Liver X receptors in cardiac hypertrophy
Cannon, Megan Valerie

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Chapter 4

The liver X receptor agonist AZ876 protects against pathological cardiac hypertrophy and fibrosis without lipogenic side effects

Megan V. Cannon¹, Hongjuan Yu¹, Wellington M. Candido¹, Martin M. Dokter¹, Eva-Lotte Lindstedt², Herman H.W. Silljé¹, Wiek H. van Gilst¹, Rudolf A. de Boer¹

¹ University Medical Center Groningen, University of Groningen, Department of Cardiology, Groningen, The Netherlands
² AstraZeneca R&D, MöIndal, Sweden

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Chapter 4

ABSTRACT

Background. Liver X receptors (LXR) transcriptionally regulate inflammation, metabolism, and immunity. Synthetic LXR agonists have been evaluated for their efficacy in the cardiovascular system, however, they elicit pro-lipogenic side effects which substantially limit their therapeutic use. AZ876 is a novel high-affinity LXR agonist. Herein, we aimed to determine the cardioprotective potential of LXR activation with AZ876.

Methods and Results. Cardiac hypertrophy was induced in C57BL6/J mice via transverse aortic constriction (TAC) for 6 weeks. During this period, mice received chow supplemented with or without AZ876 (20 µmol/kg/day). In murine hearts, LXRα protein expression was upregulated approximately 7-fold in response to TAC. LXR activation with AZ876 attenuated this increase, and significantly reduced TAC-induced increases in heart weight, myocardial fibrosis, and cardiac dysfunction without affecting blood pressure. At the molecular level, AZ876 suppressed upregulation of hypertrophy- and fibrosis-related genes and further inhibited pro-hypertrophic and pro-fibrotic transforming growth factor β (TGFβ)-Smad2/3 signaling. In isolated cardiac myocytes and fibroblasts, immunocytochemistry confirmed nuclear expression of LXRα in both these cell types. In cardiomyocytes, phenylephrine-stimulated cellular hypertrophy was significantly decreased in AZ876-treated cells. In cardiac fibroblasts, AZ876 prevented TGFβ- and angiotensin II-induced fibroblast collagen synthesis, and inhibited upregulation of the myofibroblastic marker, αSMA. Plasma triglycerides and liver weight were unaltered following AZ876 treatment.

Conclusion. AZ876 activation of LXR protects from adverse cardiac remodeling in pathological pressure overload, independently of blood pressure. LXR may thus represent a putative molecular target for anti-hypertrophic and anti-fibrotic therapies in heart failure prevention.
INTRODUCTION

Cardiac hypertrophy is a major predictor for heart failure, arrhythmia, and sudden death (1). It ensues in response to a plenitude of pathological stimuli such as ischemic injury, elevated hemodynamic load, and abnormal adrenergic and neurohormonal signal transduction (2). Intrinsically, the heart adapts by inducing hypertrophic growth of cardiomyocytes in order to withstand elevated myocardial wall stress. However, when stress is prolonged, these initial compensatory responses yield to maladaptive changes, including increased interstitial myocardial fibrosis and remodeling of the extracellular matrix. As a result, cardiac function progressively deteriorates, and over time, heart failure is inevitable. Thus, cardiac remodeling is a critical determinant of heart failure progression, and its prevention or regression still remains an elusive and salient target of therapy.

Liver X receptors (LXRs) belong to the nuclear receptor superfamily and mediate their functions via two subtypes, LXRα (NR1H3) and LXRβ (NR1H2), which share 77% sequence homology, and are also highly conserved between humans and rodents (3). Whereas LXRβ expression is ubiquitous, LXRα is predominantly expressed in liver, adipose, intestine, and macrophages, but also in heart, kidney, adrenal gland, and lung (4). In the nucleus, LXRs are bound to cognate LXR response elements (LXREs) in regulatory regions of target genes. Upon ligand activation, either physiologically by cholesterol metabolites or synthetically with agonists, LXRs modulate target gene transcription. LXRs are central in the regulation of genes controlling various biological pathways such as cholesterol homeostasis, lipid and glucose metabolism, inflammation, and immunity (5,6).

LXR agonists have been studied for their cardioprotective potential, however, systemic LXR activation elicits undesirable side effects that pose several challenges in discerning their efficacy on the myocardium. Pharmacological activation with T0901317 (T09) attenuated cardiac hypertrophy induced in mice, but this was associated with adverse increases in liver weight and hypertriglyceridemia (7), an effect mediated via hepatic Srebp1c induction (8). LXR agonists have also been implicated in blood pressure regulation, thereby influencing hemodynamic load. Administration of T09 was shown to modulate components of the renin-angiotensin system (RAS) by directly increasing renin expression in vivo (9), as well as reducing renin-, ACE-, and angiotensin II receptor type I (AT1R)-coding genes in kidneys and heart in response to adrenergic stimulation (10). Moreover, the LXR agonist, GW3965, abrogated angiotensin II (Ang II)-mediated increases in blood pressure in rats, coincident with reduced vasculature ATR expression (11).

Currently, there is an increased interest in developing new LXR modulators designed to overcome these deleterious side effects that limit their therapeutic use (12). AZ876 is a dual partial LXR agonist that has been shown to reduce atherosclerosis in mice without affecting liver or plasma triglyceride levels when administered in low dose (13). Compared
to GW3965, AZ876 proved to be a more potent binder and activator of both human and murine LXRα and LXRβ (13). The purpose of the present study was to therefore investigate the cardioprotective potential of AZ876 in a murine model of pathological cardiac hypertrophy. Our aim was to determine effects of LXR activation with AZ876 on LXR target gene expression and pathological cardiac remodeling.

METHODS

Detailed description of methods is provided in the Data Supplement.

Experimental protocol
Cardiac hypertrophy was induced in male C57BL6/J mice via transverse aortic constriction (TAC) for 6 weeks. During this period, sham- and TAC-operated mice were randomized to receive either regular chow (control), or chow supplemented with AZ876 (20 µmol/kg/day). Cardiac function was assessed with echocardiography and invasive hemodynamics. In vitro studies were performed in isolated neonatal rat ventricular myocytes (NRVMs) and adult rat cardiac fibroblasts. Leucine and proline tracer assays were used to measure protein and collagen synthesis, respectively.

Statistics
Data are presented as means ± standard error of the mean (SEM). Statistical analysis was performed with IBM SPSS Statistics 22 software (Chicago, IL, USA). Student’s paired 2-tailed t-test was used for two-group comparisons, and one-way ANOVA for multi-group comparisons, followed by Bonferroni post hoc correction. Statistically significant differences were considered if P<0.05.

