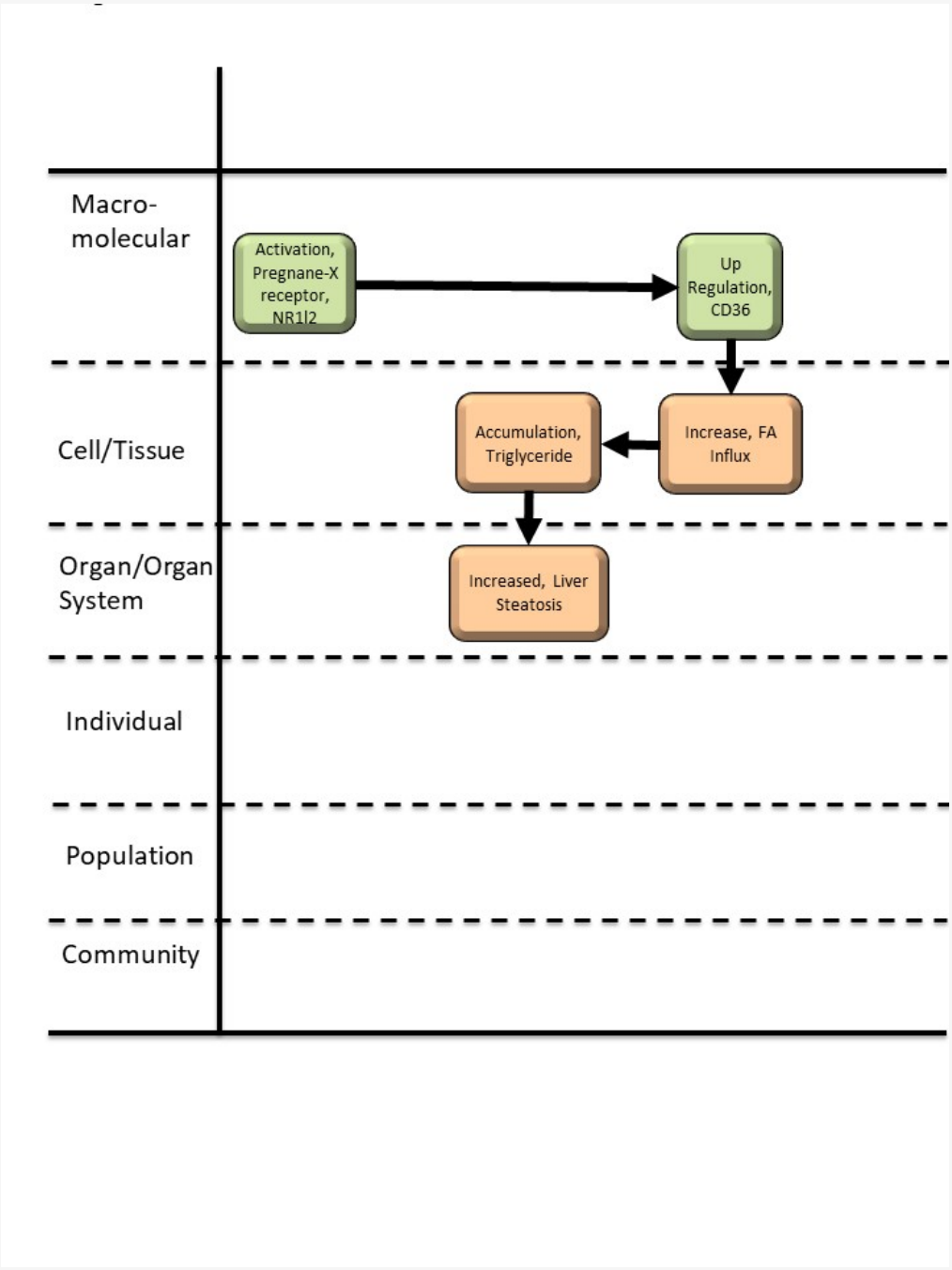
AOP ID and Title:

