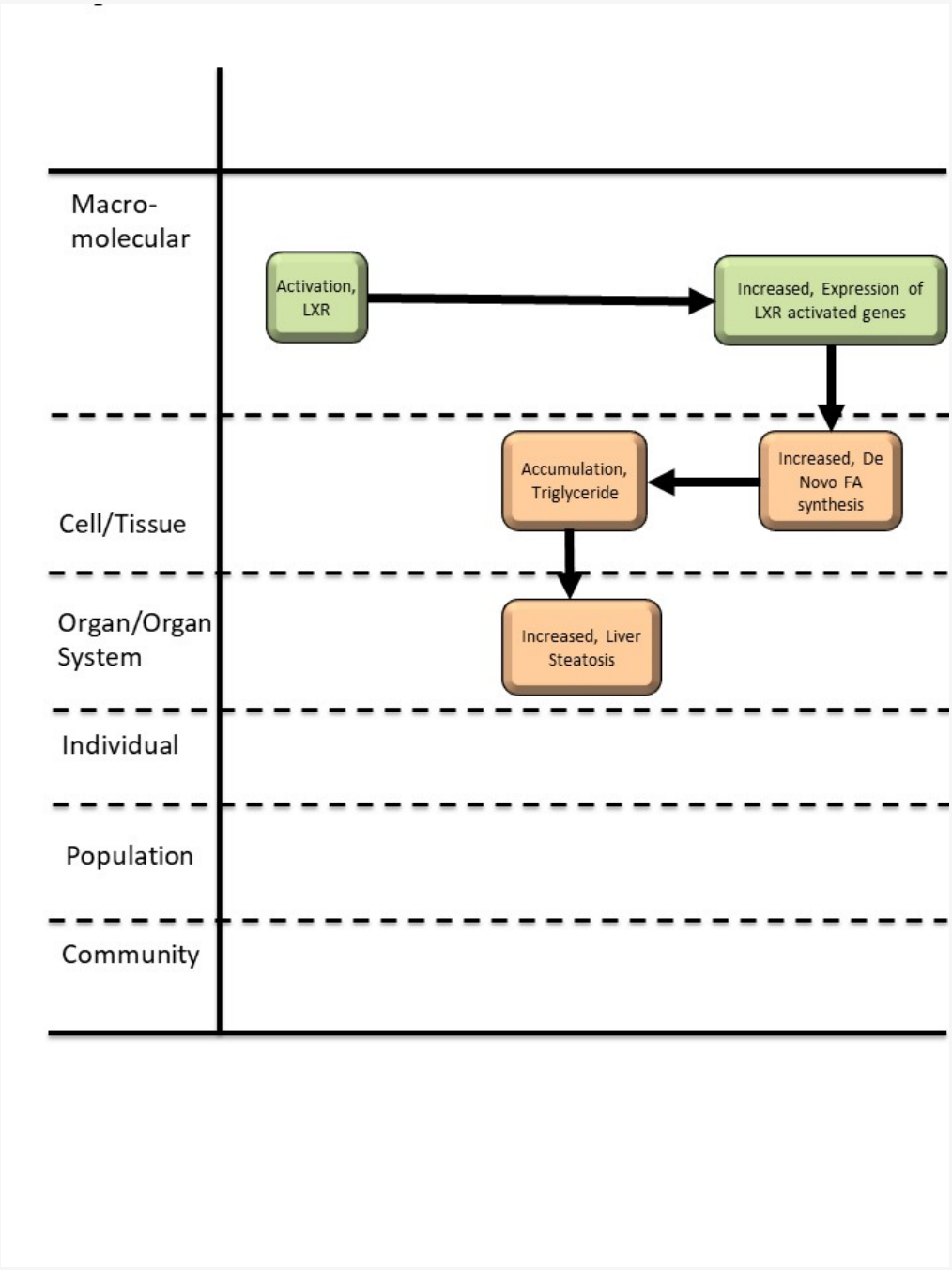
AOP ID and Title:

AOP 518: Liver X Receptor (LXR) activation leads to liver steatosis
Short Title: LXR activation leads to liver steatosis

Graphical Representation



Authors

Of the originating work: Brigitte Landesmann, Marina Goumenou, Sharon Munn and Maurice Whelan, Joint Research Centre, European Commission, Ispra, Italy

Of the content populated in the AOP-Wiki: John R. Frisch and Travis Karschnik, General Dynamics Information Technology, Duluth, Minnesota; Daniel L. Villeneuve, US Environmental Protection Agency, Great Lakes Toxicology and Ecology Division, Duluth, MN

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite			

Author status	OECD status	OECD project	SAAOP status

Abstract

Liver X receptor (LXR) belongs to a class of nuclear receptors [Arhyl hydrocarbon receptor (AHR), Constitutive androstane receptor (CAR), Oestrogen receptor (ER), Farnesoid X receptor (FXR), Glucocorticoid receptor (GR), Peroxisome proliferator-activated receptor (PPAR), Pregnane X receptor (PXR), Retinoic acid receptor (RAR)] that are needed for normal liver function, but for which increased expression (i.e. activation by binding by chemical stressors) lead to liver injury, including steatosis (Mellor *et al.* 1996). An increasing number of chemical stressors have been shown to increase LXR expression (Moya *et al.* 2020). Activation of LXR has been linked to increased expression of a group of genes (ChREBP, SREBP-1c, FAS and SCD1) involved in increasing *de novo* fatty acid synthesis (Mellor *et al.* 1996, Schultz *et al.* 2000, Postic and Girard 2008). Increases in *de novo* fatty acid synthesis is one of the main pathways for increases in triglycerides in livers (Angrish *et al.* 2016). Increases in triglycerides can result in decreased mitochondrial biochemical function or histological changes in mitochondria structure, ultimately resulting in steatosis as a primary adverse outcome (Angrish *et al.* 2016; Mellor *et al.* 1996).

