological cardiac hypertrophy and remodeling ensue in response to sustained elevations in hemodynamic workload (Frey & Olson, 2003). A hallmark of this remodeling process is the alteration in myocardial energy metabolism which is necessitated by increased energy demand (Neubauer, 2007; Ventura-Clapier et al., 2011). Under normal physiological conditions, the heart predominantly consumes fatty acids (FA), whereas various stressors trigger a compensatory shift toward glucose, the preferred substrate for its oxygen-sparing effect in ATP production (Stanley et al., 2005; Opie & Knuuti, 2009). Despite the success of current pharmacological strategies which aim to reduce afterload and hypertrophic growth using beta blockers and inhibitors of the renin–angiotensin–aldosterone system, heart failure nevertheless remains a progressive disease with high morbidity and mortality. Interventions in metabolic remodeling therefore represent a promising therapeutic adjunctive for targeting pathological cardiac hypertrophy and development of heart failure (Ardehali et al., 2012).

Liver X receptors (LXRs) are nuclear receptors that have emerged as central metabolic regulators in various organ systems and cell types. At the molecular level, LXRs function as ligand-activated transcription factors that intricately regulate and coordinate cholesterol homeostasis, glucose and lipid metabolism, and inflammatory signaling. As such, the importance of LXRs in the cardiovascular system is mainly attributed to their atheroprotective effects in accelerating reverse cholesterol transport (Naik et al., 2006; Zhang et al., 2012), and reducing atherosclerotic lesion size and inflammation (Schuster et al., 2002; Joseph et al., 2003; Giannarelli et al., 2012). The biological functions of LXRs are mediated via two subtypes,
LXRα (NR1H3), which is predominantly expressed in liver, adipose, intestine, and macrophages, but also in heart, kidney, adrenal gland, and lung, and LXRβ (NR1H2), expressed ubiquitously (Chen et al., 2005). LXRs reside in the nucleus where they heterodimerize with retinoid X receptor and are bound to cognate LXR response elements (LXREs) in regulatory regions of target genes (Peet et al., 1998).

Less is known regarding the role of LXR in pathological cardiac hypertrophy. LXRs have been implicated in blood pressure control by regulating renin gene expression in vivo (Morello et al., 2005). In blood pressure-independent models, the LXR agonist, T0901317 (T09), attenuated the hypertrophic response to pressure overload in mice (Kuipers et al., 2010), whereas this effect was exacerbated in LXRα-null mice (Wu et al., 2009). These studies, however, are limited by confounding systemic effects of pharmacological LXR activation and by the lack of agonist specificity. Therefore, it remains unclear whether LXRs directly affect the pathogenesis of hypertrophy. The purpose of the present study was to investigate the cardiосpecificity of LXRα in modulation of myocardial metabolism and pathological hypertrophy by generating a murine model of cardiac-specific LXRα overexpression.

Results

Mice with cardiac-specific LXRα overexpression exhibit no overt cardiac phenotype at baseline or with aging

To study LXRα activation in the heart, we generated transgenic (LXRα-Tg) mice with cardiac-specific overexpression of murine LXRα driven by the cardiomyocyte-specific αMHC promoter (Fig 1A). LXRα mRNA and protein expression were increased 130-fold and 9-fold, respectively (Fig 1B, C and D). Heart weight in LXRα-Tg mice, as assessed by LV weight normalized to tibia length (LV/tibia), was marginally less compared to Wt (Fig 1E). Mean arterial pressure (MAP) was unaltered in LXRα-Tg mice (Fig 1F). Echocardiography-determined LV dimensions and function were comparable to Wt, and no differences in indices of contractility and intracardiac pressures, measured in situ with microtip catheterization, were observed (Supplementary Table S1). Histological analyses of ventricular sections stained with WGA-FITC or Masson’s trichrome displayed no evidence of abnormal cardiomyocyte morphology or collagen deposition in LXRα-Tg hearts (Fig 1G). To verify whether overexpression indeed induced functionally active LXRα, we determined mRNA levels of well-described LXRα target genes (Tontonoz & Mangelsdorf, 2003) including Srebp1c, Scd1, and Abca1. These were significantly increased in LXRα-Tg mice (Fig 1H). Furthermore, Pgc1α, a co-activator of LXR (Oberkoffer et al., 2003), was also upregulated, plausibly to maintain LXRα in a constitutively active state. Lxrβ expression was not changed. The long-term effects of cardiac-specific LXRα activation were also assessed in mice up to 12 months of age. Chronic LXRα activation did not impair gross cardiac morphology or function in aged mice (Supplementary Table S1). In summary, LXRα-Tg mice displayed normal physiologic development, and all structural and functional cardiac parameters were within normal range.

