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LXR inverse agonists demonstrate liver lipid lowering effects through multiple mechanisms in rodent models of NASH and in human hepatocytes

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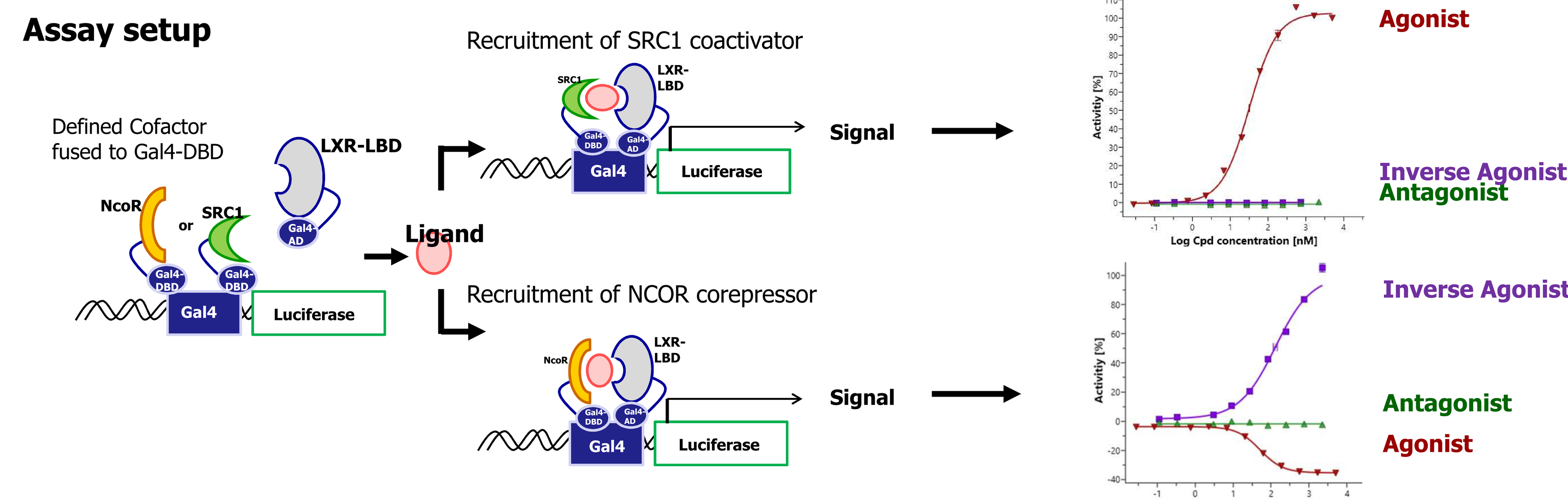
BACKGROUND & AIMs

Several mechanisms are currently evaluated as potential pharmacotherapies for the spectrum of non-alcoholic fatty liver disease (NAFLD), including modulators of nuclear receptors such as PPARα/γ/δ or FXR. Activation of Liver X Receptor (LXR) in the liver by potent, synthetic agonists is known to result in severe steatosis and hypertriglyceridemia in various animal models[1,2] and in humans[3]. Thus, we have designed and synthesized LXR inverse agonists with the aim to inhibit LXR´s pro-steatotic transcriptional activity. The pharmacological effects of these LXR inverse agonists were evaluated in human hepatocytes and in a mouse and a rat steatosis model. These first results confirm the findings by another group[4,5], that synthetic LXR inverse agonists can reduce liver fat content which may provide a new mechanism for the treatment of NAFLD / NASH.