Background

This Adverse Outcome Pathway (AOP) focuses on the pathway in which activation of Liver X receptor (LXR) leads to liver steatosis through increased *de novo* fatty acid synthesis. Environmental stressors result in activation of nuclear receptors linked to increases in triglyceride accumulation through several pathways. One of the primary pathways linked to triglyceride accumulation, and focus of this AOP, is through activation of the LXR gene and coordinated molecular responses leading to increased fatty acid synthesis. This pathway has been particular well studied in mammals (humans, lab mice, lab rats).

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	167	Activation, LXR	Activation, LXR
	KE	2199	Increased, Expression of LXR activated genes	Increased, Expression of LXR activated genes
	KE	89	Synthesis, De Novo FA	Synthesis, De Novo FA
	KE	291	Accumulation, Triglyceride	Accumulation, Triglyceride
	AO	459	Increased, Liver Steatosis	Increased, Liver Steatosis

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Activation, LXR	adjacent	Increased, Expression of LXR activated genes	Moderate	Not Specified
Increased, Expression of LXR activated genes	adjacent	Synthesis, De Novo FA	Moderate	Not Specified
Synthesis, De Novo FA	adjacent	Accumulation, Triglyceride	Moderate	Not Specified
Accumulation, Triglyceride	adjacent	Increased, Liver Steatosis	Moderate	Not Specified

Overall Assessment of the AOP

1. Support for Biological Plausibility of Key Event Relationships: Is there a mechanistic relationship between KEup and KEdown consistent with established biological knowledge?	
Key Event Relationship (KER)	Level of Support Strong = Extensive understanding of the KER based on extensive previous documentation and broad acceptance.
Relationship 3103: Activation, LXR leads to Increased, Expression of LXR activated genes	**Moderate support.** The relationship between activation of Liver X receptor and genes linked to regulation of *de novo* fatty acid synthesis is broadly accepted and consistently supported across taxa.
Relationship 3104: Increased, Expression of	**Moderate support.** The relationship between Increased, Expression of LXR activated genes

Relationship 3164: Increased, Expression of LXR activated genes leads to Synthesis, De Novo FA	**Moderate support.** The relationship between Increased, Expression of LXR activated genes and Increased *de novo* fatty acid synthesis is broadly accepted and consistently supported across taxa.
Relationship 110: Synthesis, De Novo FA leads to Accumulation, Triglyceride	**Strong support.** Increased *de novo* fatty acid synthesis is broadly recognized as a major pathway leading to accumulation of triglycerides, and consistently supported across taxa.
Relationship 2265: Accumulation, Triglyceride leads to Increased, Liver Steatosis	**Strong support.** The relationship between accumulation of triglycerides and liver steatosis is broadly accepted and consistently supported across taxa.
Overall	**Strong support**. Extensive understanding of the relationships between events from empirical studies from a variety of taxa, including frequent testing in lab mammals.

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adults	High
Juvenile	Moderate

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Sex Applicability

Sex	Evidence
Unspecific	High

Life Stage: The life stage applicable to this AOP is all life stages with a liver. Older individuals are more likely to manifest this adverse outcome pathway (adults > juveniles) due to accumulation of triglycerides.

Sex: This AOP applies to both males and females.

Taxonomic: This AOP appears to be present broadly in vertebrates, with most representative studies in mammals (humans, lab mice, lab rats).

Essentiality of the Key Events

2. Essentiality of Key Events: Are downstream KEs and/or the AO prevented if an upstream KE is blocked?	
Key Event (KE)	Level of Support Strong = Direct evidence from specifically designed experimental studies illustrating essentiality and direct relationship between key events. Moderate = Indirect evidence from experimental studies inferring essentiality of relationship between key events due to difficulty in directly measuring at least one of key events.
MIE 167 Activation, LXR	**Moderate support.** Activation of Liver X receptor is a primary activator for increases in genes linked to regulation of *de novo* fatty acid synthesis. However, expression of these genes can be elicited by other nuclear receptors and molecular processes.
KE 2199 Increased, Expression of LXR activated genes	**Moderate support.** Increased, expression of LXR activated genes is one pathway linked to increases in *de novo* fatty acid synthesis. However, a variety of molecular signals and corresponding cellular changes are required in order for *de novo* fatty acid synthesis to increase.
KE 89 Synthesis, De Novo FA	**Moderate support.** Increase in *de novo* fatty acid synthesis is a primary factor in increased triglyceride levels in cells. However, triglycerides increase in cells via a number of pathways, including increased triglyceride influx into cells.
KE 291 Accumulation, Triglyceride	**Strong support.** Accumulation of triglyceride is linked to liver steatosis. Evidence is available from toxicant, gene-knockout, and high lipid diet studies.
AO 459 Increased, Liver Steatosis	**Strong support.** Liver steatosis occurs due to a variety of stressors and breakdown of multiple biochemical pathways and physiological changes with resulting increases in triglyceride levels. Evidence is available from toxicant and high lipid diet studies.