AOP 517: Pregnane X Receptor (PXR) activation leads to liver steatosis
Short Title: PXR activation leads to liver steatosis

Graphical Representation



Authors

Of the originating work: Chander Negi, Lola Bajard, Jiri Kohoutek, and Ludek Blaha, Faculty of Science, Masaryk University, Brno, Czech Republic

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 Do not cite			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

Pregnane X receptor (PXR) 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), Liver X receptor (LXR), Peroxisome proliferator-activated receptor (PPAR), 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). Pregnenolone and progesterone are ligands in normal molecular activation of PXR (Mellor *et al.* 1996), while an increasing number of chemical stressors have been shown to increase PXR expression (Bajard *et al.* 2019; Moya *et al.* 2020). Activation of PXR has been linked to increased gene expression of CD36 (Zhou *et al.* 2006). The transmembrane protein CD36 has been shown to have a central role in fatty acid influx (Glatz *et al.* 2010), with fatty acid influx one of the main pathways for increase 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 Pregnane X Receptor (PXR) leads to liver steatosis through increased fatty acid influx. 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 PXR gene and coordinated molecular responses leading to increased fatty acid influx. 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	239	Activation, Pregnane-X receptor, NR1I2	Activation, Pregnane-X receptor, NR1I2
	KE	54	Up Regulation, CD36	Up Regulation, CD36
	KE	115	Increase, FA Influx	Increase, FA Influx
	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, Pregnane-X receptor, NR1I2	adjacent	Up Regulation, CD36	Moderate	Not Specified
Up Regulation, CD36	adjacent	Increase, FA Influx	Moderate	Not Specified
Increase, FA Influx	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 3100: Activation, Pregnane-X receptor, NR1I2 leads to Up Regulation, CD36	**Strong support.** The relationship between activation of Pregnane-X receptor and Up Regulation of CD36 is broadly accepted and consistently supported across taxa.

Relationship 66: Up Regulation, CD36 leads to Increase, FA Influx	**Strong support.** The relationship between Up Regulation of CD36 and Increase, FA Influx is broadly accepted and consistently supported across taxa.
Relationship 132: Increase, FA Influx leads to Accumulation, Triglyceride	**Strong support.** Increase, FA Influx 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 239: Activation, Pregnane-X receptor, NR1I2	**Strong support.** Activation of Pregnane-X receptor is a primary activator for increases in CD36 gene expression. Evidence is available from toxicant and gene-knockout studies.
KE 54 Up Regulation, CD36	**Strong support.** Up Regulation of CD36 expression is one gene linked to increases in fatty acid influx. Evidence is available from toxicant, gene-knockout, and high lipid diet studies.
KE 115 Increase, FA Influx	**Moderate support.** Increase in fatty acid influx is a primary factor in increased triglyceride levels in cells. Evidence is available from toxicant and gene-knockout studies.
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.
Overall	**Moderate to strong support.** Direct evidence from empirical studies from laboratory mammals for most key events, with more inferential evidence for fatty acid influx.

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 3100: Activation, Pregnane-X receptor, NR1I2 leads to Up Regulation, CD36	**Strong support.** Increases in Pregnane X-receptor expression lead to increases in upregulation of CD36 expression, primarily from studies examining TOXCAST data, as well as changes in gene expression levels after exposure to chemical stressors.
Relationship 66: Up Regulation, CD36 leads to Increase, FA Influx	**Strong support.** Increases in upregulation of CD36 expression lead to increases in fatty acid influx, primarily through measured increases in CD36 gene expression and increased triglyceride levels. Increased fatty influx is inferred from increased triglyceride levels rather than directly observed.
Relationship 132: Increase, FA Influx leads to Accumulation, Triglyceride	**Strong support.** Increases in fatty acid influx 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.

Bajard, L., Melymuk, L., and Blaha, L. 2019. Prioritization of hazards of novel flame retardants using the mechanistic toxicology information from ToxCast and Adverse Outcome Pathways. Environmental Sciences Europe 31:14.