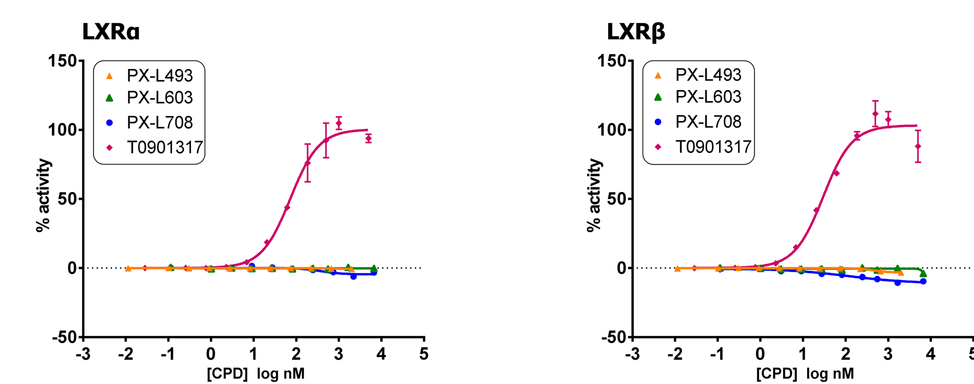
IN VITRO ACTIVITIES

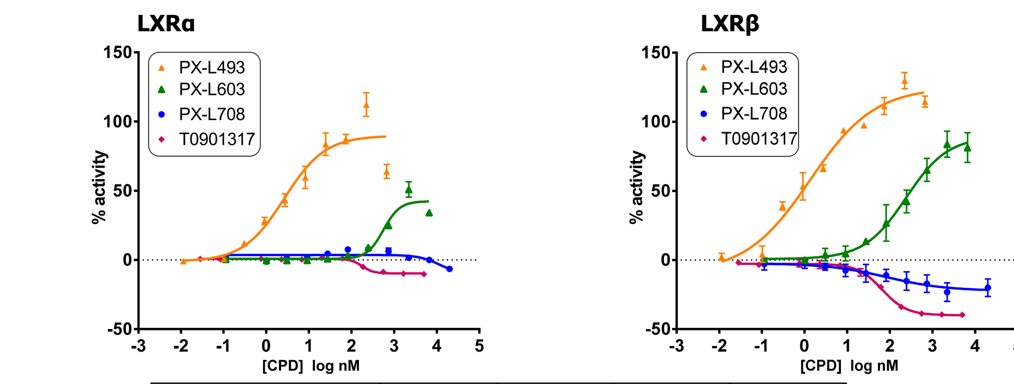
Cellular reporter assay that differentiates between agonists, antagonists and inverse agonists of LXRα or LXRβ



Gal4-LXR-2-hybrid assay using a Gal4 driven luciferase. Corepressors or coactivators were transfected as fusions to Gal4 DNA-binding domain, LXR-LBD as fusion to transactivation domain.

Recruitment of SRC1 coactivator peptide





SCR1 recruitement	PX-L493	PX-L603	PX-L708	T0901317
AC_{50} [nM] LXRalpha	inactive	inactive	inactive	72.7
AC_{50} [nM] LXRbeta	inactive	inactive	inactive	29.9

