



HAL
open science

Rexinoid bexarotene modulates triglyceride but not cholesterol metabolism via gene-specific permissivity of the RXR/LXR heterodimer in the liver.

Fanny Lalloyer, Thomas Åskov Pedersen, Barbara Gross, Sophie Lestavel, Saïd Yous, Emmanuelle Vallez, Jan-Åke Gustafsson, Susanne Mandrup, Catherine Fiévet, Bart Staels, et al.

► To cite this version:

Fanny Lalloyer, Thomas Åskov Pedersen, Barbara Gross, Sophie Lestavel, Saïd Yous, et al.. Rexinoid bexarotene modulates triglyceride but not cholesterol metabolism via gene-specific permissivity of the RXR/LXR heterodimer in the liver.. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2009, 29 (10), pp.1488-95. 10.1161/ATVBAHA.109.189506 . inserm-00410108

HAL Id: inserm-00410108

<https://inserm.hal.science/inserm-00410108>

Submitted on 8 Jan 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Rexinoid bexarotene modulates triglyceride but not cholesterol metabolism via gene-specific permissivity of the RXR/LXR heterodimer in the liver

Fanny Lalloyer ¹, Thomas Åskov Pedersen ², Barbara Gross ¹, Sophie Lestavel ¹, Saïd Yous ³, Emmanuelle Vallez ¹, Jan-Åke Gustafsson ^{4,5}, Susanne Mandrup ², Catherine Fiévet ¹, Bart Staels ^{1*}, Anne Tailleux ¹

¹ Récepteurs nucléaires, lipoprotéines et athérosclérose INSERM : U545, Institut Pasteur de Lille, Université du Droit et de la Santé - Lille II, Institut Pasteur 1, Rue du Professeur Calmette 59019 Lille cedex,FR

² Department of Biochemistry and Molecular Biology University of Southern Denmark, 5230 Odense,DK

³ ICPAL, Institut de Chimie Pharmaceutique Albert Lespagnol Univ Lille Nord de France, Lille, UDSL, Lille, Faculté des Sciences Pharmaceutiques 59019 Lille,FR

⁴ Department of Biosciences and Nutrition Karolinska Institutet, S-141 86 Huddinge,SE

⁵ Department of Biology and Biochemistry University of Houston, Center for Nuclear Receptors and Cell Signaling, Houston, TX 77204-5008,US

* Correspondence should be addressed to: Bart Staels <Bart.Staels@pasteur-lille.fr >

Abstract **Objective**

Bexarotene (Targretin®) is a clinically used anti-tumoral agent which exerts its action through binding to and activation of the Retinoid-X-Receptor (RXR). The most frequent side effect of bexarotene administration is an increase in plasma triglycerides, an independent risk factor of cardiovascular disease. The molecular mechanism behind this hypertriglyceridemia remains poorly understood.

Methods and Results

Using wild-type and LXR α / β -deficient mice, we show here that bexarotene induces hypertriglyceridemia and activates hepatic LXR-target genes of lipogenesis in an LXR-dependent manner, hence exerting a permissive effect on RXR/LXR heterodimers. Interestingly, RNA analysis and Chromatin Immunoprecipitation assays performed in the liver reveal that the *in vivo* permissive effect of bexarotene on the RXR/LXR heterodimer is restricted to lipogenic genes without modulation of genes controlling cholesterol homeostasis.

Conclusion

These findings demonstrate that the hypertriglyceridemic action of bexarotene occurs via the RXR/LXR heterodimer and show that RXR heterodimers can act with a selective permissivity on target genes of specific metabolic pathways in the liver.

MESH Keywords Animals ; Cholesterol ; metabolism ; DNA-Binding Proteins ; chemistry ; physiology ; Dimerization ; Female ; Homeostasis ; Lipogenesis ; Liver ; metabolism ; Mice ; Mice, Inbred C57BL ; Orphan Nuclear Receptors ; Receptors, Cytoplasmic and Nuclear ; chemistry ; physiology ; Retinoid X Receptors ; chemistry ; physiology ; Tetrahydronaphthalenes ; pharmacology ; Triglycerides ; metabolism

Author Keywords Retinoid-X-Receptor ; rexinoid ; hypertriglyceridemia ; Liver-X-Receptor ; murine model ; ChIP

INTRODUCTION

RXR α , RXR β and RXR γ (Retinoid-X-Receptors) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. Three subtypes (RXR α , RXR β and RXR γ) have been identified, which can be activated by natural (9-cis retinoic acid, docosahexaenoic acid) or synthetic ligands, called rexinoids¹. RXRs are regulators of a plethora of pathophysiological processes with potential clinical implications. For instance, bexarotene (LGD1069, Targretin®), a rexinoid with potent and selective activity for RXR α , β and γ ², is clinically used in the treatment of cutaneous T cell lymphoma³ and has promising effects in other cancer forms or dermatological disorders like psoriasis⁴. In addition, synthetic RXR ligands exert beneficial glucose-lowering and insulin-sensitizing effects as well as anti-obesity actions in animal models of insulin-resistance and diabetes⁵, and bexarotene improves cholesterol homeostasis and inhibits the development of atherosclerosis in a mouse model of mixed dyslipidemia⁶. However, similar to other rexinoids, bexarotene administration induces an undesirable increase in plasma triglycerides^{7, 8}, an independent risk factor of cardiovascular disease (CVD)^{9, 10}. Until now, the molecular mechanism behind bexarotene-induced hypertriglyceridemia remains unknown.

RXR is a unique nuclear receptor since it can modulate gene transcription by forming either homodimers or heterodimers with several other nuclear receptors, such as the PPARs (Peroxisome Proliferator-Activated Receptors), LXRs (Liver-X-Receptors), FXR

(Farnesoid-X-Receptor), PXR (Pregnane-X-Receptor), RARs (Retinoic-Acid-Receptors), VDR (Vitamin D-Receptor) and TR (Thyroid Hormone Receptor). These heterodimers have been further subclassified as permissive or non-permissive heterodimers. This concept states that permissive heterodimers formed by RXR and binding partners are activatable by both RXR-specific and partner specific ligands, whereas non-permissive heterodimers can only be activated by ligands specific for the binding partner. Among the nuclear receptors which require RXR as an obligate partner for their action and form permissive heterodimers are the PPARs, FXRs and LXRs. As PPARs¹¹, FXRs¹² and LXRs¹³ are involved in the regulation of genes implicated in triglyceride metabolism, permissive ligand activation of RXR by rexinoids could elicit responses of the dimerization partner and as such modulate triglyceridemia. However, whereas PPAR α and FXR activation leads to decreased plasma triglyceride levels, LXR activation increases triglycerides¹³, presenting the permissive RXR/LXR heterodimer as a potential mediator of bexarotene-induced hypertriglyceridemia.