RESULTS

AZ876 agonism induces LXR target gene expression in murine hearts
Dose-findings studies were performed with administration of AZ876 in dosages of 5, 10, and 20 µmol/kg/day over a 14-day period to test tissue-specific induction of gene expression for Lxrα and Lxrβ, as well as previously described LXR target genes, Srebp1c and the ABC transporter-encoding genes, Abca1 and Abcg1 (14). In the left ventricle (LV), the lowest dose of 5 µmol induced a marginal increase of 1.6-fold in Lxrα and Abcg1 mRNA levels, whereas the higher doses of 10 and 20 µmol caused more marked increases for Abca1 (1.6- and 1.8-fold, respectively), Abcg1 (2.4- and 2.3-fold, respectively), and Lxrβ (1.7- and 2.0-fold, respectively) (Suppl. Fig. 1A). In the liver, we observed no effect with 5 and 10 µmol, but 20 µmol had an appreciable effect for Lxrβ and Abcg1 (both 1.4-fold, respectively) (Suppl. Fig. 1B). Interestingly, Srebp1c appeared downregulated by AZ876 treatment. Administration of AZ876 over the 14-day period did not affect body weight (Suppl. Fig. 1C), nor liver weight.
AZ876 treatment attenuated the development of pathological cardiac hypertrophy

The experimental protocol for AZ876 prevention study is depicted in Figure 1A. This study employed a model of compensated cardiac hypertrophy with the aim of evaluating the preventative effect of AZ876 on hypertrophic remodeling, as opposed to disease regression where treatment is initiated after development of hypertrophic growth (reversal study). In both treated and untreated mice, TAC caused a significant gain in heart weight compared to sham-operated mice, however, this was significantly reduced with AZ876 treatment, 68% for control mice versus 45% for AZ876-treated mice (Figure 1B, Suppl. Table I). Cardiomyocyte hypertrophy was also decreased by AZ876, albeit not significantly different from TAC control mice (Suppl. Fig. 2). At the molecular level, we observed greater induction of hypertrophic-related genes in control mice compared to AZ876-treated mice following TAC (Figure 1C). Expression levels of the natriuretic peptides, Anp and Bnp, were significantly higher in TAC control mice than in TAC-AZ876 mice. Contractile proteins such as βMhc and Acta1, as well as Rcan1, central in calcium handling, were less expressed with AZ876 treatment. To assess whether cardiac LXR expression is modulated in response to hypertrophy, Western blot was performed and analysis revealed substantial upregulation of LXRα protein abundance (6.7-fold), but not in AZ876-treated hearts. We observed a moderate 3.3-fold increase in LXRβ following TAC (Figure 1D and 1E). In summary, upregulation of the LXRα isoform is more prominent in the adaptation to cardiac hypertrophy, and targeted LXR activation with AZ876 counteracts the hypertrophic response to pressure overload.

Pressure overload-induced cardiac dysfunction is mitigated by AZ876

Echocardiography was used to assess cardiac function in vivo. TAC caused a marked decline in percent fractional shortening, but this was attenuated by AZ876, -11% versus -7% (Figure 1F). Assessment of intracardiac pressures with in situ catheterization revealed that LV end-diastolic pressure was more significantly elevated in untreated hearts subjected to TAC, 146% versus 55% (Suppl. Table I), indicating a role for LXR in preserving LV compliance. These improvements in functional outcome occurred irrespective of differences in heart rate (Suppl. Table I), or mean arterial pressure (MAP) which, evidently, was elevated in both TAC groups (Figure 1G).

AZ876 agonism suppresses myocardial fibrosis in hypertrophic murine hearts

Cardiac pressure overload led to excessive development of myocardial fibrosis which was diminished in AZ876-treated hearts. Representative histological sections stained with Massons trichrome for the detection of collagen infiltration are shown (Figure 2A). When quantified, TAC markedly increased fibrosis by 4.5-fold in control mice, but AZ876 treatment reduced this to 2.8-fold (Figure 2B). Genes implicated in fibrogenesis were also assessed...
LXR agonist AZ876 attenuates cardiac hypertrophy and fibrosis

Figure 1
Cardiac hypertrophy and dysfunction is attenuated in AZ876-treated mice following 6 weeks transverse aortic constriction (TAC). (A) Experimental outline for AZ876 prevention study performed in mice. (B) Heart weight (HW/BW) ratios in sham- and TAC-operated mice treated with or without 20 µmol AZ876/kg/day; n=7-10/group. (C) Measurement of hypertrophic-associated gene expression for natriuretic peptides, Anp and Bnp, contractile proteins, βMhc and Acta1, and Rcan1 in the LV, values are normalized to 36b4, expressed as fold change; n=7-8/group. (D-E) Western blot analysis of liver X receptor (LXR) α and β protein expression in the left ventricle (LV) of mice subjected to TAC, glyceraldehyde phosphate dehydrogenase (GAPDH), expressed as fold change; n=4-6/group. (F) Echocardiographic assessment of percent fractional shortening; n=5-8/group. (G) Mean arterial pressure (MAP) measured with in situ catheterization; n=4-8/group. Data presented are means ± SEM; *P<0.05, **P<0.01, ***P<0.001 versus respective sham, #P<0.05, ##P<0.01 versus AZ876 treatment.

with RT-PCR (Figure 2C-J, Suppl. Table II). Pro-fibrotic Tgfβ and downstream mediators such as the matricellular proteins, Ctgf and fibronectin, were significantly increased by TAC, but to a lesser extent in AZ876-treated hearts (Figure 2C-E). The follistatin-like 3 (Fstl3) gene, associated with TGFβ signaling, was also decreased by AZ876 treatment (Figure 2F). AZ876
activation of LXR resulted in suppression of extracellular matrix constituents such as the fibrillar collagens, Col1a1 and Col3a1 (Suppl. Table II, Figure 2G), as well as those encoding several regulatory proteins involved in ECM turnover, Timp1, Mmp2, and Mmp14 (Figure 2H-J).

We further investigated TGFβ signaling pathways as a possible mechanism of cardiac remodeling since it is an instigator of both pathological hypertrophy and tissue fibrosis. Compared to AZ876-treated mice, TGFβ protein expression was significantly induced in the untreated group (Figure 3A and 3B) with subsequent elevations in downstream phosphorylated-Smad2/Smad3 levels (Figure 3A and 3C), which are direct TGFβ signaling effectors of target gene transcription. We also assessed non-canonical TGFβ pathways such as p38 and downstream phosphorylated Akt signaling as well as phosphorylated Erk, but did not observe an effect of AZ876 on these pathways (Suppl. Fig. 3). Therefore, AZ876 attenuation of TGFβ signaling in association with suppressed myocardial collagen

Figure 2
AZ876 treatment blunts excessive development of TAC-induced myocardial fibrosis in mice. (A) Representative Massons trichrome-stained LV sections for the detection of collagen (bar = 100 µm), and (B) quantification of whole area; n=6-10/group. (C-J) Relative mRNA expression of LV genes involved in fibrosis pathways, normalized to 36b4 and expressed as fold change; n=7-8/group. Data presented are means ± SEM; **P<0.01, ***P<0.001 versus respective sham, *P<0.05, #P<0.01 versus AZ876 treatment.
AZ876 activation of LXRα in cardiomyocytes decreases cellular hypertrophy