LXRα overexpression attenuates pathological development of cardiac hypertrophy, fibrosis, and dysfunction

To evaluate specific effects of LXRs in pathological cardiac hypertrophy, mice were subjected to pressure overload by TAC for 5 weeks. Heart weights were similar between sham-operated Wt and LXRα-Tg groups (Fig 2A). TAC caused significant increases in LV/tibia ratios; however, LXRα-Tg mice exhibited 24% less hypertrophy compared to Wt, which was further evidenced by reduced cardiomyocyte size (Fig 2B and C). In comparison with LXRα-Tg mice, the greater degree of hypertrophy observed in Wt was attributable to larger increases in interventricular septal and LV free wall thicknesses, while no marked dilatation of the LV chamber was observed for either TAC group (Supplementary Table S2). To further assess the impact of LXRα on other parameters of myocardial remodeling, fibrosis was quantified in cross-sectional LV. Collagen deposition was only marginally detected in LXRα-Tg hearts following TAC, whereas this increased 4-fold in Wt (Fig 2C and D). These anti-fibrotic effects were associated with less induction of genes involved in fibrogenesis, Col1a1 and Ctgf (Fig 2E). Following TAC, typical reactivation of the fetal gene program occurred in both groups, but to a lesser extent in LXRα-Tg mice (Fig 2E). Interestingly, we observed elevated basal levels of natriuretic peptides, ANP and BNP, in LXRα-Tg mice. In contrast, other gene markers representative of fetal gene activation were more strongly induced in Wt following TAC: zMHC to βMHC isoform transition (Myh6/Myh7 ratio), Acta1, as well as the hypertrophic gene, Rcn1.

Functional evaluation revealed that Wt mice subjected to TAC achieved significantly less systolic LV thickening and demonstrated greater acceleration toward heart failure as fractional shortening declined 11% versus only 6% in LXRα-Tg (Fig 2A). These functional improvements in LXRα-Tg mice were further accompanied by reduced intracardiac pressures (Fig 3C and D). No changes in MAP or HR were recorded (Fig 3B, Supplementary Table S2).

To determine whether cardiac LXRα overexpression affects early hypertrophic remodeling processes, mice were subjected to 1 week of TAC-induced pressure overload. Cardiac hypertrophy was present in Wt mice after 1 week of TAC; however, this was significantly attenuated in LXRα-Tg mice (Supplementary Fig S1A). Assessment of cardiac function with echocardiography indicated that function remained relatively compensated in TAC-operated mice (Supplementary Fig S1B). The effect of cardiac LXRα on hypertrophy, including expression of fetal genes (Supplementary Fig S1C), is comparable to what we observed in TAC after 5 weeks. Molecular determinants of inflammation were more strongly upregulated in Wt hearts compared to LXRα-Tg (Supplementary Fig S1D-G), whereas the anti-apoptotic factor, Bcl2, was significantly induced with LXRα overexpression (Supplementary Fig S1I and J). These data implicate an anti-inflammatory role for LXRα in the initial phase of hypertrophic pathogenesis, and possible protection against anti-apoptotic triggers.

To further verify that our observations were not model dependent, we conducted experiments with Ang II infusion over 10 days. Consistent with our findings from TAC experiments, LXRα-Tg mice showed reduced Ang II-induced myocardial hypertrophy and fibrosis with moderate improvements in hemodynamic parameters (Supplementary Fig S2). Taken together, these data demonstrate
that cardiac-specific LXRα activation ameliorates adverse cardiac remodeling and dysfunction in mice in response to diverse pathological hypertrophic stimuli.

**LXRα overexpression induces transcriptional alterations in metabolic pathways**

Gene profiling of the LV transcriptome was performed to uncover the molecular basis for the cardioprotective phenotype observed in LXRα-Tg mice (Supplementary Fig S3, Supplementary Table S3). Basal LXRα overexpression induced substantial changes in genes relating to metabolism (Fig 4A). To further investigate microarray pathway analysis, mRNA levels of several key genes regulating FA and glucose metabolism were determined (Supplementary Fig S4). In LXRα-Tg hearts, glycolysis-related genes were more differentially expressed, including upregulation of the glucose transporters, *Glut1* and *Glut4*, as well as *Pdk4*, regulating pyruvate oxidation in mitochondria.
TAC provoked parallel transcriptional alterations in Wt and LXRα-Tg mice, downregulating FA metabolism and similarly upregulating pathways pertaining to extracellular remodeling and cardiovascular disease. However, the comparison between differentially expressed genes in hypertrophic hearts was most striking for LXRα-Tg where more than 50% of upregulated genes clustered into
metabolic pathways, for example, glutathione metabolism. Collectively, these expression data convey that LXRα activation transcriptionally reprograms metabolic pathways in the heart, specifically glucose metabolism.