LXRs (LXR α and LXR β) are nuclear receptors that are activated by oxysterols or synthetic ligands such as T0901317, and bind DNA as obligate heterodimers with RXR at specific LXR response elements (LXREs) composed of a direct repeat of 5'-AGGTCA-3' spaced by four bases. LXR activation regulates triglyceride metabolism and induces hepatic steatosis and hypertriglyceridemia due, in part, to increased hepatic fatty acid synthesis and VLDL secretion^{13, 14} by upregulation of both sterol regulatory element-binding protein 1c (SREBP1c)^{13, 15, 16} and carbohydrate response element-binding protein (ChREBP)¹⁷ expression. Moreover, LXRs play an important role in the regulation of cholesterol homeostasis^{18, 19} and their activation leads to the induction of several genes implicated in reverse cholesterol transport and mobilization of cholesterol, such as the ATP binding cassette (ABC) transporters ABCA1, ABCG1, ABCG5, ABCG8 and apolipoprotein E20.

Using wild-type mice and mice deficient for both isoforms of LXR (LXR α/β -deficient mice), we show that the permissive heterodimer RXR/LXR mediates bexarotene-induced hypertriglyceridemia and hepatic lipogenesis *in vivo*. Surprisingly, RNA levels and Chromatin Immunoprecipitation (ChIP) assays reveal that this LXR-mediated effect of bexarotene in the liver is restricted to genes implicated in triglyceride homeostasis and lipogenesis, without modulation of genes controlling cholesterol homeostasis. These findings demonstrate that RXR heterodimers can act with a selective permissivity on target genes of specific metabolic pathways.

RESULTS

Bexarotene increases plasma and hepatic triglyceride levels in an LXR α/β -dependent manner

To determine whether LXRs are implicated in the bexarotene-induced hypertriglyceridemia, wild type mice and mice lacking both LXR α and LXR β (LXR α/β -deficient mice) were treated with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (figure 1A). In wild type mice, the LXR agonist induced a hypertriglyceridemia, an effect which was not observed in LXR α/β -deficient mice. Interestingly, the RXR agonist bexarotene also induced a robust dose-dependent hypertriglyceridemia, which was strongly blunted in LXR α/β -deficient mice. Thus, the RXR/LXR heterodimer appears to be the principal mediator of the bexarotene-induced hypertriglyceridemia.

Since LXR is a lipogenic transcription factor in the liver, we analyzed whether bexarotene influences triglyceride metabolism in the liver and whether these effects are mediated by LXR (figure 1B). As previously shown¹³, the LXR agonist T0901317 increased hepatic triglyceride levels in wild type, but not in LXR α/β -deficient mice. Interestingly, bexarotene induced a dose-dependent increase in hepatic triglyceride concentration in wild type but not in LXR α/β -deficient mice. Thus, the bexarotene-induced hypertriglyceridemia and hepatic triglyceride accumulation are dependent on the RXR/LXR pathway.

Bexarotene induces hepatic lipogenic genes in an LXR α/β -dependent manner

To further explore the role of LXR in the effects of bexarotene on hepatic triglyceride metabolism, the expression of LXR-target genes implicated in lipogenesis (SREBP1c, FAS and SCD1) was measured by quantitative PCR (figure 2). As expected, LXR agonist administration robustly increased the expression of SREBP1c, FAS and SCD1 in wild type mice, an effect which was completely blunted in LXR α/β -deficient mice. Interestingly, the mRNA levels of SREBP1c, FAS, and SCD1 also displayed a strong dose-dependent increase upon bexarotene treatment in wild type, but not in LXR α/β -deficient mice. To determine whether bexarotene could act via other transcription factor pathways and nuclear receptor heterodimers, RNA levels of ChREBP, a glucose-activated lipogenic transcription factor, acylCoA oxidase (ACO, PPAR α target gene) and BSEP (FXR target gene) (supplemental figure 1A) as well as Cyp3A11, CD36 and FAE (PXR target genes) (supplemental figure 1B) were measured. Bexarotene treatment increased ChREBP, ACO and BSEP mRNA both in wild type and in LXR-deficient mice (supplemental figure 1A), suggesting that ChREBP gene regulation as well as the permissive heterodimers RXR/PPAR and RXR/FXR do not participate in bexarotene-induced lipogenesis which occurs only in wild type mice. Moreover, we measured the expression of 5 α -deiodinase type 1 (DIO1), a RXR/TR target gene implicated in the metabolism of thyroid hormones (supplemental figure 1A). Bexarotene treatment decreased DIO1 expression in the liver to a similar extent in wild type and in LXR α/β -deficient mice, suggesting that potential alterations in the thyroid axis do not contribute to bexarotene-induced hypertriglyceridemia. Finally, Cyp3A11 was not modified in bexarotene-treated wild type mice, indicating that PXR does not appear to be implicated in bexarotene-induced lipogenesis (supplemental figure 1B). By contrast to Cyp3A11, CD36 and FAE mRNA levels were

strongly increased in T091317-treated wild type but not LXR-deficient mice, suggesting that these genes are activated in a LXR- but not PXR-dependent manner by T091317 (supplemental figure 1B). CD36 expression was induced by bexarotene treatment both in wild type and LXR-deficient mice, suggesting that CD36 is not implicated in bexarotene-induced lipogenesis which is only observed in wild type mice. FAE expression was strongly increased in wild type bexarotene-treated mice, an effect which was strongly reduced in LXR-deficient mice, suggesting that the lipogenic enzyme FAE could be implicated in bexarotene-induced lipogenesis, similar as SREBP-1c, SCD1 and FAS.

These data show that bexarotene upregulates specific hepatic LXR-target lipogenic genes via the permissive heterodimer RXR/LXR *in vivo* .