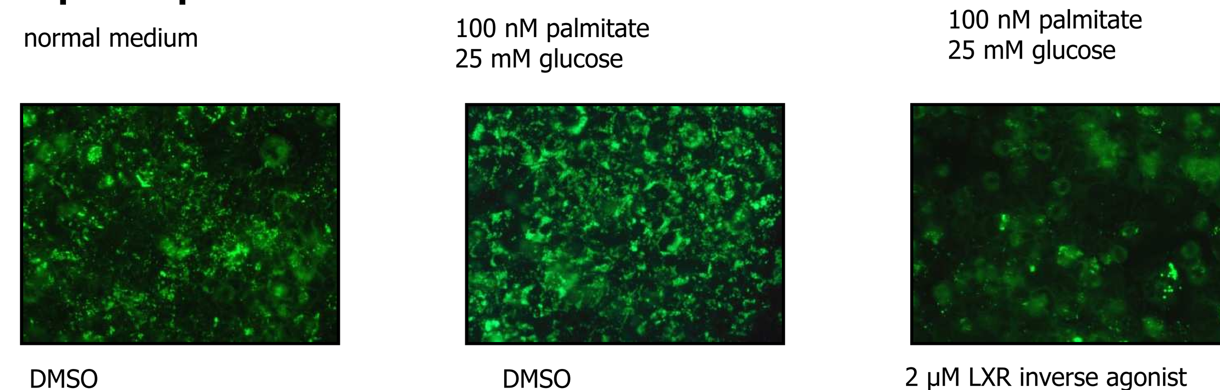
Recruitment of NCOR coactivator peptide

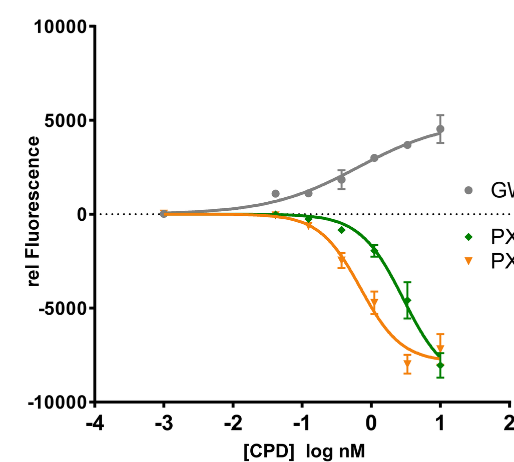
NCOR recruitement	PX-L493	PX-L603	PX-L708	T0901317
AC_{50} [nM] LXRalpha	5.3	966	inactive	177.1
AC_{50} [nM] LXRbeta	1.4	326	inactive	66.2

Compounds PX-L493 and PX-L603 were characterized in cellular reporter assays as inverse agonists of LXRα and LXRβ. Obtained AC_{50} for LXR(α/β) in NCoR recruitment Gal4 2-hybrid assay: PX-L493 (5.3/1.4 nM) ; PX-L603 (966/326 nM)

Anti steatotic effects in primary human hepatocytes

Lipid droplet accumulation





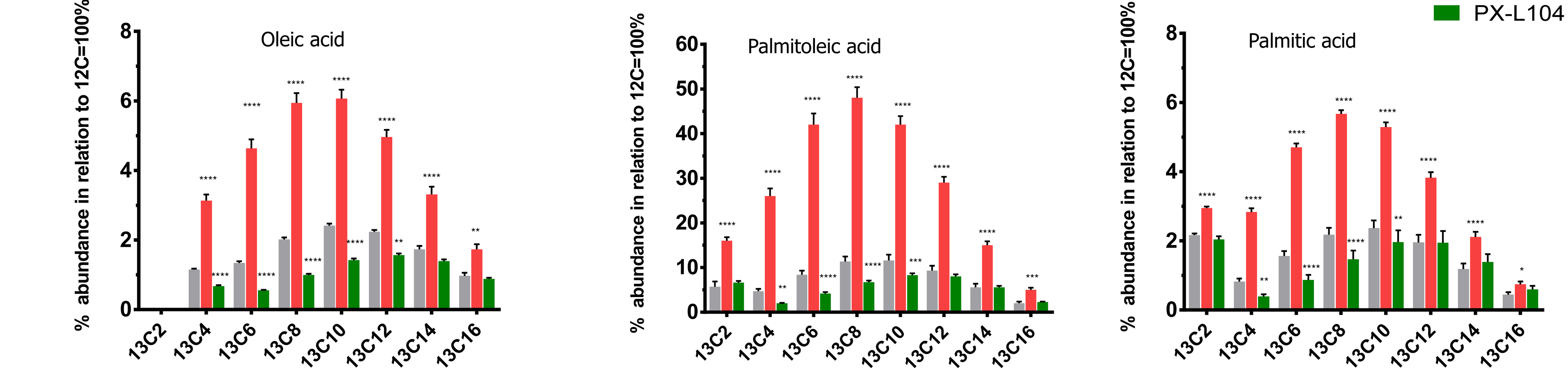
	PX-L493	PX-L603	GW3965
AC_{50} [µM]	0.7	2.9	0.7

Human primary Upcyte® hepatocytes were cultivated in the presence of 100 nM palmitate and 25 mM glucose for 5 days. The addition of LXR inverse agonists PX-L603 and PX-L493 dose-dependently reduced the hepatocyte lipid load as determined by Bodipy™ staining and fluorescence measurements.