Weight of Evidence Summary

3. Empirical Support for Key Event Relationship: Does empirical evidence support that a change in KEup leads to an appropriate change in

KEdown?

Key Event Relationship (KER)	Level of Support Strong = Experimental evidence from exposure to toxicant shows consistent change in both events across taxa and study conditions.
Relationship 3103: Activation, LXR leads to Increased, Expression of LXR activated genes	**Moderate support.** Increases in Liver X receptor expression lead to increases in genes linked to regulation of *de novo* fatty acid synthesis, primarily from studies examining TOXCAST data, as well as changes in gene expression levels after exposure to chemical stressors.
Relationship 3104: Increased, Expression of LXR activated genes leads to Synthesis, De Novo FA	**Weak support.** Increases in expression of LXR activated genes lead to increases in *de novo* fatty acid synthesis, primarily through measured increases in gene expression and increased triglyceride levels. Increased *de novo* fatty acid synthesis is inferred from increased triglyceride levels rather than directly observed.
Relationship 110: Synthesis, De Novo FA leads to Accumulation, Triglyceride	**Strong support.** Increases in *de novo* fatty acid synthesis is recognized as a primary pathway to accumulation of triglycerides.
Relationship 2265: Accumulation, Triglyceride leads to Increased, Liver Steatosis	**Strong support**. Increases in accumulation of triglyceride is recognized as a primary pathway to liver steatosis.
Overall	**Strong support**. Exposure from empirical studies shows consistent change in both events from a variety of taxa, including frequent testing in lab mammals.

References

Angrish, M.M., Kaiser, J.P., McQueen, C.A., and Chorley, B.N. 2016. Tipping the Balance: Hepatotoxicity and the 4 Apical Key Events of Hepatic Steatosis. Toxicological Sciences 150(2): 261-268.

Mellor, C.L., Steinmetz, F.P., and Cronin, T.D. 2016. The identification of nuclear receptors associated with hepatic steatosis to develop and extend adverse outcome pathways. Critical Reviews in Toxicology, 46(2): 138-152.

Moya, M., Gomez-Lechon, M.J., Castell, J.V., and Jovera, R. 2010. Enhanced steatosis by nuclear receptor ligands: A study in cultured human hepatocytes and hepatoma cells with a characterized nuclear receptor expression profile. Chemico-Biological Interactions 184: 376–387.

Landesmann, B., Goumenou, M., Munn, S., and Whelan, M. 2012. Description of Prototype Modes-of-Action Related to Repeated Dose Toxicity. European Commission Report EUR 25631, 49 pages. https://op.europa.eu/en/publication-detail/-/publication/d2b09726-8267-42de-8093-8c8981201d65/language-en

Postic, C. and Girard, J. 2008. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. The Journal of Clinical Investigation 118(3): 829-838.

Schultz, J.R., Tu, H., Luk, A., Repa, J.J., Media, J.C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D.J., Lustig, K.D., and Shan, B. 2000. Role of LXRs in control of lipogenesis. Genes and Development 14:2831–2838.

Appendix 1

List of MIEs in this AOP

Event: 167: Activation, LXR

Short Name: Activation, LXR

Key Event Component

Process	Object	Action
signaling	oxysterols receptor LXR-beta	increased
signaling	oxysterols receptor LXR-alpha	increased

AOPs Including This Key Event

AOP ID and Name	Event Type

AOP ID and Name	Event Type
Aop:34 - LXR activation leading to hepatic steatosis	MolecularInitiatingEvent
Aop:58 - NR1I3 (CAR) suppression leading to hepatic steatosis	MolecularInitiatingEvent
Aop:518 - Liver X Receptor (LXR) activation leads to liver steatosis	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

hepatocyte

Key Event Description

The LXR receptor

Liver X receptors are ligand-activated transcription factors of the nuclear receptor superfamily first identified in 1994 in rat liver (Apfel et al. 1994, Song 1994). There are two LXR isoforms termed a and ß (NR1H3 and NR1H2) which upon activation form heterodimers with retinoid X receptor (RXR) and bind to the LXR response element found in the promoter region of the target genes (Baranowski 2008). LXRs were shown to function as sterol sensors protecting the cells from cholesterol overload by stimulating reverse cholesterol transport and activating its conversion to bile acids in the liver (Baranowski 2008).

LXRa expression is restricted to liver, kidney, intestine, fat tissue, macrophages, lung, and spleen and is highest in liver, hence the name liver X receptor a (LXRa). LXRβ is expressed in almost all tissues and organs, hence the early name UR (ubiquitous receptor) (Ory 2004). The different pattern of expression suggests that LXRa and LXRβ have different roles in regulating physiological function. This is also supported from the observation that LXRa deficient mice do not develop hepatic steatosis when treated with LXR agonist that activates both types (Lund et al. 2006) and consequently the role of the two isoforms in relation to adverse effects could be different.

The molecular initiating event

Generally speaking chemicals that are able to act through NRs are usually specific ligands. These chemicals are mainly lipophilic and they mimic the action of natural hormones. However, in some cases hydrophilic chemicals (like phthalates) are also capable to act as ligands in NRs due to the molecular structure of the proteins and the pocket sites of the receptors.