Glatz, J.F.C., Luiken, J.J.F.P., and Bonen, A. 2010. Membrane Fatty Acid Transporters as Regulators of Lipid Metabolism: Implications for Metabolic Disease. Physiological Reviews 90: 367–417.

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.

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.

Zhou, J., Zhai, Y., Mu, Y., Gong, H., Uppal, H., Toma, D., Ren, S., Evans, R.M., and Xie, W. 2006. A Novel Pregnane X Receptor-mediated and Sterol Regulatory Element-binding Protein-independent Lipogenic Pathway. Journal of Biological Chemistry 281(21): 15013-15020.

Appendix 1

List of MIEs in this AOP

Event: 239: Activation, Pregnane-X receptor, NR1I2

Short Name: Activation, Pregnane-X receptor, NR1I2

Key Event Component

Process	Object	Action
signaling	nuclear receptor subfamily 1 group I member 2	increased
signaling	nuclear receptor subfamily 1 group I member 3	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:8 - Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals	MolecularInitiatingEvent
Aop:517 - Pregnane X Receptor (PXR) activation leads to liver steatosis	MolecularInitiatingEvent

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
eukaryotic cell

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Rattus norvegicus	Rattus norvegicus	High	NCBI
Mus musculus	Mus musculus	High	NCBI
Homo sapiens	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adult	High
Juvenile	Moderate

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).

List of Key Events in the AOP

Event: 54: Up Regulation, CD36

Short Name: Up Regulation, CD36

Key Event Component

Process	Object	Action
gene expression	platelet glycoprotein 4	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:34 - LXR activation leading to hepatic steatosis	KeyEvent
Aop:58 - NR1I3 (CAR) suppression leading to hepatic steatosis	KeyEvent
Aop:57 - AhR activation leading to hepatic steatosis	KeyEvent
Aop:60 - NR1I2 (Pregnane X Receptor, PXR) activation leading to hepatic steatosis	KeyEvent
Aop:517 - Pregnane X Receptor (PXR) activation leads to liver steatosis	KeyEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

hepatocyte

Key Event Description

Fatty acid translocase CD36 (FAT/CD36) is a scavenger protein mediating uptake and intracellular transport of long-chain fatty acids (FA) in diverse cell types [1], [2]. In addition, CD36 can bind a variety of molecules including acetylated low density lipoproteins (LDL), collagen and phospholipids [3]. CD36 has been shown to be expressed in liver tissue [4], [5]. It is located in lipid rafts and non-raft domains of the cellular plasma membrane and most likely facilitates LCFA transport by accumulating LCFA on the outer surface [6], [7], [8].

FAT/CD36 gene is a liver specific target of LXR activation [9]. Studies have confirmed that the lipogenic effect of LXR and activation of FAT/CD36 was not a simple association, since the effect of LXR agonists on increasing hepatic and circulating levels of triglycerides and free fatty acids (FFAs) was largely abolished in FAT/CD36 knockout mice suggesting that intact expression and/or activation of FAT/CD36 is required for the steatotic effect of LXR agonists [10], [11]. In addition to the well-defined pathogenic role of FAT/CD36 in hepatic steatosis in rodents the human up-regulation of the FAT/CD36 in NASH patients is confirmed [12]. There are now findings that can accelerate the translation of FAT/CD36 metabolic functions determined in rodents to humans [13] and suggest that the translocation of this fatty acid transporter to the plasma membrane of hepatocytes may contribute to liver fat accumulation in patients with NAFLD and HCV [14]. In addition, hepatic FAT/CD36 up-regulation is significantly associated with insulin resistance, hyperinsulinaemia and increased steatosis in patients with NASH and HCV G1 (Hepatitis C Virus Genotype1) with fatty liver. Recent data show that CD36 is also increased in the liver of morbidly obese patients and correlated to free FA levels [15].