Bexarotene increases RXR binding to the LXREs of hepatic lipogenic genes

To investigate whether the activation of hepatic lipogenic LXR-target genes was associated with an increased ability of RXR complexes to bind LXREs in the promoters of these genes, ChIP assays were performed on livers of mice treated with the highest dose of the RXR agonist, the LXR agonist or vehicle alone (figure 3). Antibodies directed against RXR (figure 3A) and RNA polymerase II (figure 3B) were used, and input as well as immunoprecipitated DNA was quantified by real-time PCR using primers positioned around the LXREs of these genes (figure 3A) or within the gene (figure 3B), respectively. The antibody against RNA polymerase II recognizes both the non-phosphorylated and the phosphorylated extending forms of RNA polymerase II, and occupancy within the gene can be used as a measure of transcriptional activity²¹ . In wild type mice, the RXR ChIP-PCR revealed that both LXR and RXR agonist treatment increased the recruitment of RXR on the LXREs of SREBP1c, SCD1 and FAS genes (figure 3A). RNA polymerase II ChIP-PCR showed that this bexarotene and T0901317-induced RXR recruitment was associated with increased RNA polymerase II occupancy in the genes encoding SREBP1c, FAS and SCD1 in wild type mice (figure 3B). By contrast, bexarotene-induced RXR recruitment to the FAS and SREBP1c LXREs was abolished in the LXR α / β -deficient mice, upon bexarotene and T0901317 treatment, whereas a small, albeit significant, increase was still observed on the SCD1 LXRE (figure 3A). These data suggest that RXR can bind independent of LXR in the vicinity of this LXRE. Moreover, in LXR α / β -deficient mice, neither bexarotene nor T0901317 lead to significant increase of RNA polymerase II occupancy on the SREBP1c and FAS genes. In keeping with the modest LXR independent binding of RXR to the SCD1 gene, a modest bexarotene-induced recruitment of RNA polymerase II was observed after bexarotene treatment. Altogether, both LXR and RXR agonist treatment increased RXR binding to LXREs and increased RNA polymerase II occupancy on hepatic lipogenic genes in wild type mice but not in LXR α / β -deficient mice.

Plasma cholesterol concentrations are increased after LXR but not RXR agonist treatment

Because LXR is a major regulator of cholesterol homeostasis, plasma cholesterol concentrations were also measured in wild type and LXR α / β -deficient mice treated with the LXR and RXR agonists, respectively (figure 4A). The LXR agonist T0901317 increased plasma total cholesterol by increasing both HDL-C and non-HDL-C in wild type, but not in LXR α / β -deficient mice (figure 4B and 4C). Hepatic cholesterol content was not modified after T0901317 treatment (data not shown). Interestingly, plasma total cholesterol, HDL-C and non-HDL-C did not change upon treatment of wild type and LXR α / β -deficient mice with the RXR agonist bexarotene. Moreover, bexarotene treatment did not influence hepatic cholesterol content (data not shown). Thus, bexarotene-mediated RXR activation of the RXR/LXR heterodimer does not influence plasma cholesterol concentrations, whereas T0901317-induced LXR activation of RXR/LXR increases plasma cholesterol concentrations.

Hepatic LXR-target genes implicated in cholesterol homeostasis are induced by LXR but not RXR agonist treatment

LXR influences cholesterol homeostasis in the liver by regulating genes encoding proteins of the ATP Binding Cassette Transporter family^{22, 23} . Therefore, the expressions of these LXR-target genes were measured in the liver. In wild type mice, LXR agonist treatment induced a strong increase in the expression of ABCG1, ABCA1, ABCG5 and ABCG8, an effect which was totally absent in LXR α / β -deficient mice (figure 5). Notably, in contrast to lipogenic LXR target genes, the hepatic mRNA levels of these LXR target genes of cholesterol metabolism only displayed minor or no responsiveness to RXR agonist treatment. Indeed, bexarotene treatment only slightly influenced the mRNA level of these genes and these effects were comparable between wild type and LXR α / β -deficient mice. These data show that, in contrast to hepatic lipogenic genes, only T0901317 induces hepatic LXR-target genes implicated in cholesterol homeostasis via RXR/LXR *in vivo* , demonstrating that RXR/LXR heterodimers can act with a selective permissivity on target genes of specific pathways in the liver. To analyze whether bexarotene can modulate these LXR-target genes of cholesterol homeostasis in another organ such as the intestine, the expression of ABCG5 and ABCG8 as well as NPC1L1 was measured in the jejunum. In contrast to T091317, the bexarotene-induced decrease of NPC1L1 expression was not LXR-dependent. However, both bexarotene and T091317 increased intestinal ABCG5 and ABCG8 mRNA levels in an LXR-dependent manner (supplemental figure 2). These LXR-dependent increases of ABCG5 and ABCG8 expression by bexarotene in the intestine differ from the absence of LXR-dependent effects of bexarotene on these genes in the liver, identifying tissue-specific mechanisms of RXR/LXR heterodimer permissivity.

RXR recruitment on the LXREs of hepatic genes implicated in cholesterol homeostasis is induced by LXR but not RXR agonist treatment

To determine how T0901317 and bexarotene affect RXR recruitment to the LXREs of genes implicated in cholesterol homeostasis, RXR ChIP-PCR was performed on livers of mice treated with the highest dose of the RXR agonist, the LXR agonist or vehicle alone (figure 6A). LXR agonist treatment increased RXR occupancy on a putative LXRE sequence adjacent to the ABCG1 gene in wild type mice but not in LXR α / β -deficient mice. Even though ABCA1 has a high basal LXRE occupancy by RXR, RXR recruitment on the reported LXRE in the proximal promoter of ABCA1 also tended to increase in wild type mice but not in LXR α / β -deficient mice. Since the mouse LXREs in ABCG5 and ABCG8 genes have not yet been characterized, we used computational analysis to identify the DR4 elements in the vicinity of these genes and we identified two putative LXREs in the first intron of the ABCG8 gene. Interestingly, LXR agonist treatment increased the recruitment of RXR to these putative LXREs only in wild type mice, indicating that these sequences are indeed functional LXREs. In keeping with ABCG1, ABCA1, ABCG5 and ABCG8 being LXR target genes in the liver, RNA polymerase II ChIP-PCR demonstrated LXR agonist dependent polymerase II occupancy in wild type but not in LXR α / β -deficient livers (figure 6B). Interestingly, in contrast to the LXR agonist, RXR agonist did not induce major changes in RXR binding to the examined LXREs adjacent to genes involved in cholesterol homeostasis neither in wild type nor in LXR α / β -deficient liver. Moreover, recruitment of RNA polymerase II to the ABCG1, ABCA1, ABCG5 and ABCG8 genes was not influenced by bexarotene in wild type and in LXR α / β -deficient mice (figure 6B). These data demonstrate that LXR and RXR agonists differently regulate RXR binding on hepatic genes implicated in cholesterol homeostasis, an effect that may contribute to their distinct effects on plasma and hepatic lipid levels.