Based on the premise that LXRs exert beneficial effects on the heart, it is presumable that LXRs are expressed in cardiac cell types and exert cell-specific functions, however, this remains unclear. Having established cardioprotective effects for AZ876 physiologically in vivo, we next investigated LXR function in a cell-specific manner. Immunofluorescence microscopy identified LXRα expression in isolated NRVMs (Figure 4A). RT-PCR was also used to confirm the expression levels of both LXRα and LXRβ in NRVMs (Suppl. Fig. 4). When NRVMs were stimulated with phenylephrine (PE) to promote cellular hypertrophy, cell size (Figure 4B) as well as protein synthesis (Figure 4C) increased 1.4-fold and 1.7-fold, respectively, but this was attenuated in AZ876-treated cells. Knockdown of LXRα in NRVMs resulted in a significant induction of hypertrophy both in the absence and presence of PE (1.4-fold and 2.3-fold, respectively), which was relatively unaltered by AZ876 treatment (1.2- and 2.0-fold, respectively), suggesting that the antagonizing effect of LXRs on hypertrophy is primarily mediated via the LXRα isoform (Figure 4C). Genes responsive to hypertrophic perturbation were evidently less upregulated in AZ876-treated cells (Figure 4D-G).
LXR agonist AZ876 attenuates cardiac hypertrophy and fibrosis

Cardiac fibroblasts are direct targets of LXR agonism in preventing fibrogenesis

We next determined LXR expression in cardiac fibroblasts as well as their functional response to pro-fibrotic stimulation. Immunocytochemistry revealed the nuclear expression of LXRα in fibroblasts isolated from the adult rat heart (Figure 5A), which was also confirmed by RT-PCR for both LXRα and LXRβ (Suppl. Fig. 5). Moreover, these cells proved to be targets for LXR activation since AZ876 abolished the 1.5-fold increase in collagen synthesis caused by both TGFβ and Ang II (Figure 5B). TGFβ is a major promoter of myofibroblast differentiation, evidenced by induction of α-smooth muscle actin (αSMA). Upregulated αSMA mRNA alongside matricellular proteins, Ctgf and periostin, were substantially diminished with increasing doses of AZ876 (Figure 5C-E). Taken together, these data indicate that cardiomyocytes and cardiac fibroblasts are direct targets of LXR agonism, and the LXR
LXR agonist AZ876 attenuates cardiac hypertrophy and fibrosis.

AZ876 does not adversely affect lipid profile

The lipogenic profile of AZ876 treatment was investigated for a systemic interaction with and without hypertrophic stress. First-generation LXR agonists are culpable in inducing hypertriglyceridemia and liver steatosis. Consistent with the dose-findings studies (Suppl. Fig. 1D), chronic administration of AZ876 did not cause increased liver weight (Figure 6A), nor were plasma triglycerides elevated, although levels tended to be lower in the TAC-AZ876 group (Figure 6B). Pooled plasma samples fractionated by fast protein liquid chromatography (FPLC) for lipoprotein lipid analysis did not reveal major discrepancies among cholesterol distribution with drug treatment (Figure 6C, Suppl. Fig. 6). Lastly, AZ876 analyte concentrations were measured in plasma to confirm the bioavailability of AZ876 in

Figure 5
AZ876 treatment antagonizes pro-fibrotic stimuli in cardiac fibroblasts. (A) Immunofluorescence was used to identify LXRα expression in adult rat cardiac fibroblasts in the absence (top panels) or presence (bottom panels) of AZ876 treatment. LXRα (green), 4',6-diamidino-2-phenylindole (DAPI) for nuclei (blue), and rhodamine-phalloidin for F-actin (red); bars = 10 µm. (B) Relative proline incorporation to assess effect of 10 nM AZ876 on collagen synthesis in presence of pro-fibrotic stimuli, TGFβ and angiotensin II (Ang II); n=3. (C-E) Effect of 10 and 15 nM AZ876 on mRNA levels of fibrosis-related gene markers, (C) α-smooth muscle actin (αSMA) for myofibroblastic differentiation, and matricellular proteins (D) Ctgf and (E) peristin; values presented as fold change; n=6-7. Data presented are means ± SEM; *P<0.05, **P<0.01, ***P<0.001.
treated groups (Figure 6D). In conclusion, chronic administration of AZ876 did not adversely affect lipid homeostasis in mice.

Figure 6
Chronic AZ876 administration does not adversely affect lipogenic profile in mice. (A) Liver weight ratios, normalized to body weight (BW). (B) Fasted plasma triglyceride levels. (C) FPLC profiles of pooled plasma samples (n=7-10) for the distribution of total cholesterol. (D) Plasma AZ876 analyte levels. Data presented are means ± SEM, n=7-10/group.
DISCUSSION

This study demonstrates that chronic administration of the LXR agonist, AZ876, attenuates pathological cardiac hypertrophy in a murine model of chronic pressure overload without altering systemic blood pressure, implicating heart-specific effects. AZ876 treatment diminished myocardial fibrosis and suppressed induction of pro-fibrotic gene expression. At the cellular level, both cardiac myocytes and fibroblasts, the two major cell types in the heart, expressed LXRs, and furthermore, these cells were direct targets in AZ876-mediated cellular protection from hypertrophic and fibrotic stimuli. Overall, the salutary effects of AZ876 on cardiac remodeling were associated with a trend toward an improved functional outcome, which, importantly, occurred in the absence of adverse lipogenic side effects typical of current LXR agonists such as T09 and GW3965.

The LXR agonist, T09, has been evaluated for its cardioprotective potential, but it remains unclear whether these effects are heart-specific. T09 attenuated LV hypertrophy in aortic-constricted mice, but this occurred in concert with reductions in MAP, which was elevated as a consequence of the hypertrophic perturbation (7). Moreover, both T09 and GW3965 have been implicated in blood pressure control via modulation of the renin-angiotensin system (9-11), and therefore the reduction in hypertrophy by pharmacological LXR activation with T09 may be a consequence of alleviated hemodynamic load. In the current study, we show that MAP was unaffected by AZ876 treatment while cardiac hypertrophy was reduced, indicating a direct mode of action for AZ876 on the pressure overloaded myocardium. The discrepancy between these current agonists and AZ876 on blood pressure regulation remains to be clarified, yet we speculate that AZ876 may be a more selective agonist with respect to other nuclear receptors (13). Nevertheless, this study underscores the myocardial-specific effects of synthetic LXR activation in protection from pathological hypertrophy.