References

1. ↑ Su & Abumrad 2009 - Su X., Abumrad N.A., Cellular fatty acid uptake: a pathway under construction. Trends Endocrinol. Metab., 20 (No 2), 72-77, 2009
2. ↑ He et al. 2011 - He J. et al, The emerging roles of fatty acid translocase/CD36 and the aryl hydrocarbon receptor in fatty liver disease, Exp. Med. And Biology, 236, 1116-1121, 2011
3. ↑ Krammer 2011 - Krammer J. et al, Overexpression of CD36 and Acyl-CoA Synthetases FATP2, FATP4 and ACSL1 Increases Fatty Acid Uptake in Human Hepatoma Cells, Int. J. Med. Sci., 8(7), 599-614, 2011
4. ↑ Pohl et al. 2005 - Pohl J., et al, FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts, Mol. Biol. Cell., 16 (No 1), 24-31, 2005
5. ↑ Cheung et al. 2007 - Cheung L., et al, Hormonal and nutritional regulation of alternative CD36 transcripts in rat liver--a role for growth hormone in alternative exon usage, BMC Mol. Biol., 8, 60, 2007
6. ↑ Ehehalt et al. 2008 - Ehehalt R., et al, Uptake of long chain fatty acids is regulated by dynamic interaction of FAT/CD36 with cholesterol/sphingolipid enriched microdomains (lipid rafts). BMC Cell. Biol., 9, 45, 2008
7. ↑ Pohl et al. 2005 - Pohl J., et al, FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts, Mol. Biol. Cell., 16 (No 1), 24-31, 2005
8. ↑ Krammer 2011 - Krammer J. et al, Overexpression of CD36 and Acyl-CoA Synthetases FATP2, FATP4 and ACSL1 Increases Fatty Acid Uptake in Human Hepatoma Cells, Int. J. Med. Sci.,

8(7), 599-614, 2011
9. ↑ Zhou 2008 - Zhou J., Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPAR gamma in promoting steatosis, Gastroenterology, 134 (No 2),556-567, 2008
10. ↑ Febbraio et al. 1999 - Febbraio M., et al, A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism, J Biol Chem, 274, 19055–19062, 1999
11. ↑ Lee et al. 2008 - Febbraio M., et al, A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism, J Biol Chem, 274, 19055–19062, 1999
12. ↑ Zhu et al. 2011 - Zhu L., et al, Lipid in the livers of adolescents with non-alcoholic steatohepatitis: combined effects of pathways on steatosis, Metabolism Clinical and experimental, 30, 1001-1011, 2011
13. ↑ Love-Gregory et al. 2011 - Love-Gregory L., Abumrad N.A., CD36 genetics and the metabolic complications of obesity, Current Opinions in Clinical Nutition and Metabolic Care, 14 (No 6), 527-534, 2011
14. ↑ Miquilena-Colina et al. 2011 - Miquilena-Colina M.E., et al, Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in nonalcoholic steatohepatitis and chronic hepatitis C, Gut., 60 (No 10), 1394-1402 , 2011
15. ↑ Bechmann et al. 2010 - Bechmann L.P., et al, Apoptosis is associated with CD36/fatty acid translocase upregulation in non-alcoholic steatohepatitis, Liver Int., 30 (No 6), 850-859, 2010

Event: 115: Increase, FA Influx

Short Name: Increase, FA Influx

Key Event Component

Process	Object	Action
positive regulation of fatty acid transport	fatty acid	increased

AOPs Including This Key Event

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

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
hepatocyte

Key Event Description

Fat influx to the liver is usually increased under condition like obesity. Free fatty acids (FFA) increase in blood leads to an increase of FFA uptake in the liver. Especially the long chain fatty acids (LCFAs) are translocated across the plasma membrane, reassembled to triglycerides and stored in lipid droplets causing hepatic steatosis [1].

As mentioned above CD36 has consistently been shown to be expressed at the plasma membrane and to enhance LCFA uptake upon over-expression [2], [3].