DISCUSSION

In the present study, we show that bexarotene, a rexinoid used clinically for the treatment of certain cancers and dermatological disorders, induces hypertriglyceridemia in an LXR-dependent manner *in vivo*. This observation is of particular importance since rexinoids have been proposed for the treatment of metabolic diseases and insulin-resistance syndromes. However, side effects such as hypertriglyceridemia^{24, 25} which is now widely recognized to be an independent risk factor of CVD⁹, have limited their use. Until now, the mechanism underlying this effect was not understood. In a study performed in ZDF rats treated for 14 days with a broad range of bexarotene doses, the authors proposed that the bexarotene-induced dose-dependent hypertriglyceridemia was due to elevated VLDL caused by a primary defect in LPL activity mainly in the muscle²⁶. Another study performed in wild-type and PPAR α -deficient mice suggested two separate pathways to explain bexarotene action on plasma triglyceride concentrations, a PPAR α -dependent pathway by activation of the RXR/PPAR α -heterodimer for a TG-decreasing effect of bexarotene and a PPAR α -independent pathway for the TG-raising effect of bexarotene but without identifying the heterodimer implicated²⁷. As a previous study performed in our laboratory in the apolipoprotein E2 knock-in mouse model showed a bexarotene-induced hypertriglyceridemia associated with an increase in the hepatic expression of FAS and SCD1, two direct LXR-target genes implicated in lipogenesis⁶, we assessed whether hypertriglyceridemia induced by the RXR agonist bexarotene could involve the RXR/LXR pathway. Indeed, the heterodimer RXR/LXR is known to be permissive and the activation of this heterodimer by LXR agonists induces hepatic steatosis and hypertriglyceridemia^{13, 14}. By measuring plasma triglyceride concentrations in wild type and LXR α / β -deficient mice, we demonstrated that bexarotene-induced hypertriglyceridemia occurred in a dose-dependent manner through the RXR/LXR pathway. This effect was associated with an upregulation of hepatic lipogenic genes (SREBP1c, FAS, FAE and SCD1) in a dose- and LXR-dependent manner. The implication of other heterodimers as mediators of the effects of bexarotene in the liver was also analyzed. Bexarotene activation of the permissive heterodimers RXR/PPAR or RXR/FXR was not affected by LXR-deficiency since both ACO (RXR/PPAR target gene) and BSEP (RXR/FXR target gene) expression was strongly increased by bexarotene in wild type and LXR α / β -deficient mice. The potential role of the thyroid hormone axis in the triglyceride response to bexarotene was also dismissed due to the fact that bexarotene decreased DIO1 (RXR/TR target gene) expression to a similar extent in wild type and LXR α / β -deficient mice. Cyp3A11 (RXR/PXR target gene) expression was not modified in the liver of bexarotene-treated wild type mice, suggesting that RXR/PXR heterodimers do not mediate bexarotene-induced lipogenesis in wild type mice.

The fact that the RXR/LXR heterodimer mediates bexarotene-induced hypertriglyceridemia suggests that it could also be implicated in hypertriglyceridemia reported *in vivo* with many other RXR agonists^{26, 28}. It is, however, difficult to extrapolate to other rexinoids because each rexinoid selectively activates its own pattern of heterodimers^{29, 30}. However, LG10506, a new rexinoid shown to selectively activate RXR/PPAR but not RXR/LXR, RXR/RAR and RXR/FXR heterodimers, has beneficial metabolic effects without inducing hypertriglyceridemia³¹, reinforcing the hypothesis that, in case of RXR agonist-induced hypertriglyceridemia, LXR seems to be the main partner implicated.

Notably, the results of this study support a new interesting concept concerning the molecular mechanisms of RXR heterodimer action. Indeed, we identify the existence of heterodimer-specific selective permissivity on target genes of specific metabolic pathways in one given tissue, *i.e.* the liver. The concept of selective permissivity linked to tissue-specific activity has previously been introduced with respect to RXR heterodimers³². The heterodimer RXR/TR, which was shown to be non-permissive, displays a permissive activity depending on the cell type^{33–35}. Conversely, the heterodimer RXR/FXR, shown to be permissive in different assays *in vitro*, can be antagonized by RXR agonists³². Taken together, these studies have demonstrated tissue-specific differences in permissivity of certain RXR heterodimers. This concept has been extended in our study to the LXR/RXR heterodimer, since bexarotene treatment increased

ABCG5 and ABCG8 mRNA levels in an LXR-dependent manner in the jejunum, but not in the liver, suggesting the existence of distinct molecular mechanisms of LXR target gene regulation in the intestine vs the liver. Moreover, we show that, in liver, RXR heterodimers can also act with a selective permissivity on specific metabolic pathways. Indeed, similar to the LXR agonist T0901317, bexarotene increased the recruitment of RXR on the LXREs of specific LXR-target genes implicated in hepatic lipogenesis (SREBP1c, SCD1 and FAS), leading to their upregulation and hepatic accumulation of triglycerides. Thus, the RXR/LXR heterodimer is permissive for lipogenesis in the liver. By contrast, whereas the LXR agonist induces the recruitment of RXR on the LXREs of hepatic genes encoding proteins implicated in cholesterol homeostasis, leading to a strong increase of their expression in an LXR-dependent manner, the RXR agonist bexarotene only slightly increased the expression of these genes and did not influence liver and plasma cholesterol homeostasis, either in wild-type or in LXR α / β -deficient mice. Taken together, these results demonstrate that the heterodimer RXR/LXR is non-permissive for cholesterol homeostasis in the liver. Thus, these data show that, in the same organ (ie the liver in our study), an RXR heterodimer (in this case RXR/LXR) may be permissive for target genes of specific metabolic pathways. These observations reinforce the concept that retinoids can have molecule-, inter-tissue-and intra-tissue specific effects, which may find application in drug development by synthesizing RXR modulators with appropriate tissue and gene selective profiles for a potential clinical use.

In conclusion, this study shows that the molecular mechanism of bexarotene-induced hypertriglyceridemia is dependent on the RXR/LXR pathway and that in liver the *in vivo* permissive effect of bexarotene on the RXR/LXR heterodimer is restricted to specific lipogenic LXR-target genes but does not affect LXR-target genes implicated in cholesterol homeostasis. In addition, our study supports the notion of selective permissivity on target genes of specific metabolic pathways for the mode of action of RXR heterodimers.