**Constitutive LXRα activation enhances myocardial glucose uptake and utilization**

We next evaluated whether global transcriptional changes relating to glucose metabolism translated into a functional metabolic outcome. To this end, in vivo glucose uptake measurements were performed in a separate sham/TAC cohort (n = 22) by injecting mice with FDG and using microPET imaging modality. Basal myocardial FDG-glucose uptake was 1.9-fold higher in LXRα-Tg mice compared to WT, indicating a greater propensity for glucose utilization. Consistent with previous reports showing a substrate shift to glucose following hypertrophic perturbation (Liao et al., 2002; Voelkl et al., 2012), TAC lead to a substantial increase in FDG-glucose uptake in both groups. WT mice achieved 90% increase, which subsequently matched basal LXRα-Tg levels. On the other hand, TAC-operated LXRα-Tg mice exhibited comparatively even greater capacity for glucose uptake that was augmented 50% above basal LXRα-Tg levels and that of WT TAC cohorts (Fig 4B and C). Increased glucose uptake in LXRα-Tg hearts did not impact systemic blood glucose levels as no significant differences in basal levels prior to scan, nor under fasted conditions (Supplementary Table S1), were observed. Furthermore, enhanced glucose uptake was not stored, but rather utilized since myocardial glycogen content was unaltered (Fig 4D).

Expression levels of several key proteins involved in substrate metabolism and regulation were measured. In concert with FDG-glucose uptake levels, GLUT1 and GLUT4 protein levels were increased in LXRα-Tg hearts (Fig 4E), suggesting their membrane translocation and functionality. No appreciable differences between LXRα-Tg and WT were observed for hexokinase 2 (HK2), the enzyme catalyzing the first step in glycolysis, nor phosphorylated-AMPK, a key metabolic regulator in response to increased workload and energetic stress, as well as for CD36 regulating myocardial FA uptake (Supplementary Fig S5). In summary, enhanced myocardial glucose uptake evidenced in LXRα-Tg mice in the stressed and non-stressed state is associated with induction of GLUT1 and GLUT4.

**LXRα-deficient mice manifest reduced myocardial glucose uptake capacity in response to TAC**

To gain further insight into LXRα regulation of myocardial metabolism in cardiac hypertrophy, loss-of-function studies were performed in LXRα−/− mice (Supplementary Table S4). Following 5 weeks of pressure overload, both WT and LXRα−/− mice developed LV hypertrophy (Fig 5A). The relative increases in LV/tibia ratios were considerably higher for LXRα−/− compared to WT, 50% versus 30%, albeit not significantly different from each other. Furthermore, LXRα-deficient hearts exhibited a greater tendency toward cardiac dysfunction (Fig 5B and C). Activation of the fetal gene program occurred to a similar extent in both LXRα−/− and WT mice (Fig S5–G).

The metabolic response of LXRα-null hearts to hypertrophic perturbation was assessed by evaluating myocardial glucose uptake and GLUT expression. Both GLUT1 and GLUT4 were evidently less upregulated in LXRα−/− hearts in response to TAC (Fig 5H). Furthermore, FDG-glucose analysis with microPET revealed that LXRα−/− mice demonstrated an inability to normalize the increases in glucose uptake levels achieved in pressure overload-induced hypertrophy (Fig S1 and J, Supplementary Fig S6). Myocardial glucose uptake increased 1.6-fold in WT, but only 1.3-fold in LXRα−/− mice. Overall, loss of LXRα resulted in a more progressive deterioration of function following TAC that was associated with a compromised adaptive capacity to augment glucose uptake.

**Mitochondrial oxidative capacity of pyruvate is unaltered by chronic LXRα overexpression**

To assess whether LXRα-Tg mice displayed increased mitochondrial capacity to oxidize glucose-derived substrates, we determined oxidative phosphorylation in permeabilized LV muscle fibers in the presence of pyruvate in an additional sham/TAC cohort (n = 26). Basal (state 2) and maximal ADP-stimulated (state 3) oxygen consumption rates did not differ among stressed and unstressed LXRα-Tg and WT hearts (Supplementary Fig S7A), indicating that the capacity for pyruvate oxidation was neither impaired nor enhanced by LXRα overexpression or by TAC. Citrate synthase activity, a marker of mitochondrial density to which all
respirometry measurements were normalized, was similar for all groups (Supplementary Fig S7B).