The molecular initiating event in the presented MoA is the binding to the LXR or the permissive RXR of the LXR-RXR dimer leading to activation. LXR activation can be achieved via a wide range of endogenous neutral and acidic ligands as shown by crystallographic analysis (Williams et al. 2003). There are known endogenous but also synthetic ligands that can act as agonists. Endogenous agonists for this receptor are the oxysterols (oxidized cholesterol derivatives like 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 27-hydroxycholesterol, and cholestenoic acid) mainly with similar affinity for the two isoforms (Baranowski 2008). Oxysterols bind directly to the typical hydrophobic pocket in the C-terminal domain (Williams et al. 2003). Other endogenous ligands are the D-glucose and D-Glucose-6-phosphate (Mitro 2007). However, the hydrophilic nature of glucose and its low affinity for LXR present a challenge to the central dogma about the nature of the NR-ligand interaction (Lazar & Wilson 2007). Unsaturated fatty acids have also been shown to bind and regulate LXRa activity in cells. However, in contrast to the role of oxysterols, the biological relevance of this observation has not been established in vivo (Pawar et al. 2003). The function of LXRs is also modulated by many currently used drugs such as statins, fibrates, and thazolidinedione derivatives (Jamroz-Wiśniewska et al. 2007). Some synthetic LXR agonists have been developed like the non-steroidal agonists T0901317 and GW3965 (Schultz et al 2000, Collins et al. 2002). LXR forms a permissive dimer with the RXR which means that chemicals that can activate this receptor can trigger the same pathway as the LXR agonists. The endogenous RXR agonist is 9-cis-retinoic acid (Heyman et al. 1992) while synthetic agonists include LGD1069 and LG100268 (Boehm et al. 1994 and 1995).

In addition to the agonist binding in the LXR there are other mechanisms for its control. LXRa gene promoter contains also functional peroxisome proliferator response element (PPRE) and peroxisome proliferator-activated receptor (PPAR) a and γ agonists were shown to stimulate LXRa expression in human and rodent (Baranowski 2008). Control of the LXRa expression is also dependent on insulin and post-translationally by protein kinase A that phosphorylates receptor protein at two sites thereby impairing its dimerization and DNA-binding (Baranowski 2008).

Identification of the site of action

As already mentioned above LXR isoforms are expressed in various tissues but in relation to the presented MoA we refer to LXRs that are expressed in the hepatocytes.

Nuclear receptors may be classified into two broad classes according to their sub-cellular distribution in the absence of ligand. Type I NRs (like ER and AhR) are located in the cytosol (and they are translocated into the nucleus after ligand binding) while type II NRs like LXRs (but also PXR, PPARa and PPARγ) are located in the nucleus of the cell.

The specific site of binding and the affinity of a ligand for the LXRs depend on the structure of the ligand.

Binding in the LXREs and target genes transcription

Upon ligand-induced activation both isoforms form obligate heterodimers with the retinoid X receptor (RXR) and regulate gene expression through binding to LXR response elements (LXREs) in the promoter regions of the target genes (Fig. 1). The LXRE consists of two idealized hexanucleotide sequences (AGGTCA) separated by four bases (DR-4 element).

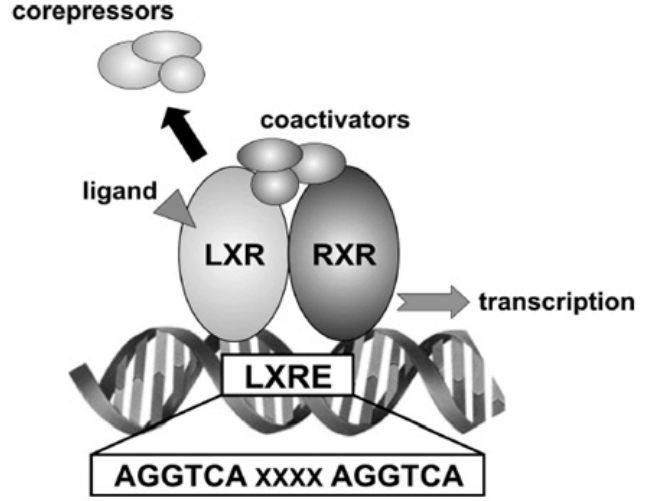


Figure 1. Mechanism of transcriptional regulation mediated by LXRs. RXR - retinoid X receptor, LXRE - LXR response element (Baranowski 2008)

Target genes of LXRs are involved in cholesterol and lipid metabolism regulation ([1], [2]) including:

- ABC - ATP Binding Cassette transporter isoforms A1, G1, G5, and G8
- ApoE - Apolipoprotein E
- CETP - Cholesterylester Transfer Protein
- CYP7A1 - Cytochrome P450 isoform 7A1 - cholesterol 7a-hydroxylase
- FAS - Fatty Acid Synthase
- LPL - Lipoprotein Lipase
- LXR-a - Liver X Receptor-a
- SREBP-1c - Sterol Response Element Binding Protein 1c
- ChREBP - Carbohydrate Response Element Binding Protein
- FAT/CD36 – Fatty acid uptake transporter (liver)

Auto-regulation of the LXRa

Human specific auto-regulated expression specifically of the LXRa has been demonstrated from several studies (Laffitte et al. 2001, Whitney et al. 2001, Li et al. 2002, Kase et al. 2007). Human LXRa gene promoter has a functional LXRE activated by both LXRa and β. In addition human liver LXRa expression is induced by both natural and synthetic LXR agonists.