MATERIALS AND METHODS

Animal experiments

All experiments were performed with the approval of the Pasteur Institute review board, Lille France. Bexarotene was synthesized in the Laboratoire de Chimie Thérapeutique (Faculté des Sciences Pharmaceutiques, Université de Lille 2, France).

Female C57BL6 (13 weeks of age) wild type (n=28) or LXR α / β -deficient (n=28) mice³⁶, 37 obtained from Jan-Ake Gustafsson's laboratory were divided into four groups (n=7/group) and were treated with the RXR agonist bexarotene (suspended in 1% carboxymethylcellulose at the doses of 30 or 100 mg/kg body weight (mpk)), the LXR agonist T0901317 (30 mpk) or vehicle alone by oral gavage once daily for 14 days. At the end of the treatment, blood was collected by retro-orbital venipuncture under isoflurane anesthesia after 4h of fasting (9am–1pm). Plasma was separated by centrifugation (15 min, 4°C, 4 000 rpm) and used within 3 days for biochemical analysis. Animals were weighed and sacrificed by cervical dislocation. Livers were removed immediately, frozen in nitrogen and stored at -80°C for further analysis.

For expanded Material and Methods, please see supplemental information .

Acknowledgements:

We thank Véronique Touche for excellent technical assistance.

Sources of funding

This work was supported by grants from the Swedish Research Council and European FP6 STREP project X-TRA-NET (018882), the Foundation Coeur et Artères, the Danish Natural Science Research Council, Conseil Régional Nord-Pas de Calais and Fond Européen de Développement.

Footnotes:

Disclosure

None

References:

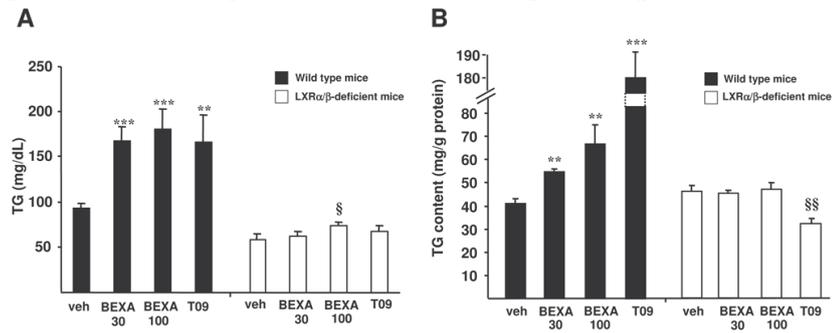
- 1 . Boehm MF , Zhang L , Zhi L , McClurg MR , Berger E , Wagoner M , Mais DE , Suto CM , Davies JA , Heyman RA . Design and synthesis of potent retinoid X receptor selective ligands that induce apoptosis in leukemia cells . *J Med Chem* . 1995 ; 38 : 3146 - 3155
- 2 . Hamann LG . An efficient, stereospecific synthesis of the dimer-selective retinoid X receptor modulator (2E,4E,6Z)-7-[5,6,7,8- tetrahydro-5,5,8,8-tetramethyl-2-(n-propyloxy)naphthalen-3-yl]-3-methyl octa-2,4, 6-trienoic acid . *J Org Chem* . 2000 ; 65 : 3233 - 3235
- 3 . Querfeld C , Nagelli LV , Rosen ST , Kuzel TM , Guitart J . Bexarotene in the treatment of cutaneous T-cell lymphoma . *Expert Opin Pharmacother* . 2006 ; 7 : 907 - 915
- 4 . Farol LT , Hymes KB . Bexarotene: a clinical review . *Expert Rev Anticancer Ther* . 2004 ; 4 : 180 - 188
- 5 . Pinaire JA , Reifel-Miller A . Therapeutic potential of retinoid x receptor modulators for the treatment of the metabolic syndrome . *PPAR Res* . 2007 ; 2007 : 94156 -
- 6 . Lalloyer F , Fievet C , Lestavel S , Torpier G , van der Veen J , Touche V , Bultel S , Yous S , Kuipers F , Paumelle R , Fruchart JC , Staels B , Tailleux A . The RXR agonist bexarotene improves cholesterol homeostasis and inhibits atherosclerosis progression in a mouse model of mixed dyslipidemia . *Arterioscler Thromb Vasc Biol* . 2006 ; 26 : 2731 - 2737
- 7 . Hurst RE . Bexarotene ligand pharmaceuticals . *Curr Opin Investig Drugs* . 2000 ; 1 : 514 - 523