References

1. ↑ Amacher 2011 - Amacher D.E., The mechanistic basis for the induction of hepatic steatosis by xenobiotics, Expert Opinion on Drug Metabolism and Toxicology, 7 (No 8), 949-965, 2011

2. ↑ Baranowski 2008 - Baranowski, Biological role of liver X receptors, Journal of Physiology and Pharmacology, 59 (Suppl 7), 31–55, 2008
3. ↑ Su & Abumrad 2009 - Su X., Abumrad N.A., Cellular fatty acid uptake: a pathway under construction. Trends Endocrinol. Metab., 20 (No 2), 72-77, 2009

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 ID and Name	Event Type
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: 3100: Activation, Pregnane-X receptor, NR1I2 leads to Up Regulation, CD36

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Pregnane X Receptor (PXR) 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
Adults	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

Activation of Pregnane-X receptor (PXR) gene expression has been shown to lead to increased gene expression and protein levels of CD36. CD36 is a transmembrane protein, and is of scientific interest because of the role of CD36 in fatty acid influx into cells.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking increased PXR expression to CD36 expression is moderate. Gene expression studies in mammalian systems have linked activation of PXR to increased gene expression and protein levels of CD36.

Empirical Evidence

Species	Duration	Dose	Activation PXR?	Upregulation CD36?	Summary	Citation
Lab mice (*Mus musculus)*	5 weeks	Wild-type versus transgenic-	yes	yes	Transgenic-human PXR	Zhou *et al.* (2006)

		human PXR mice.			mice showed increased expression of PXR genes and correlated increased expression of CD36 genes compared to null mice.	
Human *(Homo sapiens)*	24 hours	20 um efavirenz in vitro	Yes	Yes	Increased PXR gene expression vs control in hepatocytes exposed to 20 um efavirenz for 24 hours and increased CD36 gene expression in hepatocytes exposed to 20 um efavirenz for 24 hours.	Gwag *et al.* (2009)

References

Gwag, T., Meng, Z., Sui, Y., Helsley, R.N., Park, S.-H., Wang, S., Greenberg, R.N., and Zhou, C. 2019. Non-nucleoside reverse transcriptase inhibitor efavirenz activates PXR to induce hypercholesterolemia and hepatic steatosis Journal of Hepatology 70: 930–940.

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.

Zhou, J., Zhai, Y., Mu, Y., Gong, H., Uppal, H., Toma, D., Ren, S., Evans, R.M., and Xie, W. 2006. A Novel Pregnane X Receptor-mediated and Sterol Regulatory Element-binding Protein-independent lipogenic pathway. The Journal of Biological Chemistry 281(21): 15013–15020.

Relationship: 66: Up Regulation, CD36 leads to Increase, FA Influx

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
LXR activation leading to hepatic steatosis	adjacent	Not Specified	
Pregnane X Receptor (PXR) 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
Sex **Evidence**

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

CD36 gene expression has been shown to be a key regulator of fatty acid influx, primarily in mammal studies. CD36 is a transmembrane protein, and increased CD36 gene expression can result in increased fatty acid influx. Chemical stressors or high fat diets can help trigger fatty acid influx.

Evidence Supporting this KER

Biological Plausibility

The biological plausibility linking increased CD36 expression to increased fatty acid uptake is moderate. CD36 is a transmembrane protein, and upregulation of CD36 has been linked to increased fatty acid uptake, primarily in mammalian systems.

Empirical Evidence

Since the link between upregulation of CD36 and increased fatty acid influx has been established, empirical studies often measure increased CD36 gene expression and increased lipid content in cells and infer that the mechanism was increased fatty acid influx (Moya et al. 2010).

Species	Duration	Dose	Upregulated CD36?	Increase FA influx?	Summary	Citation
Lab mice (*Mus musculus)*	5 weeks	Wild-type versus transgenic-human PXR mice.	yes	yes	Transgenic-human PXR mice showed increased expression of CD36 genes in livers and increased lipid accumulation versus wild-type mice. FA influx was inferred as there was no increase in gene expression of SREBP, which would be expected to be upregulated if de novo fatty acid synthesis was the mechanism for increased triglycerides.	Zhou *et al.* (2006)
Human *(Homo sapiens)*		Children and adolescents exhibiting steatosis versus children and adolescents without steatosis	Yes	Yes	CD36, FABPpm, SLC27A2, SLC27A5 gene expression were upregulated and CD36 and CPT-1 protein expression was upregulated in subjects	Zhu *et al.* (2011)