Identification of the isoform via which LXRs exert their cardiac effects is an important consideration given the increased effort devoted to designing ligands that emphasize isoform and target tissue specificity (12). In the heart, evidence suggests that the cardioprotective effects are conferred by LXRα since the hypertrophic attenuation afforded by T09 treatment was not rescued by the LXRβ isofom in LXRα knockout (KO) mice, despite a compensatory increase in LXRβ mRNA expression following aortic constriction (7). Similarly, Wu et al found hypertrophy and ANP expression to be further exacerbated in LXRα KO mice compared to wild-type (15), while assessment of LXR protein in the current study showed substantial increases for LXRα over LXRβ. Using cultured NRVMs, we demonstrate that AZ876 antagonized cardiomyocyte hypertrophy primarily in an LXRα-dependent manner since knockdown of LXRα resulted in more robust PE-induced cellular growth that was not completely normalized by AZ876. Collectively, these data suggest that LXRα is the more responsive isoform in myocardial hypertrophy.
To our knowledge, this is the first study to demonstrate a role for LXRs in cardiac fibrosis. AZ876-treated hearts subjected to TAC manifested reduced interstitial fibrosis and pro-fibrotic molecular markers in association with less elevated LV end-diastolic pressure. We found TGFβ-Smad2/3 signaling to be activated in TAC hearts, but to a lesser extent in mice treated with AZ876. TGFβ is critically involved in the pathogenesis of both hypertrophic and fibrotic remodeling by stimulating hypertrophic growth of cardiomyocytes, proliferation of cardiac fibroblasts, including their transition to myofibroblasts, as well as the deposition of extracellular matrix proteins (16). A cross-talk between LXRs and TGFβ signaling via Smad2/3 interaction has been proposed (17), and T09 activation of LXRs has been shown to decrease Tgfβ1 in other diseased organs susceptible to fibrotic remodeling, including diabetic nephropathy (18) and chronic asthma-induced airway remodeling (19).

Since AZ876 agonism imparted dual effects on myocardial hypertrophy and fibrosis, mechanistically, it is plausible that paracrine signaling from cardiomyocytes responding to hypertrophic stimuli influences fibroblast activation (20). In support of this notion, we found marked attenuation of fibrosis in transgenic mice with cardiomyocyte-specific LXRα overexpression subjected to TAC and Ang II stimulation (Cannon et al. unpublished data). In this study, AZ876 modulated several genes in vivo such as Tgfβ, Ctgf, and Fstl3, which are known to be secreted by myocytes and cause paracrine activation of adjacent fibroblasts (16,21,22). It is difficult to dissect in vivo whether, LXRs directly target cardiac fibroblasts and affect fibrogenesis. To address this, we isolated cardiac fibroblasts and herewith demonstrated that LXRs are indeed expressed in this cell type, and moreover, AZ876 activation prevented TGFβ- and Ang II-induced collagen synthesis and myofibroblast differentiation. So although TGFβ-Smad signaling may be an autocrine/paracrine mechanism by which LXRs exert their anti-fibrotic, and anti-hypertrophic, effects in the heart, we nevertheless have elucidated cardiac fibroblasts to be an independent target of LXR agonism in reducing fibrotic remodeling. Furthermore, we identified putative LXREs in Ctgf and Fstl3 promoter regions by in silico bioinformatic analysis, suggesting that LXR-induced repression of cardiac fibrotic genes through a negative LXRE may be a direct mechanism of LXR activation.

In conclusion, our data implicate a role for LXRα in the adaptive response to chronic pressure overload, and subsequent synthetic activation of LXR with AZ876 protects from pathological cardiac remodeling, independent of blood pressure or lipogenic systemic effects. Herein, we establish a novel function for LXR in countervailing myocardial fibrosis, and the potential mechanism may involve attenuated TGFβ-Smad2/3 signaling. LXR therefore represents a putative molecular target for anti-hypertrophic and anti-fibrotic therapies, and AZ876 should be further explored for its cardioprotective potential.

**Perspectives**
LXRs have emerged as important therapeutic targets in cardiovascular disease given their anti-inflammatory functions as well as their atheroprotective effects in activating the reverse
cholesterol transport system. Since hypertriglyceridemia is a risk factor for heart failure (23), current LXR agonists are therefore not suitable in cardiology practice due to the increased levels of circulating triglycerides they elicit via hepatic lipogenesis. In addition, studies are hindered due to lack of an LXR-specific agonist. AZ876 is a newly developed high-affinity LXR agonist that is more selective for LXR with respect to other nuclear receptors (13) and consequently lacks the adverse lipogenic profile typical of current LXR agonists. Thus, it allows for more specific evaluation of LXR, which herein was used to assess the efficacy of LXR agonism on adverse cardiac remodeling processes in a murine model of chronic pressure overload. LXR activation with AZ876 antagonized the development of pathological cardiac hypertrophy and dysfunction. Moreover, activation of LXR reduced myocardial fibrosis, which has important implications considering fibrosis is a central pathological mechanism underlying heart disease of various etiologies, as well as other diseased organ systems associated with fibrosis. Therefore, LXR agonists with greater binding potency and selectivity for LXRs represent promising therapeutic agents for intervening in cardiac pathophysiology.

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CONFLICT OF INTEREST

E.L.L. is an employee of AstraZeneca, R&D, Mölndal, Sweden.
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SUPPLEMENTAL METHODS

Animals and AZ876 treatment
The investigation was conducted in accordance with the US National Institutes of Health, the eighth edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011), and the AstraZeneca Bioethics Policy. All experimental protocols were approved by the Animal Care and Use Committee of the University of Groningen, Groningen, The Netherlands, and by AstraZeneca, Sweden. Male C57BL6/J mice, approximately 8 weeks of age, were obtained from Harlan, The Netherlands. The animals were housed at the central animal facility in Groningen, and maintained in a temperature-controlled environment with alternating 12hr:12hr light:dark cycles. Following a two week acclimation period, studies with AZ876 (provided by AstraZeneca, Mölndal, Sweden) were initiated. Mice were randomized to receive either regular chow (control group), or chow supplemented with three doses of AZ876: 5 µmol/kg/day (2.2 µg/g/day), 10 µmol/kg/day (4.4 µg/g/day), or 20 µmol/kg/day (8.8 µg/g/day). Access to water and chow was provided ad libitum. After 14 days, mice were sacrificed, blood was sampled for plasma lipid analyses, and the hearts and livers were harvested, weighed, and frozen in liquid nitrogen in preparation for RNA analysis. Results of these studies indicated that 20 µmol AZ876/kg/day induced LXR target gene expression most effectively without affecting liver weight or plasma lipid profile (Suppl. Fig. 1), and was therefore selected for subsequent experiments.