Interestingly, we recorded increased state 2 and state 3 respiration rates with palmitoyl (C16)-carnitine in LXRα-Tg, suggesting a trend toward increased capacity to oxidize FA (Supplementary Fig S7C). This may be due to a reciprocal effect on pyruvate oxidation by Pdk4, which was induced in LXRα-Tg (Supplementary Fig S4A).

The respiratory control ratio, indicative of overall mitochondrial function (Brand & Nicholls, 2011), tended to be higher for palmitoyl-carnitine in stressed and unstressed hearts overexpressing LXRα (Supplementary Fig S7D). Further lipid profiling revealed increased myocardial leanness in LXRα-Tg hearts (Supplementary Fig S8), despite induction of several lipogenic gene targets (Srebp1c, Scd1, Fasn) (Fig 1H, Supplementary Fig S4B).

**LXRα-mediated glucose uptake increases O-GlcNAc signaling in cardiomyocytes**

Since no differences in mitochondrial capacity to utilize pyruvate was identified, we postulated that beneficial effects derived from
Figure 5. LXRα-null mice manifest reduced myocardial glucose uptake capacity in response to TAC.

A LV/tibia ratios in sham- and TAC-operated WT and LXRα−/− mice; n = 10 WT sham, n = 8 LXRα−/− sham, n = 12 WT TAC, n = 10 LXRα−/− TAC. *P = 0.006 versus WT sham, **P = 0.00008 versus LXRα−/− sham.

B Cardiac functional assessment at 5 weeks post-TAC. (B) Percent fractional shortening determined with echocardiography; n = 10 WT sham, n = 7 LXRα−/− sham, n = 12 WT TAC, n = 10 LXRα−/− TAC. *P < 0.00001 versus respective sham, §P = 0.06. (C) LV end-diastolic pressure (LVEDP) recorded in situ; n = 10 WT sham, n = 7 LXRα−/− sham, n = 10 WT TAC, n = 9 LXRα−/− TAC. *P = 0.001 versus WT sham, **P = 0.00005 versus LXRα−/− sham.

D–G Relative mRNA gene expression as determined by RT-PCR, normalized to 36B4; n = 8/group. Anp: no significant differences; Bnp: *P = 0.01 versus WT sham, **P = 0.0003 versus LXRα−/− sham; Myh6/Myh7: *P = 0.0002 versus WT sham, **P = 0.0003 versus LXRα−/− sham; Acta1. *P = 0.001 versus respective sham.

H Western blot detection of GLUT protein expression in LV tissue normalized to GAPDH, expressed as fold change, shows no significant differences among groups; n = 6–8/group.

I Representative 18F-FDG-microPET scans.

J Myocardial FDG uptake quantified as standard uptake value (SUV); n = 6/group. *P = 0.0001 versus WT sham, #P = 0.047.

Data information: Data are means ± SEM; one-way ANOVA with Bonferroni’s multiple comparison test was used to compare groups. Source data are available online for this figure.
LXRα-mediated enhanced glucose uptake involved alternate pathways of glycolytic metabolism. One such pathway is the hexosamine biosynthesis pathway (HBP) which culminates in the formation of O-GlcNAc, a posttranslational modifier of numerous proteins. The HBP has been demonstrated to be an essential signaling system in the failing heart (Watson et al., 2010), and accumulating evidence from in vitro and ex vivo systems shows that augmented O-GlcNAc levels via the HBP potentiates cytoprotection (Ngoh et al., 2010; Darley-Usmar et al., 2012). Using NRVMs, we tested the hypothesis that LXRα-mediated increases in glucose uptake would enhance substrate availability for O-GlcNAc. NRVMs transfected with Ad-LXRα showed significantly elevated 2-deoxyglucose (2-DC) levels of 1.8-fold compared to Ad-cont cells, which was further augmented 40% following PE stimulation (Fig 6A). Glut4 and Glut1 mRNA levels were correspondingly increased (Fig 6B and C). Next, we assessed whether enhanced glucose availability led to HBP activation and downstream formation of O-GlcNAc. Ad-LXRα cells displayed increased global protein O-GlcNAcylation that was further enhanced with PE (Fig 6D). Administration of DON to inhibit HBP flux attenuated Ad-LXRα-increased O-GlcNAcylation (Fig 6D), confirming the link between LXRα and HBP-O-GlcNAc signaling.