References

1. ↑ Peet 1998 - Peet D.J., Cholesterol and Bile Acid Metabolism Are Impaired in Mice Lacking the Nuclear Oxysterol Receptor LXRa in mammals, Cell, 93, 693–704, 1998
2. ↑ Edwardsa et al. 2002 - Edwardsa P.A., et al, LXRs; Oxysterol-activated nuclear receptors that regulate genes

controlling lipid homeostasis, (Oxidized Lipids as Potential Mediators of Atherosclerosis), Vascular Pharmacology, 38 (No 4), 249–256, 2002

List of Key Events in the AOP

Event: 2199: Increased, Expression of LXR activated genes

Short Name: Increased, Expression of LXR activated genes

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:518 - Liver X Receptor (LXR) activation leads to liver steatosis	KeyEvent

Biological Context

Level of Biological Organization
Molecular

Event: 89: Synthesis, De Novo FA

Short Name: Synthesis, De Novo FA

Key Event Component

Process	Object	Action
fatty acid biosynthetic process	fatty acid	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:34 - LXR activation leading to hepatic steatosis	KeyEvent
Aop:518 - Liver X Receptor (LXR) activation leads to liver steatosis	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
hepatocyte

Key Event Description

A number of pathways and a great number of enzymes like GK, L-PK, ACC, FAS and SCD-1 are involved in the de novo FA synthesis [1]. As it is already discussed above these enzymes are induced by LXR agonists (FAS, SCD1), the SREBP-1c (GK, ACC, FAS) and the ChREBP (L-PK, ACC, FAS) leading to enhancement of the de novo FA synthesis.

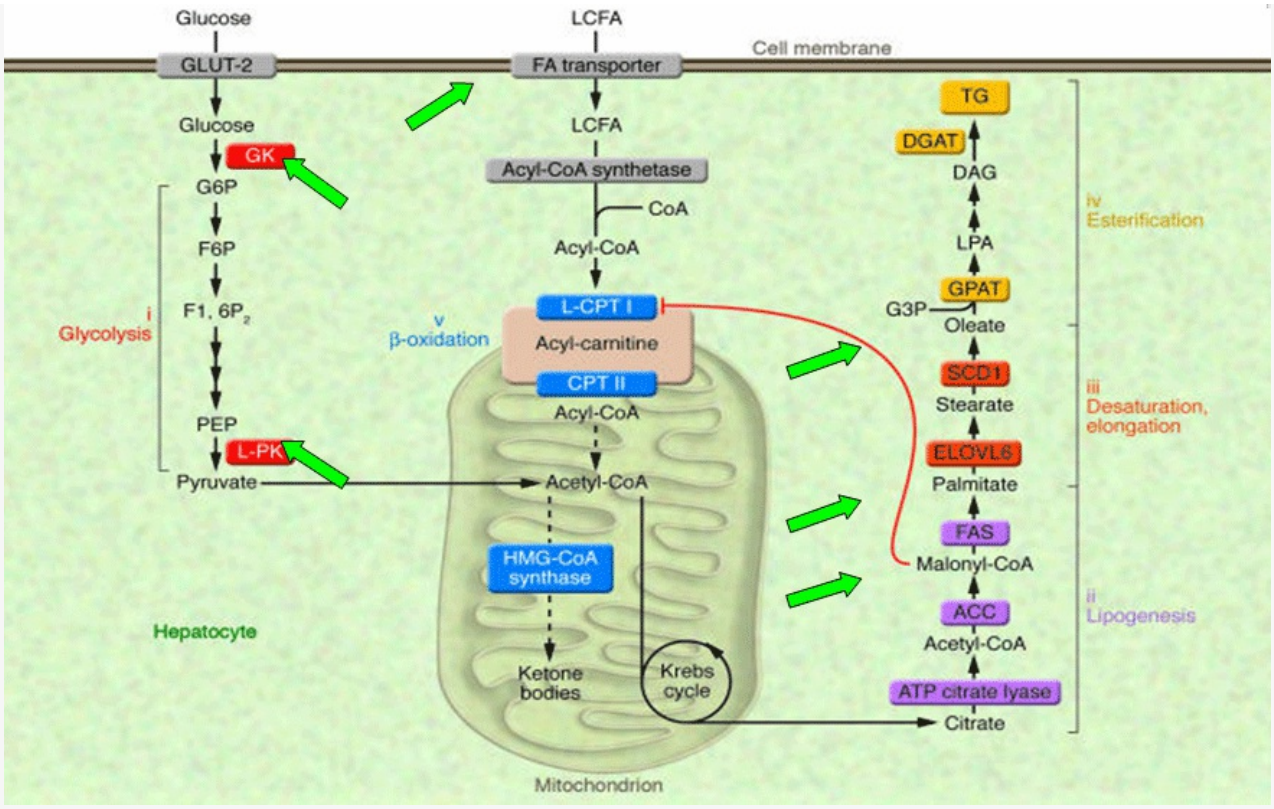


Figure 1. Metabolic pathway for de novo FA synthesis and TG formation [1]

As proposed from Diraison et al 1997 the de novo FA synthesis contributes maximum 5% to the synthesis of FA and TG under normal conditions. Conditions associated with high rates of lipogenesis, such as low fat - high carbohydrate (LF/HC) diet, hyperglycemia, and hyperinsulinemia are associated with a shift in cellular metabolism from lipid oxidation to TG esterification, thereby increasing the availability of TGs derived from VLDL synthesis and secretion.