Pressure overload-induced cardiac hypertrophy
Transverse aortic constriction (TAC) is a well established procedure for inducing cardiac hypertrophy via chronic pressure overload (1). At day 0, mice underwent either sham or TAC surgery, and randomly assigned to receive either regular chow (control) or chow supplemented with 20 µmol AZ876/kg/day for a period of six weeks. In brief, mice were anesthetized with 2% isoflurane, intubated, and placed supine on a heated pad for mechanical ventilation. The skin was incised and tissue between the second intercostal space was dissected to locate the aortic arch. Next, a 7-0 silk suture was tied around a blunt 27-gauge needle placed between the brachiocephalic and left carotid arteries, creating a predetermined stenosis. The needle was immediately removed following ligation and the incised skin closed. For sham surgeries, the aortic arch was not ligated. Carprofen (5.0 mg/kg) was administered subcutaneously, perioperatively, to relieve wound pain.

Cardiac function assessment
Echocardiography was performed to assess functional parameters in vivo at six weeks post TAC, as described previously (2). Briefly, M-mode and 2D transthoracic measurements were obtained under anesthesia (Vivid 7, 14-MHz linear array transducer; GE Healthcare, Chalfont St. Giles, UK). Mice were maintained on a heated pad, and a topical depilation agent was used to remove chest hair. From the parasternal short axis view, M-mode tracings were recorded to measure left ventricular (LV) dimensions and calculate percent fractional
shortening.

Hemodynamic parameters were assessed following in situ catheterization of the aorta and LV with a micromanometer-tipped pressure catheter (1.4F; Millar Instruments, Houston, TX, USA). In brief, the right carotid artery was punctured, followed by catheter insertion and 3 min were allotted for stabilization. Arterial pressures were recorded, followed by advancement of the catheter into the LV to record intracardiac pressures. Heart rate (HR), systolic and diastolic arterial and intracardiac pressures, and maximal and minimal rates of pressure change for contractility (dP/dt\textsubscript{max} and dP/dt\textsubscript{min}) were measured. Following removal of the catheter, blood was sampled via heart puncture. Hearts were flushed with 10 ml PBS to remove red blood cells, then excised, portioned into atria and ventricles, and weighed. The LV was further portioned for immunohistochemistry or frozen in liquid nitrogen for further expressional and biochemical analyses.

**RNA isolation and gene expression analysis**

Total RNA was isolated from LV and liver tissue with TRIzol reagent protocol (Invitrogen, Carlsbad, CA, USA). RNA extraction from cells was performed with Nucleospin RNA II kit (Macherey-Nagel, Duren, Germany). From total RNA, cDNA was synthesized using RNeasy Mini Kit (Qiagen Inc, Valencia, CA, USA). Quantitative real-time PCR of cDNAs were performed on a C1000 Thermal Cycler CFX384 Real-Time PCR Detection system (Bio-Rad Laboratories, Veenendaal, The Netherlands). Quantified mRNA levels were normalized to the invariant transcript, 36b4.

**Western blot analysis**

Protein lysates were prepared from frozen LV tissue homogenized in ice-cold lysis buffer (50 mM Tris pH 8.0, 1% NP40, 0.5% deoxycholate, 0.1% SDS, 150 mM NaCl, 1 mM PMSF, 15 mM Na Vanadate) supplemented with protease and phosphatase inhibitor cocktails (Sigma). Protein concentrations were measured using Bio-Rad DC Protein Assay (Bio-Rad). Equal amounts of protein (25 µg) were resolved on SDS-PAGE gels, and separated proteins were transferred onto nitrocellulose membranes (Bio-Rad). Immunoblotting was performed using primary and secondary antibodies from the following commercial suppliers: anti-human LXRα (2ZPPZ0412H, R&D Systems, Perseus Proteomics); anti-LXRβ (ab28479, Abcam); TGF-β (#3711), Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (#8828), p38 MAPK (#9212), Phospho-Akt (Ser473) (#4060), Akt (#4691), p44/42 MAPK (Erk1/2) (#4695) (Cell Signaling); P-Erk (# sc-7383) (Santa Cruz); glyceraldehyde-3-phosphate dehydrogenase (10R-G109A, Fitzgerald, USA); rabbit anti-mouse immunoglobulins/HRP (P0260, Dako, Denmark); goat anti-rabbit immunoglobulins/HRP (P0448, Dako, Denmark). Signals were detected by ECL (PerkinElmer, Waltham, MA, USA), and densitometry was quantified with ImageQuant LAS 4000 (GE Healthcare Europe GmbH, Diegem, Belgium). Fold changes are calculated and shown.
Histological assessment of fibrosis and cardiomyocyte size
Mid-transverse sections of the LV were post-fixed in 4% paraformaldehyde for paraffin embedding. Sections of 4 µm were stained with either Massons trichrome for collagen detection, or FITC-labeled wheat germ agglutinin (WGA) to quantify myocyte cross-sectional area. Whole stained sections were scanned using Nanozoomer 2.0-HT (Hamamatsu, Japan). Fibrosis was quantified as a percentage of entire section from 20X magnification (ScanScope, Aperio Technologies, Vista, CA, USA). For cardiomyocyte size quantification, five randomly selected fields from whole-stained WGA-FITC LV sections imaged at 20X magnification were used to measure cross-sectional diameter from approximately 30 cells per mouse heart (Image J, NIH, Bethesda, MD, USA), and calculated as area.

Lipid analyses and AZ876 levels
Preceding sacrifice, mice were fasted for four hours and blood was sampled via heart puncture and collected in EDTA-coated tubes. Plasma was isolated and stored at -80°C. Commercial reagents were purchased to measure triglycerides (Roche Diagnostics, Mannheim, Germany) and non-esterified fatty acids (NEFA) (DiaSys, Holzheim, Germany). For tissue triglycerides, lipids were extracted according to Bligh and Dyer methods (3), and triglycerides were measured with a commercial kit (Roche Diagnostics, Mannheim, Germany).

Pooled plasma samples were subjected to fast protein liquid chromatography (FPLC) gel filtration using a Superose 6 column (GE Healthcare, Little Chalfont, UK). Plasma was obtained from each mice per group: n=4/group for dose-findings study and n=7-10/group for TAC study. Samples were chromatographed at a flow rate of 0.5 ml/min, and lipoprotein fractions of 500 µl each were collected and individually assayed for cholesterol concentrations (Roche/Hitachi, Mannheim, Germany). Components of plasma cholesterol were estimated from fractions forming the first, second, and third peaks and were considered to be VLDL, LDL, and HDL, respectively.

AZ876 concentrations were measured in plasma. Twenty µL blood plasma samples were precipitated with 150 µL acetonitrile containing 0.2% formic acid and internal standard, and then vortex mixed for 5 min followed by centrifugation (20 min , 4000 rpm at 4°C). The supernatants were diluted 1:1 with 0.2% formic acid in 33% acetonitrile. Five µL of the dilution was injected on a reversed phase chromatographic column (Waters Atlantis T3, 3µm, 2.1x30mm) and eluted using an acetonitrile gradient with 0.2% formic acid. Detection of AZ876 was made by a Quattro Premier tripple quadropole mass spectrometer (Waters, Manchester, UK).