- 8 . Staels B . Regulation of lipid and lipoprotein metabolism by retinoids . *J Am Acad Dermatol* . 2001 ; 45 : S158 - 167
- 9 . Austin MA . Plasma triglyceride as a risk factor for cardiovascular disease . *Can J Cardiol* . 1998 ; 14 : (Suppl B) 14B - 17B
- 10 . McBride PE . Triglycerides and risk for coronary heart disease . *Jama* . 2007 ; 298 : 336 - 338
- 11 . Lefebvre P , Chinetti G , Fruchart JC , Staels B . Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis . *J Clin Invest* . 2006 ; 116 : 571 - 580
- 12 . Chiang JY . Bile acid regulation of gene expression: roles of nuclear hormone receptors . *Endocr Rev* . 2002 ; 23 : 443 - 463
- 13 . Schultz JR , Tu H , Luk A , Repa JJ , Medina JC , Li L , Schwendner S , Wang S , Thoolen M , Mangelsdorf DJ , Lustig KD , Shan B . Role of LXRs in control of lipogenesis . *Genes Dev* . 2000 ; 14 : 2831 - 2838
- 14 . Grefhorst A , Elzinga BM , Voshol PJ , Plosch T , Kok T , Bloks VW , van der Sluijs FH , Havekes LM , Romijn JA , Verkade HJ , Kuipers F . Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles . *J Biol Chem* . 2002 ; 277 : 34182 - 34190
- 15 . Yoshikawa T , Shimano H , Amemiya-Kudo M , Yahagi N , Hasty AH , Matsuzaka T , Okazaki H , Tamura Y , Iizuka Y , Ohashi K , Osuga J , Harada K , Gotoda T , Kimura S , Ishibashi S , Yamada N . Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1 c gene promoter . *Mol Cell Biol* . 2001 ; 21 : 2991 - 3000
- 16 . Repa JJ , Liang G , Ou J , Bashmakov Y , Lobaccaro JM , Shimomura I , Shan B , Brown MS , Goldstein JL , Mangelsdorf DJ . Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta . *Genes Dev* . 2000 ; 14 : 2819 - 2830
- 17 . Cha JY , Repa JJ . The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR . *J Biol Chem* . 2007 ; 282 : 743 - 751
- 18 . Janowski BA , Willy PJ , Devi TR , Falck JR , Mangelsdorf DJ . An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha . *Nature* . 1996 ; 383 : 728 - 731
- 19 . Lehmann JM , Kliewer SA , Moore LB , Smith-Oliver TA , Oliver BB , Su JL , Sundseth SS , Winegar DA , Blanchard DE , Spencer TA , Willson TM . Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway . *J Biol Chem* . 1997 ; 272 : 3137 - 3140
- 20 . Repa JJ , Berge KE , Pomajzl C , Richardson JA , Hobbs H , Mangelsdorf DJ . Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta . *J Biol Chem* . 2002 ; 277 : 18793 - 18800
- 21 . Nielsen R , Pedersen TA , Hagenbeek D , Moulos P , Siersbaek R , Megens E , Denissov S , Borgesen M , Francois KJ , Mandrup S , Stunnenberg HG . Genome-wide profiling of PPARgamma:RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis . *Genes Dev* . 2008 ; 22 : 2953 - 2967
- 22 . Wang Y , Kurdi-Haidar B , Oram JF . LXR-mediated activation of macrophage stearyl-CoA desaturase generates unsaturated fatty acids that destabilize ABCA1 . *J Lipid Res* . 2004 ; 45 : 972 - 980
- 23 . Yu L , York J , von Bergmann K , Lutjohann D , Cohen JC , Hobbs HH . Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8 . *J Biol Chem* . 2003 ; 278 : 15565 - 15570
- 24 . Miller VA , Benedetti FM , Rigas JR , Verret AL , Pfister DG , Straus D , Kris MG , Crisp M , Heyman R , Loewen GR , Truglia JA , Warrell RP Jr . Initial clinical trial of a selective retinoid X receptor ligand, LGD1069 . *J Clin Oncol* . 1997 ; 15 : 790 - 795
- 25 . Rizvi NA , Marshall JL , Dahut W , Ness E , Truglia JA , Loewen G , Gill GM , Ulm EH , Geiser R , Jaunakais D , Hawkins MJ . A Phase I study of LGD1069 in adults with advanced cancer . *Clin Cancer Res* . 1999 ; 5 : 1658 - 1664
- 26 . Davies PJ , Berry SA , Shipley GL , Eckel RH , Hennuyer N , Crombie DL , Ogilvie KM , Peinado-Onsurbe J , Fievet C , Leibowitz MD , Heyman RA , Auwerx J . Metabolic effects of rexinoids: tissue-specific regulation of lipoprotein lipase activity . *Mol Pharmacol* . 2001 ; 59 : 170 - 176
- 27 . Ouamrane L , Larrieu G , Gauthier B , Pineau T . RXR activators molecular signalling: involvement of a PPAR alpha-dependent pathway in the liver and kidney, evidence for an alternative pathway in the heart . *Br J Pharmacol* . 2003 ; 138 : 845 - 854
- 28 . Claudel T , Leibowitz MD , Fievet C , Tailleux A , Wagner B , Repa JJ , Torpier G , Lobaccaro JM , Paterniti JR , Mangelsdorf DJ , Heyman RA , Auwerx J . Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid X receptor . *Proc Natl Acad Sci U S A* . 2001 ; 98 : 2610 - 2615
- 29 . Lala DS , Mukherjee R , Schulman IG , Koch SS , Dardashti LJ , Nadzan AM , Croston GE , Evans RM , Heyman RA . Activation of specific RXR heterodimers by an antagonist of RXR homodimers . *Nature* . 1996 ; 383 : 450 - 453
- 30 . Cesario RM , Klausung K , Razzaghi H , Crombie D , Rungta D , Heyman RA , Lala DS . The rexinoid LG100754 is a novel RXR:PPARgamma agonist and decreases glucose levels in vivo . *Mol Endocrinol* . 2001 ; 15 : 1360 - 1369
- 31 . Leibowitz MD , Ardecky RJ , Boehm MF , Broderick CL , Carfagna MA , Crombie DL , D'Arrigo J , Etgen GJ , Faul MM , Grese TA , Havel H , Hein NI , Heyman RA , Jolley D , Klausung K , Liu S , Mais DE , Mapes CM , Marschke KB , Michellys PY , Montrose-Rafizadeh C , Ogilvie KM , Pascual B , Rungta D , Tyhonas JS , Urcan MS , Wardlow M , Yumibe N , Reifel-Miller A . Biological characterization of a heterodimer-selective retinoid X receptor modulator: potential benefits for the treatment of type 2 diabetes . *Endocrinology* . 2006 ; 147 : 1044 - 1053
- 32 . Kassam A , Miao B , Young PR , Mukherjee R . Retinoid X receptor (RXR) agonist-induced antagonism of farnesoid X receptor (FXR) activity due to absence of coactivator recruitment and decreased DNA binding . *J Biol Chem* . 2003 ; 278 : 10028 - 10032
- 33 . Li D , Li T , Wang F , Tian H , Samuels HH . Functional evidence for retinoid X receptor (RXR) as a nonsilent partner in the thyroid hormone receptor/RXR heterodimer . *Mol Cell Biol* . 2002 ; 22 : 5782 - 5792
- 34 . Li D , Yamada T , Wang F , Vulin AI , Samuels HH . Novel roles of retinoid X receptor (RXR) and RXR ligand in dynamically modulating the activity of the thyroid hormone receptor/RXR heterodimer . *J Biol Chem* . 2004 ; 279 : 7427 - 7437
- 35 . Castillo AI , Sanchez-Martinez R , Moreno JL , Martinez-Iglesias OA , Palacios D , Aranda A . A permissive retinoid X receptor/thyroid hormone receptor heterodimer allows stimulation of prolactin gene transcription by thyroid hormone and 9-cis-retinoic acid . *Mol Cell Biol* . 2004 ; 24 : 502 - 513
- 36 . Alberti S , Schuster G , Parini P , Feltkamp D , Diczfalusy U , Rudling M , Angelin B , Bjorkhem I , Pettersson S , Gustafsson JA . Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice . *J Clin Invest* . 2001 ; 107 : 565 - 573
- 37 . Schuster GU , Parini P , Wang L , Alberti S , Steffensen KR , Hansson GK , Angelin B , Gustafsson JA . Accumulation of foam cells in liver X receptor-deficient mice . *Circulation* . 2002 ; 106 : 1147 - 1153

Figure 1

RXR or LXR agonist treatment increases plasma and hepatic triglyceride levels in vivo in an LXR α / β -dependent manner

Plasma (A) and hepatic (B) triglyceride concentrations were measured in LXR α / β -deficient mice (\square) and wild type mice (\blacksquare) gavaged with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (N=7/group). **p<0.01, ***p<0.001 versus wild type mice treated with vehicle; §p<0.05, §§p<0.01 versus LXR α / β -deficient mice treated with vehicle.