References

1. ↑ 1.0 1.1 Postic & Girard 2008 - Postic C., Girard J., Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice, J. Clin. Invest. 118 (No 3), 829–838, 2008

Event: 291: Accumulation, Triglyceride

Short Name: Accumulation, Triglyceride

Key Event Component

Process	Object	Action
	triglyceride	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:34 - LXR activation leading to hepatic steatosis	KeyEvent
Aop:57 - AhR activation leading to hepatic steatosis	KeyEvent
Aop:318 - Glucocorticoid Receptor activation leading to hepatic steatosis	KeyEvent
Aop:517 - Pregnane X Receptor (PXR) activation leads to liver steatosis	KeyEvent
Aop:518 - Liver X Receptor (LXR) activation leads to liver steatosis	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

hepatocyte

Key Event Description

Leads to Fatty Liver Cells.

List of Adverse Outcomes in this AOP

Event: 459: Increased, Liver Steatosis

Short Name: Increased, Liver Steatosis

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:58 - NR1I3 (CAR) suppression leading to hepatic steatosis	AdverseOutcome
Aop:60 - NR1I2 (Pregnane X Receptor, PXR) activation leading to hepatic steatosis	AdverseOutcome
Aop:61 - NFE2L2/FXR activation leading to hepatic steatosis	AdverseOutcome
Aop:62 - AKT2 activation leading to hepatic steatosis	AdverseOutcome
Aop:36 - Peroxisomal Fatty Acid Beta-Oxidation Inhibition Leading to Steatosis	AdverseOutcome
Aop:213 - Inhibition of fatty acid beta oxidation leading to nonalcoholic steatohepatitis (NASH)	KeyEvent
Aop:285 - Inhibition of N-linked glycosylation leads to liver injury	KeyEvent
Aop:318 - Glucocorticoid Receptor activation leading to hepatic steatosis	AdverseOutcome
Aop:517 - Pregnane X Receptor (PXR) activation leads to liver steatosis	AdverseOutcome
Aop:518 - Liver X Receptor (LXR) activation leads to liver steatosis	AdverseOutcome

Biological Context

Level of Biological Organization

Organ

Organ term

Organ term

liver

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Vertebrates	Vertebrates	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Steatosis is the result of perturbations in well-known metabolic pathways that are well-studied and well-known in many taxa.

Key Event Description

Biological state: liver steatosis is the inappropriate storage of fat in hepatocytes.

Biological compartment: steatosis is generally an organ-level diagnosis; however, the pathology occurs within the hepatocytes.

Role in biology: steatosis is an adverse endpoint.

Description from EU-ToxRisk:

Activation of stellate cells results in collagen accumulation and change in extracellular matrix composition in the liver causing fibrosis. (Landesmann, 2016)(Koo et al 2016)

How it is Measured or Detected

Steatosis is measured by lipidomics approaches that measure lipid levels, or by histology.

Regulatory Significance of the AO

Steatosis is a regulatory endpoint and has been used as an endpoint in many US EPA assessments, including IRIS assessments.

References

Landesmann, B. (2016). Adverse Outcome Pathway on Protein Alkylation Leading to Liver Fibrosis, (2).

https://doi.org/10.1016/j.molcel.2005.08.010

Koo, J. H., Lee, H. J., Kim, W., & Kim, S. G. (2016). Endoplasmic Reticulum Stress in Hepatic Stellate Cells Promotes Liver Fibrosis via PERK-Mediated Degradation of HNRNPA1 and Up-regulation of SMAD2. *Gastroenterology*, *150*(1), 181–193.e8. https://doi.org/10.1053/j.gastro.2015.09.039

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 3103: Activation, LXR leads to Increased, Expression of LXR activated genes

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Liver X Receptor (LXR) activation leads to liver steatosis	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High
Juvenile	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Key Event Relationship Description

Activation of Liver X receptor (LXR) gene expression has been shown to lead to increased gene expression and protein levels of loci associated with fatty acid synthesis, including Sterol regulatory element-binding protein (SRBEP), Fas cell surface death receptor (FAS), stearoyl-CoA desaturase 1 (SCD1), and Carbohydrate response element binding protein (CHREBP). Elevation of these molecular components increase the rate of fatty acid synthesis.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking increased LXR expression to expression of genes associated with fatty acid synthesis is moderate. Gene expression studies in mammalian systems have linked activation of LXR to increased gene expression and protein levels of Sterol regulatory element-binding protein (SRBEP), Fas cell surface death receptor (FAS), stearoyl-CoA desaturase 1 (SCD1), and Carbohydrate response element binding protein (CHREBP), associated with fatty acid synthesis.

Empirical Evidence

Species	Duration	Dose	Activation LXR?	Upregulation LXR activated genes?	Summary	Citation
Human *(Homo sapiens)*, lab mice (*Mus musculus*)	Up to 7 days	1 µM, 5 µM, and 10 uM T0901317, T0314407 (LXR agonists) for HEK293 cells, 5, 50 mg/kg bdwt T0901317 for mice	Yes	Yes	Increased LXR gene expression vs control in HEK293 cells and C57BL/6 mice, with correlated increases in CYP7A1, SCD-1, and SREBP-1 gene expression in a dose-dependent manner.	Schultz *et al.* (2000)
Human *(Homo sapiens)*, lab rat (*Rattus norvegicus*)	96 hours	0.3, 3, 30 nm Insulin plus 2 uM GW3965 (LXR agonist)	Yes	Yes	Increased LXR gene expression vs control in human and rat cells, with correlated increases in SREBP-1c, FASN, SCD1 in a dose-dependent manner.	Kotokorpi *et al.* (2007)

References

Kotokorpi, P., Ellis, E., Parini, P., Nilsson, L.-M., Strom, S., Steffensen, K.R., Gustafsson, J.-A., and Mode, A. 2007. Physiological Differences between Human and Rat Primary Hepatocytes in Response to Liver X Receptor Activation by 3-[3-[N-(2-Chloro-3-trifluoromethylbenzyl)-(2,2-diphenylethyl)amino]propyloxy]phenylacetic Acid Hydrochloride (GW3965). Molecular Pharmacology 72(4): 947-955.