Neonatal rat ventricular myocytes and adenoviral transfection
Neonatal rat ventricular myocytes (NRVMs) were isolated from Sprague-Dawley pups (aged 1-3 days), as previously described (4). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal calf serum (FCS) and penicillin-streptomycin
LXR agonist AZ876 attenuates cardiac hypertrophy and fibrosis

Chapter 4

(100 µg/ml), and incubated in 5% CO₂ at 37°C (Invitrogen, Breda, The Netherlands). Cells treatments were performed in FCS-free DMEM for 24 hours with 50 µM phenylephrine (PE) and 10 nM AZ876 in DMSO where indicated.

Adenoviral constructs were generated with the ViraPower™ adenoviral expression system (Invitrogen) as previously described (5). siRNA oligonucleotide sequences used against LXRα were: forward GATCCCGGAGTGTCGCCTTCGCAAATTCAAGAGATTTGCGAAGGCGACACTCCTTTTTGGAAA, and reverse AGCTTTTCCAAAAAGGAGTGTCGCCTTCGCAAATCTCTTTGAATTGCGAAGGCGACACTCCGG. A GFP-expressing adenovirus (Ad-cont) was used as control. Recombinant adenovirus was produced by transfecting adenoviral constructs into HEK 293A cells using Lipofectamine 2000 (Invitrogen). Transfections of NRVMs occurred in FCS-free DMEM medium for 24 hours prior to initiation of treatments as described above.

**Adult rat cardiac fibroblasts**

Ventricles from adult rats were excised and placed in ice-cold Krebs Henseleit (KH) buffer (117.5 mM NaCl, 5.6 mM KCl, 1.18 mM MgSO₄, 1.28 mM NaH₂PO₄, 2.52 mM CaCl₂, 25 mM NaHCO₃, 11.1 mM glucose). Ventricles were minced and digested with Liberase TM (Roche Diagnostics, Mannheim, Germany) in KH buffer supplemented with 10 mM HEPES and DNase for 10 min with constant stirring. Supernatant from the first digestion was discarded. Thereafter, cells from subsequent digestions were pooled, filtered, and centrifuged. The pellet was resuspended in DMEM medium supplemented with 10% FCS and penicillin-streptomycin (50 IU/ml and 50 mg/ml, respectively) (Invitrogen, Breda, The Netherlands), and incubated in 5% CO₂ at 37°C. For experiments, cells from passage two were used and cultured for 24 hours in serum-free DMEM before 24 hour treatment with the following: 10 nM or 15 nM AZ876, 10 ng/ml TGFβ or 1 µM Ang II.

**Leucine and proline tracer assays**

Leucine incorporation assay was performed in NRVMs to assess cellular growth and hypertrophy. Proline incorporation assay was performed in isolated cardiac fibroblasts to assess collagen synthesis. In brief, serum-starved cells were cultured in 12-well plates for 24 hours. Following above-indicated transfections and treatments, L-[4,5-³H] Leucine (GE Healthcare Europe, Diemen, Belgium), or L-[2,3,4,5-³H]Proline (Perkin Elmer, Groningen, The Netherlands) was added and cells were cultured for additional 24 hours. Cells were then washed twice with PBS, followed by incubation for 1 hour at 4°C in 1 ml cold 5% trichloroacetic acid (TCA). Following wash steps with TCA then PBS, proteins were solubilized with 0.5 M NaOH for 1 hour at room temperature. Proteins were transferred to scintillation tubes containing Ultima Gold XR scintillation liquid (Perkin Elmer, Groningen, The Netherlands). The amount of radioactivity (CPM) was determined by a LS6500 Beckman Coulter scintillation counter.
Immunofluorescence staining

Cells were cultured in 12-well plates on 18 mm coverslips. For NRVMs, coverslips were laminin-coated. Cells were fixed for 10 min in 4% paraformaldehyde followed by permeabilization with ice-cold 0.3% Triton X100 for 5 min. Cells were blocked for 1 hour with a 3% bovine serum albumin (BSA) 0.1% PBS/Tween solution containing 2% goat serum, then subsequently incubated for 1 hour with a monoclonal anti-human LXRα antibody (2ZPPZ0412H, R&D Systems, Perseus Proteomics). After washing, cells were further incubated with goat anti-mouse IgG-FITC secondary antibody (sc-2010, Santa Cruz Biotechnology Inc., Heidelberg, Germany) and fluorescent phalloidin-rhodamine (Invitrogen, Breda, The Netherlands) for detection of F-actin. Coverslips were mounted using Vectashield mounting medium with 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA), and imaged with a confocal microscope (Leica Microsystems, Wetzlar, Germany). Cell size was determined from 8-10 randomly selected fields imaged at 20X magnification from which 5-8 cells per field were used to measure area (Image J, NIH, Bethesda, MD, USA).

Statistics

All data are presented as means ± standard error of the mean (SEM). Comparisons made among groups were tested by one-way ANOVA, followed by Bonferroni post hoc analysis to assess statistical significance. Two-group comparisons were determined with student’s paired 2-tailed t-test. Statistically significant differences were considered if P<0.05. Statistical analysis was performed using IBM SPSS Statistics 22 (Chicago, IL, USA).
SUPPLEMENTAL REFERENCES


LXR agonist AZ876 attenuates cardiac hypertrophy and fibrosis

Chapter 4

Supplemental Figure 1
Dose-dependent effects of AZ876 on gene expression in murine hearts and liver. (A-B) Relative mRNA levels to assess induction of LXR expression as well as known target genes Srebp1c, Abca1, Abcg1 in (A) left ventricle (LV), and (B) liver; expressed as fold change. (C) Body weight (BW), and (D) liver weight assessment post 14 days AZ876 treatment. Data presented are means ± SEM; n=4/group; *P<0.05 versus con.