**Figure 2**

RXR or LXR agonist treatment induces hepatic lipogenic gene expression in mice in vivo in an LXR α / β -dependent manner

Hepatic mRNA levels of SREBP1c, SCD1 and FAS were measured in LXR α / β -deficient mice (\square) and wild type mice (\blacksquare) gavaged with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (N=7/group). Data normalized to 28S mRNA are expressed as a percentage of the wild type mice group treated with vehicle alone, to which an arbitrary value of 100 was attributed. **p<0.01 versus wild type mice treated with vehicle, §p<0.05, §§p<0.01 versus LXR α / β -deficient mice treated with vehicle.

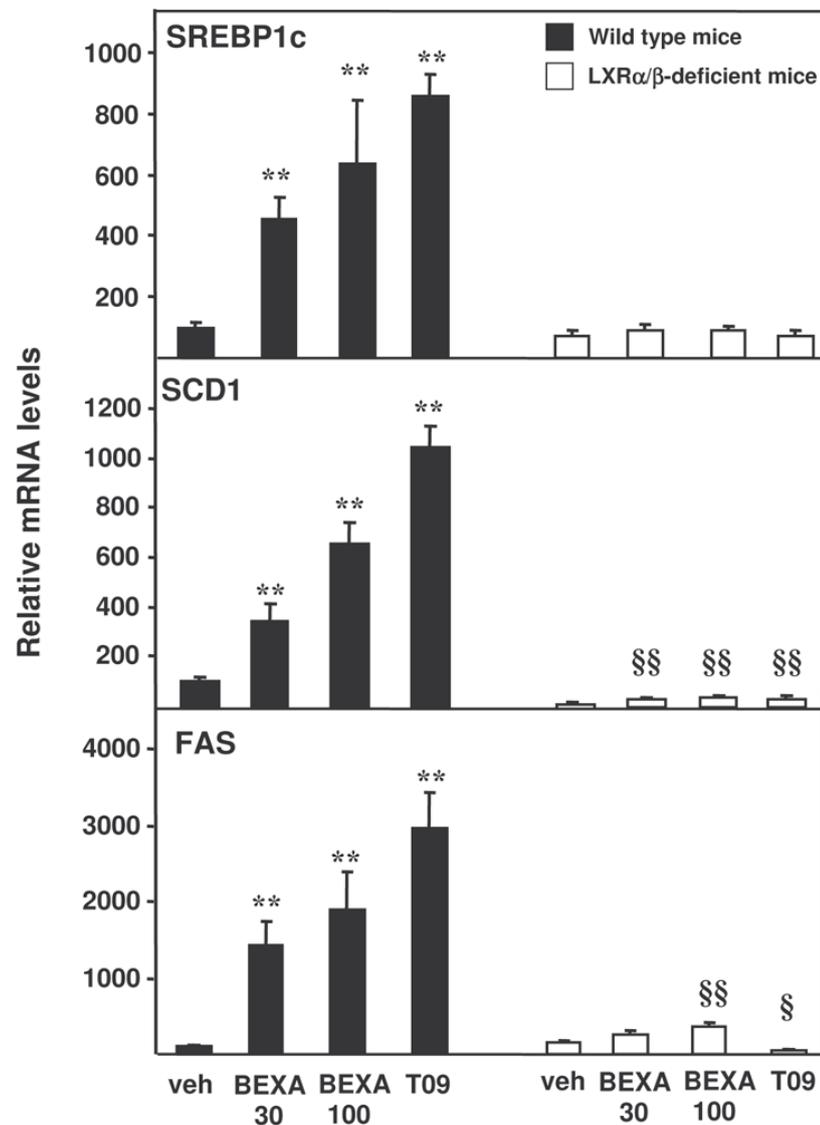
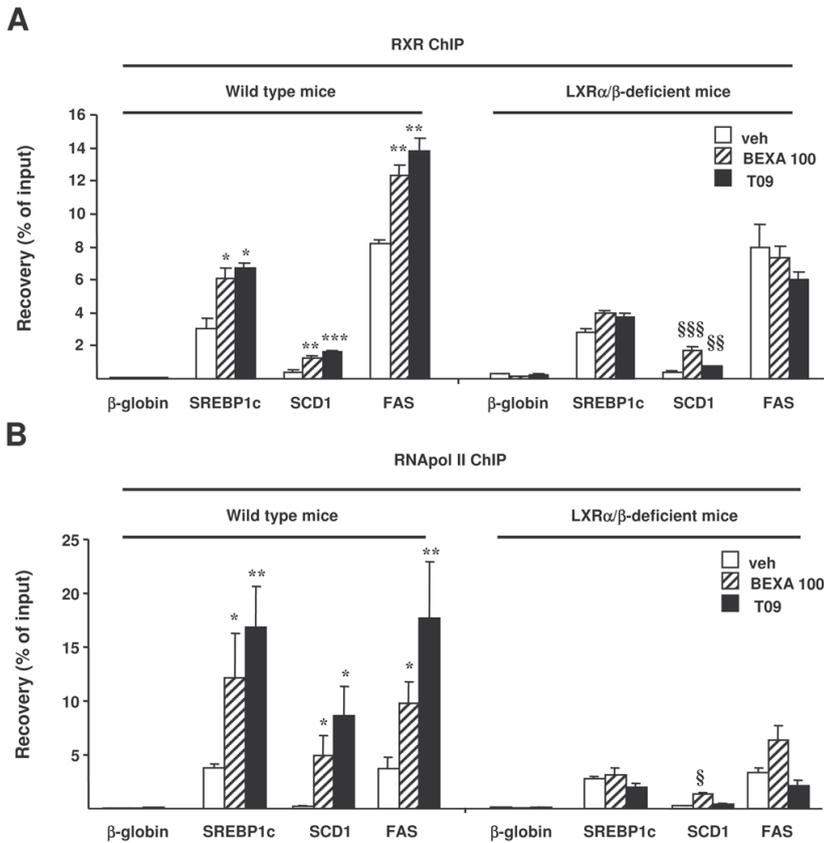