IN VITRO ACTIVITIES

De novo lipogenesis (DNL)

Mass isotope distribution analysis (MIDA)

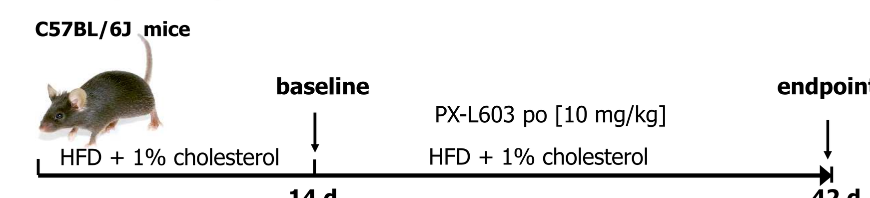


In vitro de novo lipogenesis was assed using mass isotopomer distribution analysis (MIDA) following [^{13}C]-acetate treatment of hepatocytes. Upcyte hepatocytes were cultivated in the presence of 100 mM [^{13}C]-acetate and LXR Agonist GW3965 (0.5 µM) or LXR inverse Agonist PX-L104 (0.5 µM) (AC50 for LXR(α/β) in NCoR recruitment Gal4 2-hybrid assay 78 nM/ 4,6 nM) for 72 h. To quantify the de novo lipid biosynthesis, incorporation of isotope was determined using HR-LC/MS after fatty acid isolation.

IN VIVO ACTIVITIES

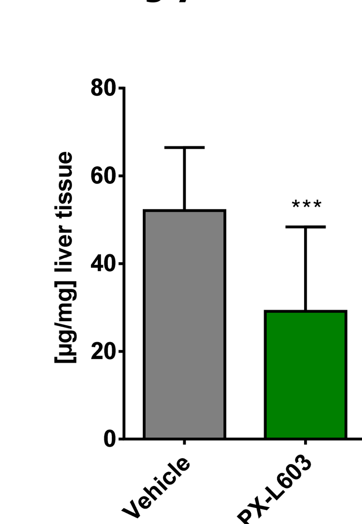
LXR inverse agonists show liver lipid lowering in high fat diet fed mice

Study design

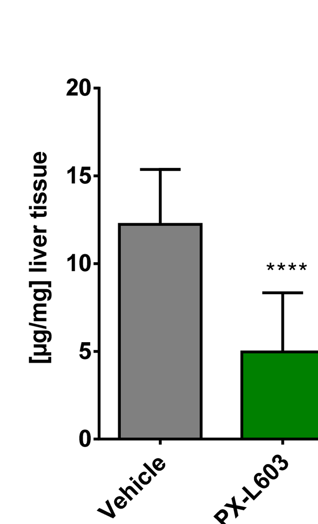


C57Bl/6 mice were maintained on a Survit-type high fat diet with 1% cholesterol for two weeks. PX-L603 (10 mg/kg, po) LXR inverse agonists or vehicle were administered for the following 4 weeks on the same diet. Hepatic triglyceride content was significantly reduced from 52.1(±19.3) µg/mg to 29.1 (±14.4) µg/mg. Total cholesterol in the livers of animals treated with PX-L603 was 5.0 (±3.4) µg/mg compared to 12.2 (±3.1) µg/mg of vehicle treated animals.