Supplemental Figure 2
Effect of AZ876 on cardiomyocyte hypertrophy in vivo following 6 weeks pressure overload. (A) Quantification of myocyte cross-sectional area from WGA-FITC-stained histological sections; n=7-8/group. (B) Representative left ventricular WGA-FITC sections; bar = 10 µm. Data presented are means ± SEM; ***P<0.001 versus respective sham.
### Supplemental Table I. Baseline Characteristics and Hemodynamic Data of Mice following Six Weeks of Transverse Aortic Constriction

<table>
<thead>
<tr>
<th></th>
<th>sham control (n=10)</th>
<th>sham AZ876 (n=8)</th>
<th>TAC control (n=8)</th>
<th>TAC AZ876 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organ weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>29.4 ± 0.6</td>
<td>29.1 ± 1.0</td>
<td>29.0 ± 0.6</td>
<td>29.2 ± 0.6</td>
</tr>
<tr>
<td>Heart weight/BW, mg/g</td>
<td>5.8 ± 0.1</td>
<td>5.9 ± 0.2</td>
<td>9.8 ± 0.4†</td>
<td>8.4 ± 0.5†</td>
</tr>
<tr>
<td>Liver weight/BW, mg/g</td>
<td>54.6 ± 1.4</td>
<td>55.2 ± 0.8</td>
<td>52.7 ± 1.5</td>
<td>52.9 ± 1.5</td>
</tr>
<tr>
<td>Kidney weight/BW, mg/g</td>
<td>13.8 ± 0.2</td>
<td>13.2 ± 0.2</td>
<td>13.1 ± 0.4</td>
<td>12.4 ± 0.3</td>
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<tr>
<td><strong>Hemodynamics</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>427 ± 18</td>
<td>399 ± 13</td>
<td>476 ± 21</td>
<td>460 ± 14</td>
</tr>
<tr>
<td>Systolic arterial pressure, mmHg</td>
<td>97.9 ± 1.7</td>
<td>96.0 ± 3.5</td>
<td>148.4 ± 7.9†</td>
<td>155.6 ± 6.3†</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mmHg</td>
<td>64.2 ± 2.3</td>
<td>61.1 ± 3.1</td>
<td>62.4 ± 3.9</td>
<td>71.4 ± 3.7</td>
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<td>LV end-systolic pressure, mmHg</td>
<td>101.1 ± 1.8</td>
<td>102.0 ± 4.2</td>
<td>144.7 ± 14.1&quot;</td>
<td>146.2 ± 7.1†</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>8.6 ± 1.4</td>
<td>11.6 ± 1.9</td>
<td>21.2 ± 4.5*</td>
<td>18.0 ± 3.7</td>
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<tr>
<td>dP/dt max, mmHg</td>
<td>7449 ± 337</td>
<td>7046 ± 358</td>
<td>7485 ± 952</td>
<td>6925 ± 576</td>
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<td>dP/dt min, mmHg</td>
<td>-7448 ± 418</td>
<td>-6726 ± 295</td>
<td>-6154 ± 1152</td>
<td>-7265 ± 870</td>
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<tr>
<td>Tau, τ, msec</td>
<td>6.4 ± 0.5</td>
<td>6.2 ± 0.7</td>
<td>8.5 ± 0.7</td>
<td>8.5 ± 0.9</td>
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</table>

Data presented are means ± SEM. * P<0.05, † P<0.01, TAC versus corresponding sham group; ‡ P<0.05, § P<0.01, control versus AZ876 treatment.
Supplemental Figure 3
Non-canonical pathways involved in TGFβ signaling in hypertrophic murine hearts. Western blot was performed in the LV of mice subjected to 6 weeks sham or TAC with or without AZ876 treatment. (A) p38 MAPK, (B) phosphorylated Akt (Ser 473) to total Akt, and (C) phosphorylated Erk to total Erk, normalized to GAPDH. Quantitative values are expressed as fold change; n=5-6/Group. Data presented are means ± SEM; *P<0.05 versus respective sham.

Supplemental Figure 4
Dose-response for AZ876 induction of LXR target gene expression in isolated cardiomyocytes. RT-PCR analysis to evaluate effect of 24-hour AZ876 treatment on (A) Lxrα, (B) Lxrβ, as well as target genes (C) Srebplc and (D) Abca1 mRNA levels in NRVMs; normalized to 36b4. Data presented are means ± SEM; n=2-5/group; *P<0.05, **P<0.01 versus con.
Supplemental Figure 5
LXR and target gene expression in isolated cardiac fibroblasts. Assessment of (A) Lxrα, (B) Lxrβ, (C) Srebplc, and (D) Abca1 mRNA with RT-PCR following 24 hour treatment with or without AZ876; expression levels normalized to 36b4. Data presented are means ± SEM; n=6/group (except 5 nmol is n=1); ***P<0.001 versus con.

Supplemental Figure 6
Total lipoprotein analysis from dose-findings studies for AZ876. Plasma samples were pooled (n=4/group) and subjected to FPLC. VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
### Supplemental Table II. Effect of AZ876 on Gene Transcription in Mice subjected to Pressure Overload