From our findings in LXRα-Tg mice, we speculated that increased myocardial natriuretic peptide expression (Fig 2E) in conjunction with preference for glucose may, in part, be evidence of an endogenous cardioprotective stress response elicited via LXRα overexpression. The anti-hypertrophic properties of ANP and BNP are well established (Nishikimi et al., 2006). Ad-LXRα cells expressed both increased Anp and Bnp (3.2-fold and 3.8-fold, respectively), which were subsequently suppressed following DON inhibition, suggesting that their induction is linked to O-GlcNAc effector signaling (Fig 6E and F). Further assessment of cellular hypertrophy indicated that DON inhibition of HBP flux also abolished the Ad-LXRα-mediated reductions in cell size that was increased upon PE stimulation (Fig 6G and H).

Alternative experiments were performed with si-LXRα to address the causal relationship among LXRα expression, protein O-GlcNAc modification, and hypertrophy. Knockdown of LXRα led to comparatively higher levels of hypertrophic growth (Fig 7A), and lower levels of O-GlcNAc following PE-induced cellular stress (Fig 7B). Interestingly, gene expression analysis (Fig 7C–F) revealed significant downregulation of Anp in LXRα-silenced cells (Fig 7C).

Transcriptional activators of natriuretic peptides are O-GlcNAc modified in LXRα-Tg hearts

Finally, to corroborate our in vitro findings, we assessed global protein O-GlcNAc levels from LV tissue lysates of mice overexpressing and deficient for LXRα. Most extensive O-GlcNAcylation was observed in LXRα-Tg hearts involving proteins between 40 and 55 kDa in size (marked in Fig 8A). In contrast, loss of LXRα resulted in attenuated O-GlcNAc signal in response to TAC (Fig 8B). To further identify specific O-GlcNAc targets, agarose wheat germ agglutinin (WGA) precipitation was performed to isolate nuclear GlcNAcylated proteins. Using antibodies specific for known transcription factors activating ANP and BNP (Morin et al., 2000; Hayek & Nemer, 2011), Western blot analysis revealed that GATA4 and MeF2c precipitated with WGA in LXRα-Tg hearts, but not with Nkx-2.5, suggesting that O-GlcNAc modification of GATA4 and MeF2c potentiates their activities (Fig 8C and D). N-acetylgalcosamine (GlcNAc), a competitor, served as a control.

In summary, these data indicate that cardiac LXRα integrates glucose metabolism and downstream O-GlcNAcylation with induction of cytoprotective natriuretic peptides to orchestrate an anti-hypertrophic response. Therefore, the energy-independent effects of glucose that, herein, are governed by LXRα may be an important salutary mechanism in modulating and preserving myocyte function (Fig 9).

Discussion

In the present study, we describe a cardiac-specific overexpression model for LXRα in mice, and herewith, elucidate the significance of LXRα in modulating myocardial metabolism in pathological hypertrophy. We established that constitutive LXRα activation in murine hearts substantially diminished LV hypertrophy, adverse cardiac remodeling, and improved overall cardiac function following chronic pressure overload and Ang II stimulation. Using this model, we identified the intrinsic transcriptional regulatory mechanisms LXRα exerts in the heart and in countering hypertrophic stress. By principally modulating glucose pathways, LXRα functionally enhanced the capacity for myocardial glucose uptake, which was conversely impaired in hypertrophic hearts deficient for LXRα. Furthermore, increased glucose utilization via an energy-independent pathway resulted in the glycosylation of transcription factors inducing natriuretic peptide expression, which we identified as a putative end effector of LXRα-mediated protective effects in the heart.

The role of LXRα in protection against cardiac pathophysiology is not well established. This has been previously addressed in pharmacological studies using the LXR agonist, T09, and LXRα-null mice (Wu et al., 2009; Kuipers et al., 2010). Conceivably, these approaches are restrained by confounding variables associated with systemic LXR activation, which include lipogenic (Peet et al., 1998), anti-inflammatory (Zelcer & Tontonoz, 2006), and blood pressure lowering (Leik et al., 2007) effects. Moreover, T09 mediates its effects indiscriminately via other nuclear receptors since it is also a co-activator of farnesoid X receptor, pregnane X receptor, and retinoic acid receptor signaling (Houck et al., 2004; Mitro et al., 2007; Kumar et al., 2010). Selectively overexpressing cardiac LXRα in mice circumvented these confounding factors and afforded a system for delineating the heart-specific effects of LXRα. Using two diverse hypertrophic perturbations, we demonstrated that constitutive LXRα activation counterbalanced pathological growth and remodeling processes in the heart, including blunting the development of myocardial fibrosis, an observation in line with previous studies demonstrating the anti-fibrotic effects of LXRα in kidney (Tachibana et al., 2012) and in liver (Beaven et al., 2011). Cardiac LXRα also appears to influence early remodeling processes since less inflammation in association with decreased hypertrophy occurred at an earlier time point of 1 week post-TAC. LXRα-Tg mice may also be less susceptible to apoptosis, which is underscored by upregulation of Bcl2. Taken together, counteraction of inflammatory signaling and myocyte death may explain the attenuated development of fibrosis remodeling we observed after 5 weeks TAC.
Figure 6. LXRα enhances glucose uptake and O-GlcNAc signaling via activation of the hexosamine biosynthetic pathway (HBP) in cultured cardiomyocytes.