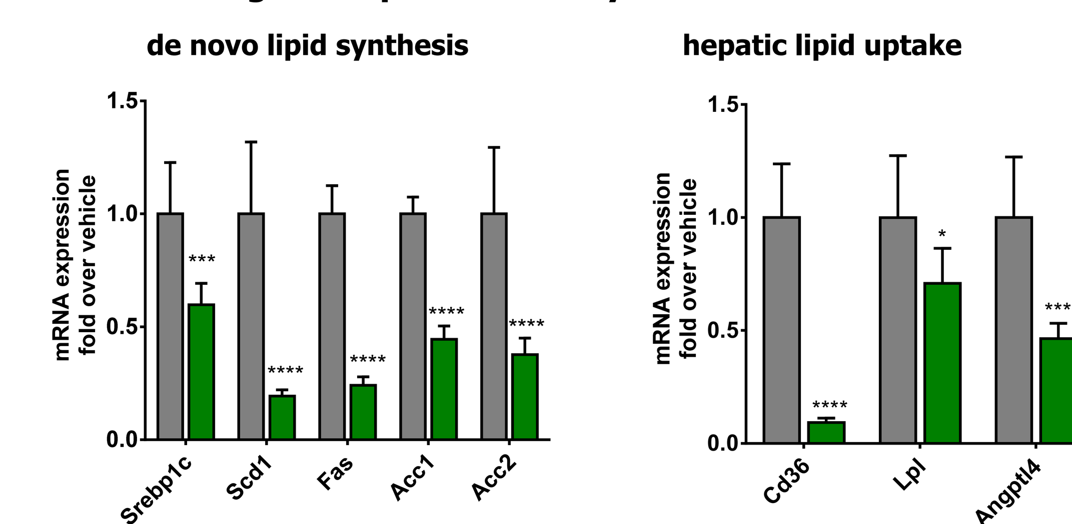
Liver triglyceride



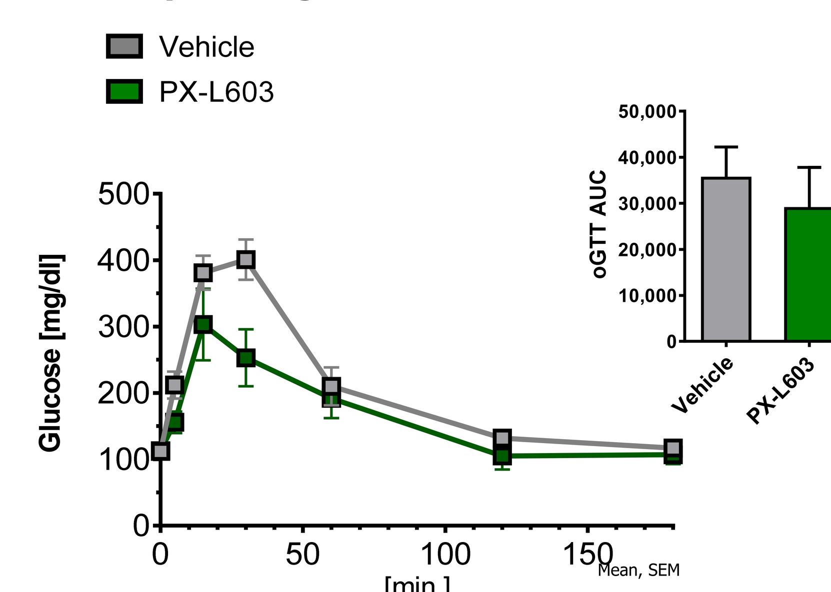
Liver cholesterol



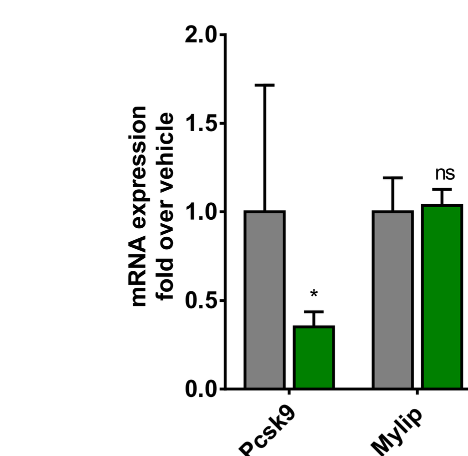
Mouse liver gene expression analysis

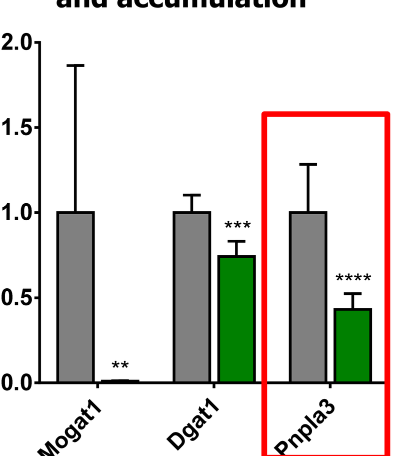


oGTT – plasma glucose levels



PX-L603 shows lowering in plasma glucose after oral challenge compared to vehicle.



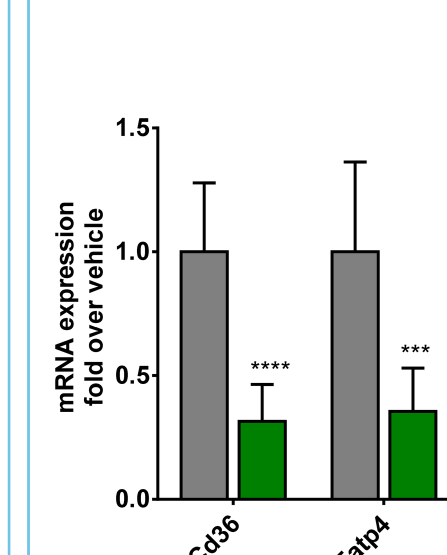


Gene expression analysis of the liver tissue from the mouse study revealed that LXR inverse agonist PX-L603 repressed known LXR target genes involved in fatty acid synthesis and lipid homeostasis such as Scd1, Fas, Cd36 or Angptl4. It is noteworthy, that PX-L603 treatment reduced the mRNA expression of **Pnpla3** with high significance. Pnpla3 expression is highly associated with human NAFLD development.