Figure 3

RXR or LXR agonist treatment increases RXR recruitment to the LXREs of lipogenic genes in vivo

ChIP assays were performed using antibodies against RXR (A) or RNA polymerase II (B) on livers of LXR α / β -deficient mice and wild type mice treated with vehicle alone, bexarotene (100 mpk) or T0901317 (30 mpk). Recovery was determined by real-time PCR using primers positioned at the LXRE of SREBP1c, FAS and SCD1 (A) or primers located in the body of the gene (B). β -globin was used as a control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus wild type mice treated with vehicle, § $p < 0.05$, §§ $p < 0.01$, §§§ $p < 0.001$ versus LXR α / β -deficient mice treated with vehicle.

**Figure 4**

LXR, but not RXR agonist treatment increases plasma cholesterol levels in vivo

TC (A), HDL-C (B) and non-HDL-C (C) were measured in LXR α / β -deficient mice (□) and wild type mice (■) gavaged, as indicated, with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (N=7/group). ** $p < 0.01$, *** $p < 0.001$ versus wild type mice treated with vehicle.

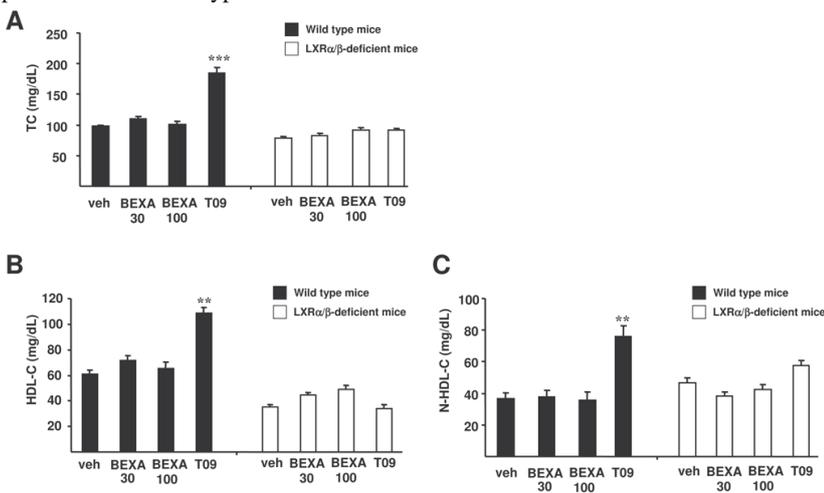
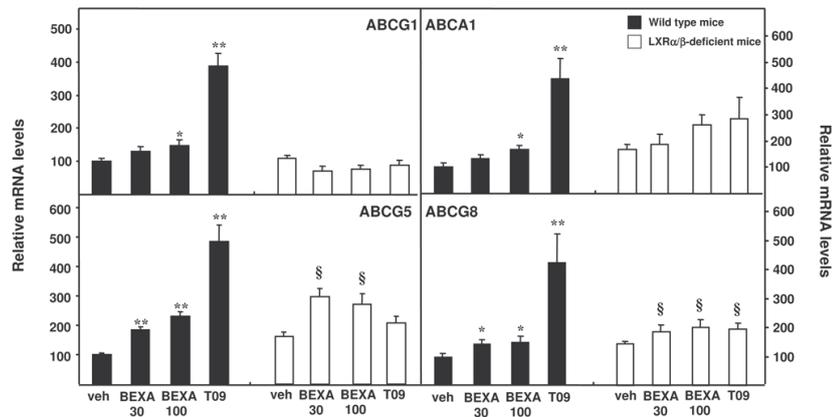


Figure 5

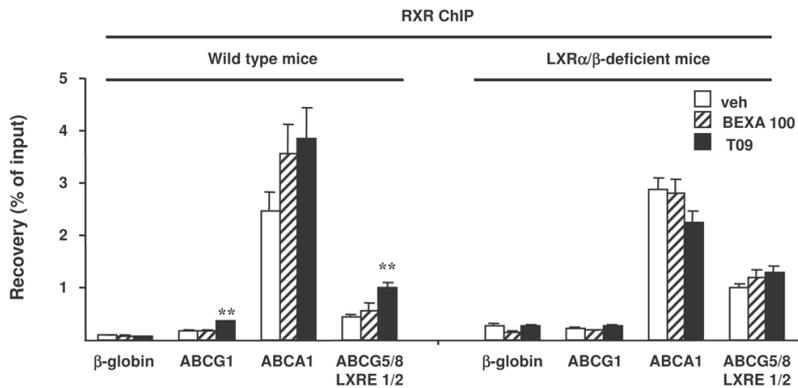
LXR, but not RXR agonist treatment upregulates hepatic cholesterol genes in vivo

Hepatic mRNA levels of ABCG1, ABCA1, ABCG5 and ABCG8 were measured in LXR α / β -deficient mice (\square) and wild type mice (\blacksquare) gavaged, as indicated, with the RXR agonist (bexarotene, 30 and 100 mpk), the LXR agonist (T0901317, 30 mpk) or vehicle alone for 14 days (N=7/group). Data normalized to 28S gene are expressed as a percentage of the wild type mice group treated with vehicle alone, to which an arbitrary value of 100 was attributed. * p<0.05, **p<0.01 versus wild type mice treated with vehicle, § p<0.05 versus LXR α / β -deficient mice treated with vehicle.

**Figure 6**

LXR, but not RXR agonist treatment increases RXR recruitment to the LXRE of cholesterol genes in vivo

ChIP assays were performed using antibodies against RXR (A) or RNA polymerase II (B) on livers of LXR α / β -deficient mice and wild type mice treated with vehicle alone, bexarotene (100 mpk) or T0901317 (30 mpk). Recovery was determined by real-time PCR using primers positioned at the LXRE of ABCA1, ABCG1 and at two putative LXREs of ABCG5 and ABCG8 (A) or primers located in the body of the gene (B). β -globin was used as a control. *p<0.05, **p<0.01 versus wild type mice treated with vehicle.

A**B**