Neonatal rat ventricular myocytes were transfected with Ad-LXRα or GL2 (Ad-cont), and treatments with phenylephrine (PE) and DON (inhibitor of HBP) were initiated for 24 h.

A Assessment of 2-deoxyglucose (2-DG) uptake from 4 independent experiments. *P = 0.03 versus Ad-cont.

B, C Glut mRNA expression determined by RT–PCR normalized to 36b4, n = 5 per condition in the absence of PE, n = 4 per condition in the presence of PE. *P = 0.02 versus Ad-cont, **P = 0.008 versus Ad-cont, *P = 0.02, ***P = 0.008.

D Western blot indicating Ad-LXRα- and PE-induced increases in global protein O-GlcNAcylation, which was abrogated following inhibition of HBP with DON. LXRα protein expression is shown, and GAPDH served as a loading control.

E, F Modulation of Anp and Bnp mRNA levels by Ad-LXRα-induced O-GlcNAc signaling. Gene expression as determined by RT–PCR normalized to 36b4, n = 5 per condition in the absence of PE, n = 4 per condition in the presence of PE. *P = 0.02 versus Ad-cont, **P = 0.008 versus Ad-cont, #P = 0.03, ##P = 0.02.

G Measurement of cell size, n = 5 per condition. *P = 0.008 versus Ad-cont, #P = 0.02, ###P = 0.03.

H Representative images for the determination of cell size. Cells were stained with an antibody specific for LXRα (green, indicated by arrow), DAPI for nuclei (blue), and rhodamine-phalloidin for F-actin (red); scale bar = 50 μm.

Data information: Data are means ± SEM and are reported as fold change with respect to control group; Kruskal–Wallis test followed by Mann–Whitney U-test was used for group comparisons.

Source data are available online for this figure.
In loss-of-function studies, LXRα−/− mice did not develop significantly greater severity of hypertrophy with respect to WT, although function was worsened in LXRα-deficient hearts. This is in contrast to a previous report showing exacerbated hypertrophic response in LXRα−/− mice (Wu et al., 2009). The discrepancy between LXRα overexpression and deficiency cannot be fully explained herein; however, our in vitro data indicate that, at the cellular level, there is a clear effect on hypertrophic growth in cardiomyocytes lacking LXRα, and thus, compensatory mechanisms may be operative in the intact heart. Interestingly, recent evidence from a genome-wide association study (GWAS) of electrocardiographic LV hypertrophy (LVH) found a genetic variant, or SNP, in the LXRα (Nr1h3) locus to be significantly associated with the LVH trait. Furthermore, expression QTL analysis showed a significant correlation between decreased expression of Nr1h3 and increased LVH (P. Van der Harst, unpublished data, 2011), supporting an anti-hypertrophic role for LXRα.

Functionally, gene profiling in our model of cardiac-specific LXRα overexpression identified primary effects for LXRα on metabolic pathways, and further investigation into this metabolic profile revealed that myocardial glucose uptake in association with GLUT expression was significantly increased in LXRα-Tg hearts and in isolated cardiomyocytes. GLUT1 and GLUT4 have previously been elucidated as targets for transcriptional regulation by LXRα in adipose tissue and skeletal muscle (Ross et al., 2002; Dalen et al., 2003; Laffitte et al., 2003; Kase et al., 2005; Griesel et al., 2010). Myocardial glucose uptake capacity was enhanced in LXRα-Tg mice, and more importantly, when challenged with hypertrophic stress, these mice demonstrated an even more robust response versus that of WT. Conversely, insufficient glucose uptake capacity that ensued in hypertrophic hearts deficient for LXRα resulted in a worsened functional outcome. The shift toward greater glucose reliance is believed to be an adaptive response that confers cardioprotection (Opie & Knuuti, 2009; Kolwicz & Tian, 2011), and evidence from other genetic mouse models renders further support for a role for glucose uptake in myocardial protection. Cardiac-specific overexpression of GLUT1 in mice increased glucose uptake and glycolysis which prevented the development of ventricular dysfunction and improved survival (Liao et al., 2002; Luptak et al., 2005), whereas reduced glucose utilization in GLUT4 knockout mice manifested greater hypertrophy and acceleration toward heart failure (Katz et al,
1995; Domenighetti et al., 2010). With cardiac insulin resistance and metabolic dysregulation known to precede the development of heart failure (Witteles & Fowler, 2008; Brouwers et al., 2013), strategies sensitizing the heart to glucose uptake may thus have clinically relevant implications in the long-term prognosis of heart failure.