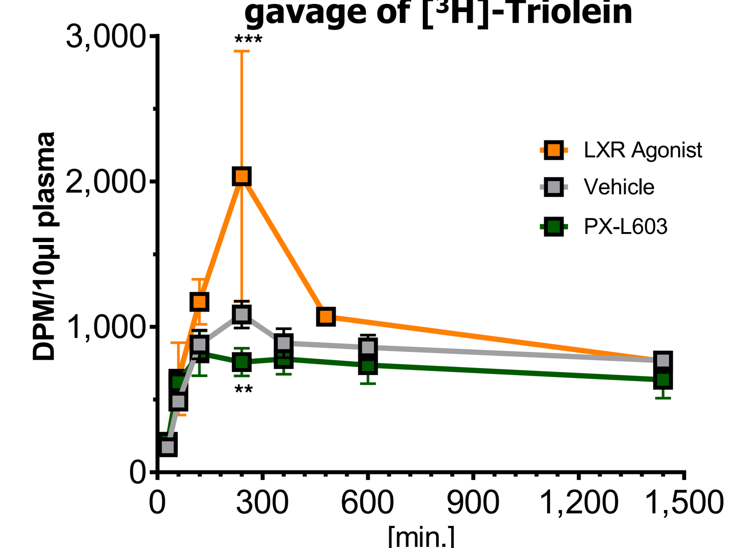
IN VIVO ACTIVITIES

Reduced intestinal fatty acid uptake upon LXR inverse agonism

Intestinal gene regulation

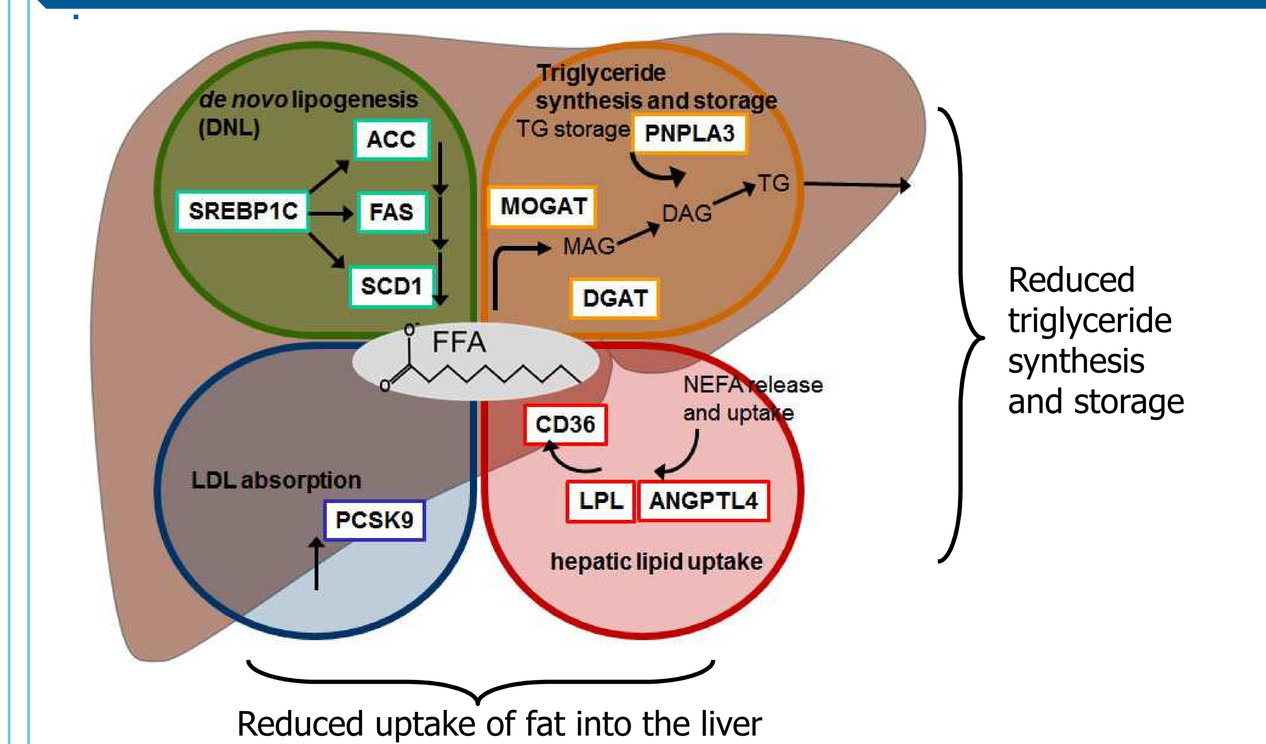


[^{3}H]-counts in plasma after oral gavage of [^{3}H]-Triolein



Gene expression analysis of mouse intestinal segments revealed that LXR inverse agonist PX-L603 repressed Cd36 and Fatp4 mRNA expression. Both are genes involved in intestinal lipid uptake.

CONCLUSION



Inhibition of LXR´s transcriptional activity by synthetic inverse agonists results in:

- Inhibition of *de novo* lipogenesis (DNL)
- Reduction of free fatty acid (FFA) release from chylomicrons and reduced FFA uptake
- Reduced triglyceride synthesis through downregulation of Mogat and Dgat
- Downregulation of Pnpla3 expression, an enzyme with proven clinical significance in NASH patients

→ ultimately resulting in reduced liver fat.

→ **This suggests that inhibition of the LXR pathway in the liver is a useful novel approach for a pharmacotherapy of NAFLD.**

REFERENCES

1: Schultz et al., *Genes Dev.* 14(22):2831-8. (2000); 2: Cha & Repa, *J Biol Chem.* 282(1):743-51. (2007);3: Kirchgessner, et al., Cell Metab. 24(2):223-33. (2016);4: Griffett et al., ACS Chem Biol. 8(3):559-67. (2013);5: Griffett et al., Mol Metab. 4(4):353-7. (2015)