<table>
<thead>
<tr>
<th>Gene</th>
<th>sham + con</th>
<th>sham + AZ876</th>
<th>TAC + con</th>
<th>TAC + AZ876</th>
</tr>
</thead>
<tbody>
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<td><strong>LXR/target genes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Lxra</td>
<td>1.00 ± 0.11</td>
<td>1.38 ± 0.08</td>
<td>1.27 ± 0.11</td>
<td>1.02 ± 0.12</td>
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<td>Lxrb</td>
<td>1.00 ± 0.14</td>
<td>1.31 ± 0.09</td>
<td>1.59 ± 0.12</td>
<td>1.17 ± 0.17</td>
</tr>
<tr>
<td>Srebp1c</td>
<td>1.00 ± 0.15</td>
<td>4.93 ± 1.20</td>
<td>1.28 ± 0.16</td>
<td>1.68 ± 0.34</td>
</tr>
<tr>
<td>Abca1</td>
<td>1.00 ± 0.14</td>
<td>2.05 ± 0.30</td>
<td>1.28 ± 0.13</td>
<td>1.17 ± 0.15</td>
</tr>
<tr>
<td>Abcg1</td>
<td>1.00 ± 0.17</td>
<td>3.10 ± 0.61</td>
<td>2.53 ± 0.36</td>
<td>1.99 ± 0.30</td>
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<tr>
<td><strong>Inflammation</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>1.00 ± 0.12</td>
<td>0.75 ± 0.11</td>
<td>7.27 ± 1.12</td>
<td>5.25 ± 0.84</td>
</tr>
<tr>
<td>Mcp1</td>
<td>1.00 ± 0.09</td>
<td>1.06 ± 0.15</td>
<td>1.20 ± 0.17</td>
<td>1.12 ± 0.18</td>
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<tr>
<td>Tnfα</td>
<td>1.00 ± 0.12</td>
<td>0.96 ± 0.07</td>
<td>0.86 ± 0.10</td>
<td>0.68 ± 0.08</td>
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<td><strong>Hypertrophy</strong></td>
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<tr>
<td>Anp</td>
<td>1.00 ± 0.12</td>
<td>1.44 ± 0.29</td>
<td>13.88 ± 2.55†</td>
<td>7.47 ± 1.75†</td>
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<td>Bnp</td>
<td>1.00 ± 0.06</td>
<td>0.80 ± 0.08</td>
<td>2.69 ± 0.50</td>
<td>1.88 ± 0.16</td>
</tr>
<tr>
<td>Myh7</td>
<td>1.00 ± 0.11</td>
<td>1.26 ± 0.06</td>
<td>7.60 ± 0.94*</td>
<td>4.90 ± 0.88*</td>
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<tr>
<td>Acta1</td>
<td>1.00 ± 0.06</td>
<td>1.06 ± 0.09</td>
<td>15.30 ± 1.63†</td>
<td>10.30 ± 1.50†</td>
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<td>Rcan1</td>
<td>1.00 ± 0.18</td>
<td>0.74 ± 0.09</td>
<td>8.84 ± 1.73‡</td>
<td>3.34 ± 0.54‡</td>
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<td><strong>Fibrosis</strong></td>
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<td>Tgfb</td>
<td>1.00 ± 0.08</td>
<td>1.06 ± 0.05</td>
<td>1.73 ± 0.13†</td>
<td>1.41 ± 0.09</td>
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<tr>
<td>Ctgf</td>
<td>1.00 ± 0.12</td>
<td>0.81 ± 0.08</td>
<td>5.26 ± 0.88‡</td>
<td>3.27 ± 0.37‡</td>
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<tr>
<td>Fibronectin</td>
<td>1.00 ± 0.13</td>
<td>1.03 ± 0.06</td>
<td>3.85 ± 0.45†</td>
<td>2.58 ± 0.41†</td>
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<td>Fstl3</td>
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<td>1.20 ± 0.05</td>
<td>4.51 ± 0.61†</td>
<td>2.52 ± 0.34‡</td>
</tr>
<tr>
<td>Acta2</td>
<td>1.00 ± 0.05</td>
<td>1.00 ± 0.08</td>
<td>1.25 ± 0.14</td>
<td>1.01 ± 0.12</td>
</tr>
<tr>
<td>Col1a1</td>
<td>1.00 ± 0.05</td>
<td>0.77 ± 0.04</td>
<td>2.34 ± 0.21†</td>
<td>1.74 ± 0.29*</td>
</tr>
<tr>
<td>Col3a1</td>
<td>1.00 ± 0.04</td>
<td>0.87 ± 0.07</td>
<td>2.39 ± 0.21†</td>
<td>1.82 ± 0.23†</td>
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<td>Timp1</td>
<td>1.00 ± 0.08</td>
<td>0.88 ± 0.10</td>
<td>7.28 ± 1.23†</td>
<td>3.24 ± 0.65†</td>
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<td>Mmp2</td>
<td>1.00 ± 0.08</td>
<td>1.15 ± 0.06</td>
<td>2.09 ± 0.15†</td>
<td>1.51 ± 0.17*</td>
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<td>Mmp14</td>
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<td>0.96 ± 0.06</td>
<td>2.28 ± 0.29†</td>
<td>1.60 ± 0.17</td>
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<td>Gal3</td>
<td>1.00 ± 0.29</td>
<td>1.32 ± 0.27</td>
<td>3.36 ± 0.43*</td>
<td>3.00 ± 0.60</td>
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<td><strong>Renin-Angiotensin System</strong></td>
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<td>AT1R</td>
<td>1.00 ± 0.09</td>
<td>1.12 ± 0.05</td>
<td>0.73 ± 0.07*</td>
<td>0.91 ± 0.03</td>
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<td>Glut1</td>
<td>1.00 ± 0.15</td>
<td>0.88 ± 0.09</td>
<td>1.48 ± 0.15</td>
<td>1.26 ± 0.10</td>
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<td>Glut4</td>
<td>1.00 ± 0.14</td>
<td>0.97 ± 0.09</td>
<td>0.83 ± 0.08</td>
<td>0.86 ± 0.05</td>
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<tr>
<td>Pfkm</td>
<td>1.00 ± 0.05</td>
<td>0.90 ± 0.07</td>
<td>0.77 ± 0.05*</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Pdk4</td>
<td>1.00 ± 0.14</td>
<td>1.97 ± 0.24†</td>
<td>1.34 ± 0.19</td>
<td>2.42 ± 0.40†</td>
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<td><strong>Fatty acid metabolism</strong></td>
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<td>Cd36</td>
<td>1.00 ± 0.09</td>
<td>1.08 ± 0.04</td>
<td>0.80 ± 0.05</td>
<td>0.90 ± 0.07</td>
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<tr>
<td>Ppara</td>
<td>1.00 ± 0.13</td>
<td>0.98 ± 0.08</td>
<td>0.84 ± 0.07</td>
<td>0.88 ± 0.07</td>
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<tr>
<td>Acc2</td>
<td>1.00 ± 0.11</td>
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<td>0.87 ± 0.05</td>
<td>1.09 ± 0.06</td>
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<td>Mcd</td>
<td>1.00 ± 0.11</td>
<td>1.08 ± 0.04</td>
<td>0.79 ± 0.06</td>
<td>0.90 ± 0.06</td>
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<td>Cpt1b</td>
<td>1.00 ± 0.15</td>
<td>1.01 ± 0.07</td>
<td>0.89 ± 0.03</td>
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<td>Pgc1a</td>
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<td>0.96 ± 0.05</td>
<td>0.66 ± 0.04*</td>
<td>0.91 ± 0.05</td>
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<tr>
<td>Ucp3</td>
<td>1.00 ± 0.16</td>
<td>1.76 ± 0.20</td>
<td>0.91 ± 0.24</td>
<td>1.47 ± 0.18</td>
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</table>

Left ventricular gene expression levels are normalized to the invariant transcript, 36b4, and are presented as fold change. Data presented are means ± SEM, n=7-8. * P<0.05, † P<0.01, ‡ P<0.001, TAC versus corresponding sham group; * P<0.05, † P<0.01, con versus AZ876 treatment.
**Supplemental Table III. Effects of AZ876 on Plasma and Liver Lipids in Sham and TAC-Operated Mice**

<table>
<thead>
<tr>
<th></th>
<th>sham control</th>
<th>AZ876</th>
<th>TAC control</th>
<th>AZ876</th>
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<tbody>
<tr>
<td><strong>Plasma analytes</strong></td>
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<td>Triglycerides, mmol/L</td>
<td>1.31 ± 0.08</td>
<td>1.11 ± 0.25</td>
<td>0.96 ± 0.10</td>
<td>0.54 ± 0.04</td>
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<tr>
<td>Non-esterified fatty acids, mmol/L</td>
<td>0.28 ± 0.02</td>
<td>0.25 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>0.34 ± 0.04</td>
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<tr>
<td>AZ876, nmol/L</td>
<td>0.0 ± 0.0</td>
<td>10.1 ± 2.8*</td>
<td>0.0 ± 0.0</td>
<td>5.0 ± 0.4</td>
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<tr>
<td><strong>Tissue triglycerides</strong></td>
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<td>Liver triglycerides, mmol/g</td>
<td>57.5 ± 9.2</td>
<td>98.7 ± 14.8</td>
<td>45.5 ± 4.6</td>
<td>103.2 ± 18.4*</td>
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</table>

Data presented are means ± SEM. * P<0.05, # P<0.001, control versus AZ876 treatment.
LXR agonist AZ876 attenuates cardiac hypertrophy and fibrosis