Since glucose uptake rates were enhanced by LXR\(\alpha\) activation, we hypothesized that this would also lead to downstream changes in energy-dependent pathways, causing increased mitochondrial oxidative capacity. However, we did not observe corresponding increases in oxidative capacity from pyruvate, suggesting that LXR\(\alpha\) does not transcriptionally reprogram pathways for the enhancement of mitochondrial glucose utilization. In essence, excess glucose uptake and glycolysis appears to be partially uncoupled from mitochondrial oxidation and ATP synthesis in LXR\(\alpha\)-Tg hearts, possibly via a regulatory effect of LXR\(\alpha\) on Pdk4, which negatively regulates pyruvate dehydrogenase complex (PDC) activity. This is in contrast to previous reports showing that, in the protection against cardiac stress, GLUT1 overexpression corrected insufficient glucose utilization and oxidation caused by PPAR\(\alpha\) deficiency in mice (Luptak et al., 2005), and preserved mitochondrial energetic status (Liao et al., 2002). That glucose oxidative capacity is not increased in LXR\(\alpha\)-Tg hearts may be due to the fact that mitochondrial oxidation rates are indeed normal and not compromised, and since myocardial contractility is unimpaired is evidence that ATP supply is sufficient to fuel contraction. Consequently, excess glucose uptake is neither stored nor oxidized, but is instead diverted into other glycolytic functions due to a modulatory effect of Pdk4.

Currently, the role of glucose signaling independent of its energy-providing effects is largely unaddressed in the hypertrophic and failing heart, but has been implicated to play an important role in myocyte function and survival (Kolwicz et al., 2013). Moreover, the fate of glucose is of interest given that increased glucose uptake and glycolysis in cardiac hypertrophy do not always result in concomitant increases in glucose oxidation (Allard et al., 1994; Wambolt et al., 1999; Doenst et al., 2013). Our data indicate that, by enhancing glucose flux, cardiac LXR\(\alpha\) activates an ancillary pathway of glycolysis, the HBP, increasing levels of the posttranslational modifier, O-GlcNAc. Further, we establish that this pathway induces transcription of natriuretic peptides via glycosylation of GATA4 and Mef2c, transcriptional activators of ANP and BNP (Morin et al., 2000). It is interesting that LXR\(\alpha\)-Tg hearts exhibit increased basal ANP and BNP mRNA levels without inducing the complete fetal gene

![Figure 8. O-GlcNACylation is increased with cardiac LXR\(\alpha\) overexpression in mice.](image-url)

A, B Western blot analyses of global protein O-GlcNAc levels in left ventricles of mice with either (A) cardiac-specific LXR\(\alpha\) overexpression or (B) LXR\(\alpha\) deficiency and subjected to 5 weeks TAC.

C, D Nuclear protein extracts were precipitated with agarose WGA in the absence or presence of GlcNAc, a competitor, and analyzed by Western blot with antibodies against Mef2c, GATA4, or Nkx-2.5, known transcription factors of natriuretic peptides; bands represent 3 pooled hearts per Wt and LXR\(\alpha\)-Tg lanes, and (D) quantification is for \(n = 2\), expressed as fold change.

Data information: Data are means ± SEM. IP, immunoprecipitation; WGA, wheat germ agglutinin; GlcNAc, N-acetylglucosamine; WB, Western blot.

Source data are available online for this figure.
response or displaying signs of cardiac dysfunction normally associated with their induction, suggesting that the expression of individual fetal genes is indeed regulated by distinct signal mechanisms. Nevertheless, the cardioprotective effects of natriuretic peptide signaling are well established (Nishikimi et al., 2006), and murine models with ablated natriuretic peptide signaling show increased propensity for cardiac hypertrophy and myocardial fibrosis (Tamura et al., 2000; Holtwick et al., 2003; Wang et al., 2003). Therefore, the anti-hypertrophic and anti-fibrotic potential of local ANP and BNP, glucose transporter; HBP, hexosamine biosynthesis pathway; Mef, enhancer factor; O-GlcNAc, O-N-acetylglucosamine; PDC, pyruvate dehydrogenase complex; Pdk4, pyruvate dehydrogenase kinase 4.