Landesmann, B., Goumenou, M., Munn, S., and Whelan, M. 2012. Description of Prototype Modes-of-Action Related to Repeated Dose Toxicity. European Commission Report EUR 25631, 49 pages. https://op.europa.eu/en/publication-detail/-/publication/d2b09726-8267-42de-8093-8c8981201d65/language-en

Negi, C.K., Bajard, L., Kohoutek, J., and Blaha, L. 2021. An adverse outcome pathway based in vitro characterization of novel flame retardants-induced hepatic steatosis. Environmental Pollution 289: 117855.

Schultz, J.R., Tu, H., Luk, A., Repa, J.J., Medina, J.C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D.J., Lustig, K.D., and Shan, B. 2000. Role of LXRs in control of lipogenesis. Genes and Development 14:2831–2838.

Relationship: 3104: Increased, Expression of LXR activated genes leads to Synthesis, De Novo FA

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Liver X Receptor (LXR) activation leads to liver steatosis	adjacent	Moderate	Not Specified

Relationship: 110: Synthesis, De Novo FA leads to Accumulation, Triglyceride

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
LXR activation leading to hepatic steatosis	adjacent	Not Specified	
Liver X Receptor (LXR) activation leads to liver steatosis	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI
Mus musculus	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High
Juvenile	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Life Stage: All life stages with a liver. Older individuals are more likely to manifest this adverse outcome pathway (adults > juveniles) due to accumulation of triglycerides.

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly in vertebrates, with most representative studies in mammals (humans, lab mice, lab rats).

Key Event Relationship Description

De novo fatty acid synthesis is a main pathway broadly accepted as a mechanism for accumulation of triglycerides in cells. Chemical stressors or alteration of gene expression levels can trigger increased fatty acid influx, as well as changes to membrane permeability and membrane proteins that facilitate fatty acid transport.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking increased fatty acid synthesis to accumulation of triglycerides is strong, as a main pathway conserved across taxa.

Empirical Evidence

Species	Duration	Dose	Increased FA synthesis?	Increased triglyceride?	Summary	Citation
Human (Homo sapiens), lab mice (Mus musculus)	Up to 7 days	1 µM, 5 µM, and 10 uM T0901317, T0314407 (LXR agonists) for HEK293 cells, 5, 50 mg/kg bdwt T0901317 for mice	Yes	Yes	Increased CYP7A1, SCD-1, and SREBP-1 gene expression vs control in HEK293 cells and C57BL/6 mice, genes linked with fatty acid synthesis, with correlated increases in triglycerides, phospholipids, and HDL cholesterol in a dose-dependent manner.	Schultz et al. (2000)
Lab mice (Mus mucsculus)	4 days	10 mg/kg/day T0901317 (LXR agonist)	Yes	Yes	Lab mice exposed to 10 mg/kg/day T0901317 had increased gene expression of SRBEP, ACC, FAS, genes linked with fatty acid synthesis, and correlated increased triglycerides, cholesterol, fatty acid.	Grefhorst et al. (2002)
Human (Homo sapiens), lab rat (Rattus norvegicus)	96 hours	0.3, 3, 30 nm Insulin plus 2 uM GW3965 (LXR agonist)	Yes	Yes	Increased SREBP-1c, FASN, SCD1 gene expression vs control in human and rat cells, with correlated increases in fatty acid synthesis, pointing to increased de novo lipogenesis, in a dose-dependent manner.	Kotokorpi et al. (2007)

In empirical studies, the link between increased fatty acid synthesis and accumulation of triglycerides is generally inferred. Increased expression of genes and/or signaling molecules known to facilitate fatty acid synthesis, and corresponding increases in triglyceride content in cells, are correlated to show evidence that increases are due to increased synthesis rather than alternative pathways. Angrish et al. (2016) review genes, signaling molecules, and chemical stressors linked to increased fatty acid synthesis, as well as other pathways leading to accumulation of triglycerides in cells.

References

Angrish, M.M., Kaiser, J.P., McQueen, C.A., and Chorley, B.N. 2016. Tipping the balance: Hepatotoxicity and the 4 apical key events of hepatic steatosis. Toxicological Sciences 150(2): 261–268.

Grefhorst, A., Elzinga, B.M., Voshol, P.J., Plösch, T., Kok, T., Bloks, V.W., van der Sluijs, F.H., Havekes, L.M., Romijn, J.A., Verkade, H.J., and Kuipers, F. 2002. Stimulation of Lipogenesis by Pharmacological Activation of the Liver X Receptor Leads to Production of Large, Triglyceride-rich Very Low Density Lipoprotein Particles. The Journal of Biological Chemistry 277(37): 34182–34190.