In conclusion, this study demonstrates that LXRα protects from pathological cardiac hypertrophy as an important cardiac transcriptional regulator that further promotes the adaptive capacity for glucose uptake and utilization in cardiac hypertrophy. Furthermore, this study highlights the under-recognized potential for non-energy-dependent pathways of glycolysis such as the HBP in promoting cytoprotection. New generation LXR agonists with less lipogenic profiles are currently being developed, and we postulate that such agonists may be useful modulators of myocyte metabolism in the prevention of pathological cardiac remodeling and heart failure.

Materials and Methods

For more detailed Methods, see the Supplementary Information.

Animal models

The murine NR1H3 gene (GeneBank: NM_013839) was obtained from German Science Centre for Genomic Research (RZPD; clone # IRAVp968B0923D). This PCR product was amplified by polymerase chain reaction (PCR) and cloned into a previously described vector containing the cardiac-specific αMHC promoter (Gulick et al., 1991). Transgenic founders were obtained by pronuclear injection of the αMHC-LXRα construct into FVB oocytes. Transgene identification was performed by a PCR-based method using the following primers: sense 5′-CGGCACCTCTAGAACCTCT-3′, antisense 5′-TGCTGACTCCAAACCTCC-3′. Mice were backcrossed for at least three generations into the C57BL/6 (The Jackson Laboratory) genetic background. αMHC-LXRα (LXRα-Tg) mice were generated by the UMCG mouse clinic in collaboration with the Mayo Clinic (Rochester, NY, USA). For all experiments, non-transgenic littermates (Wt) served as controls.

Homozygous LXRα knockout mice (LXRα−/−; gift from Dr. Gustafsson) (Alberti et al., 2001) and matching C57BL/6BomTac wild-type (WT) mice were obtained from Taconic, Denmark.

Experimental protocol

All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Groningen. Male mice (8–10 weeks) were subjected to an infusion of angiotensin II (Ang II) (1.0 mg/kg/day) for 10 days, or pressure overload by transverse aortic constriction (TAC) for either 1 or 5 weeks. In subsequent studies, a subset of mice underwent sham/TAC for 5 weeks for further assessment of myocardial 2-deoxy-2-[18F]fluoro-D-glucose (FDG)-glucose uptake with microPET, or mitochondrial oxidative phosphorylation measurements. Cardiac function was determined with echocardiography and invasive hemodynamic monitoring, as previously described (Yu et al., 2013). LV tissue samples were used to perform expression studies, immunohistochemical, and biochemical analyses.

In vitro studies

Neonatal rat ventricular myocytes (NRVMs) were isolated from 1- to 3-day-old Sprague Dawley pups, as described (Lu et al., 2012). Recombinant adenovirus containing murine LXRα (Ad-LXRα) or silenced LXRα (si-LXRα) was used to infect NRVMs, and cells were
treated with phenylephrine (PE) or 6-diazo-5-oxonorleucine (DON) (Sigma). Cellular glucose uptake and protein synthesis were assessed with 2-deoxyglucose (2-DG) and [3H]leucine assays, respectively.

Statistical analysis

All data are presented as means ± standard error of the mean (SEM). Student’s paired 2-tailed t-test was used for two group comparisons. One-way ANOVA was performed to analyze differences for multiple-group comparisons, followed by Bonferroni post hoc analysis. Kruskal–Wallis test followed by Mann–Whitney U-test was used to analyze cell experiments. All results were tested at the P < 0.05 level of significance.

Supplementary information for this article is available online: http://embomo.med.embopress.org

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Author contributions

MVC, JJHWS, PvdH, and RADB designed the study. MVC, JWAS, IVB, GJJS, and RADB performed animal experiments, PET scanning, and echocardiography. HHWS, BvdS, jvD, and JAG designed and generated transgenic mice. MVC and JC conducted and analyzed metabolic assays. MVC and HHWS conducted cell experiments; the data were analyzed and interpreted by MVC, HHWS, JWAS, JC, BvdS, and LjdW. WHvG and RADB were involved in funding and supervision. MVC and RADB drafted the manuscript. HHWS, LjdW, PvdH, WHvG, JC, BvdS, GJJS, and jvD were involved in critical evaluation and intellectual contribution to the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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LXR protects from pathological cardiac hypertrophy
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