Kotokorpi, P., Ellis, E., Parini, P., Nilsson, L.-M., Strom, S., Steffensen, K.R., Gustafsson, J.-A., and Mode, A. 2007. Physiological Differences between Human and Rat Primary Hepatocytes in Response to Liver X Receptor Activation by 3-[3-[N-(2-Chloro-3-trifluoromethylbenzyl)-(2,2-diphenylethyl)amino]propyloxy]phenylacetic Acid Hydrochloride (GW3965). Molecular Pharmacology 72(4): 947-955.

Landesmann, B., Goumenou, M., Munn, S., and Whelan, M. 2012. Description of Prototype Modes-of-Action Related to Repeated Dose Toxicity. European Commission Report EUR 25631, 49 pages. https://op.europa.eu/en/publication-detail/-/publication/d2b09726-8267-42de-8093-8c8981201d65/language-en

Negi, C.K., Bajard, L., Kohoutek, J., and Blaha, L. 2021. An adverse outcome pathway based in vitro characterization of novel flame retardants-induced hepatic steatosis. Environmental Pollution 289: 117855.

Schultz, J.R., Tu, H., Luk, A., Repa, J.J., Medina, J.C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D.J., Lustig, K.D., and Shan, B. 2000. Role of LXRs in control of lipogenesis. Genes and Development 14:2831–2838.

Relationship: 2265: Accumulation, Triglyceride leads to Increased, Liver Steatosis

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Glucocorticoid Receptor activation leading to hepatic steatosis	adjacent		
Pregnane X Receptor (PXR) activation leads to liver steatosis	adjacent	Moderate	Not Specified
Liver X Receptor (LXR) activation leads to liver steatosis	adjacent	Moderate	Not Specified

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI
Mus musculus	Mus musculus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High
Juvenile	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Life Stage: All life stages with a liver. Older individuals are more likely to manifest this adverse outcome pathway (adults > juveniles) due to accumulation of triglycerides.

Sex: Applies to both males and females.

Taxonomic: Appears to be present broadly in vertebrates, with most representative studies in mammals (humans, lab mice, lab rats).

Key Event Relationship Description

Steatosis is a key event representing increased accumulation of fat in liver cells. In this key event relationship we are focused on accumulation of triglycerides leading to steatosis. Increased accumulation of triglycerides in cells is evidence of imbalance in the influx and synthesis versus metabolism or breakdown of lipid compounds. Increased accumulation of triglycerides can be enhanced by chemical stressors, or alteration of regulation by gene expression.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking accumulation of triglycerides to steatosis is strong. Increased accumulation of triglycerides represents an imbalanced influx and synthesis of compounds versus normal function, resulting in liver steatosis.

Empirical Evidence

Species	Duration	Dose	Damaged mitochondria?	Liver steatosis	Summary	Citation
Human (*Homo sapiens)*	14 days	In vitro exposure of 20 mM amiodarone, 50 mM tetracycline.	yes	yes	HepG2 human cells showed correlated increases in triglycerides and other lipid compounds and steatosis oxidation after 14 days of tetracycline exposure and after both 1 and 14 days of amiodarone exposure.	Anthérieu *et al.* (2011)
Human (*Homo sapiens)*	24 hours	In vitro exposure of at least 6 concentrations to 28 compounds selected for steatogenic potential.	yes	yes	HepG2 human cells exposed to fialuridine, sodium valproate, doxycycline, amiodarone, tetracycline showed changes in the mitochondrial membrane potential by analysis of TMRM fluorescence and corresponding increases in lipid accumulation, with higher doses exhibiting greater lipid accumulation and correlated steatosis.	Donato *et al.* (2009)
Human (*Homo sapiens)* and mouse (*Mus musculus*)	16 weeks	Transgenic and wild-type mice with normal and high cholesterol diet.	yes	yes	Human subjects with liver steatosis had increased RBP4 gene expression. Transgenic mice with human RBP4 gene had disrupted	Liu *et al.* (2016)

					membranes, increased mitochondria dysfunction assessed by decreased citrate synthase activity, and correlated increases in triglycerides associated with steatosis, in comparison to wild-type mice.	

References

References
Antherieu, S., Rogue, A., Fromenty, B., Guillouzo, A., and Robin, M.-A. 2011. Induction of Vesicular Steatosis by Amiodarone and Tetracycline Is Associated with Up-regulation of Lipogenic Genes in HepaRG Cells. Hepatology 53:1895-1905.

Donato, M.T., Martinez-Romero, A. Jimenez, N., Negro, A., Gerrerad, G., Castell, J.V., O'Connor, J.-E., and Gomez-Lechon, M.J. 2009. Cytometric analysis for drug-induced steatosis in HepG2 cells. Chemico-Biological Interactions 181: 417–423.

Landesmann, B., Goumenou, M., Munn, S., and Whelan, M. 2012. Description of Prototype Modes-of-Action Related to Repeated Dose Toxicity. European Commission Report EUR 25631, 49 pages. https://op.europa.eu/en/publication-detail/-/publication/d2b09726-8267-42de-8093-8c8981201d65/language-en

Liu, Y., Mu, D., Chen, H., Li, D., Song, J., Zhong, Y., and Xia, M. 2016. Retinol-Binding Protein 4 Induces Hepatic Mitochondrial Dysfunction and Promotes Hepatic Steatosis. The Journal of Clinical Endocrinology and Metabolism 101: 4338–4348.

Negi, C.K., Bajard, L., Kohoutek, J., and Blaha, L. 2021. An adverse outcome pathway based in vitro characterization of novel flame retardants-induced hepatic steatosis. Environmental Pollution 289: 117855.