					exhibiting steatosis linking increased triglyceride levels to fatty acid influx; FASN, SCD1, and acyl-COA gene expression were also upregulated in subjects exhibiting steatosis linking increased triglyceride levels to de novo fatty acid synthesis; both pathways appear to be responsible for increased triglycerides.	
Mouse *(Mus musculus)*	5 weeks	High fat versus low fat diet, transgenic mice	Yes	Yes	Mouse fed high fat diet had higher CD36 expression and triglyceride accumulation than mice fed low fat diet; transgenic mice and hepatocytes with CD36 gene had higher fatty acid influx than null mice and hepatocytes measured by the fluorescent fatty acid analog BODIPY.	Koonen *et al.* (2007)
Lab mice (*Mus musculus)*	24 hours	20 um efavirenz in vitro	Yes	Yes	Increased CD36 gene expression vs control in hepatocytes exposed to 20 um efavirenz and correlated higher fatty acid transport as measured by palmitic acid uptake.	Gwag *et al.* (2009)

References

Gwag, T., Meng, Z., Sui, Y., Helsley, R.N., Park, S.-H., Wang, S., Greenberg, R.N., and Zhou, C. 2019. Non-nucleoside reverse transcriptase inhibitor efavirenz activates PXR to induce hypercholesterolemia and hepatic steatosis Journal of Hepatology 70: 930–940.

Koonen, D.P.Y., Jacobs, R.L., Febbraio, M. Young, M.E., Soltys, C.-L.M., Ong, H., Vance, D.E., and Dyck, J.R.B. 2007. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. Diabetes 56: 2863-2871.

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

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

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.

Zhu, L., Baker, S.S., Liu, W., Tao, M.-H., Patel, R., Nowak, N.J., and Baker, R.D. 2011. Lipid in the livers of adolescents with nonalcoholic steatohepatitis: combined effects of pathways on steatosis. Metabolism Clinical and Experimental 60: 1001-1011.

Zhou, J., Zhai, Y., Mu, Y., Gong, H., Uppal, H., Toma, D., Ren, S., Evans, R.M., and Xie, W. 2006. A Novel Pregnane X Receptor-mediated and Sterol Regulatory Element-binding Protein-independent lipogenic pathway. The Journal of Biological Chemistry 281(21): 15013–15020.

Relationship: 132: Increase, FA Influx leads to Accumulation, Triglyceride

AOPs Referencing Relationship

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

Increased fatty acid influx 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 influx to accumulation of triglycerides is strong, as a main pathway conserved across taxa.

Empirical Evidence

In empirical studies, the link between increased fatty acid influx and accumulation of triglycerides is generally inferred. Zhou et al. (2006) link accumulation of triglycerides to increased fatty acid influx in the livers of transgenic mice with increased Pregnane X

Receptor expression compared to wild-type mice.
Increased expression of genes and/or signaling molecules known to facilitate fatty acid influx, and corresponding increases in triglyceride content in cells, are correlated to show evidence that increases are due to increased influx rather than alternative pathways. Angrish et al. (2016) review genes, signaling molecules, and chemical stressors linked to increased fatty acid influx, as well as other pathways leading to accumulation of triglycerides in cells. For a review of membrane proteins facilitating fatty acid influx, see Glatz et al. (2010).

Species	Duration	Dose	Increased FA influx?	Increased triglyceride?	Summary	Citation
Lab mice (*Mus musculus)*	16 hours	Wild-type versus transgenic-cd36 mice.	yes	yes	Heptatocytes from transgenic-CD36 mice showed increased fatty acid influx than null mice and measured by the fluorescent fatty acid analog BODIPY and correlated increased triglycerides.	Koonen *et al.* (2007)
Lab mice (*Mus musculus)*	1 week, 24 hours	100 mg/kg/day oral or in vitro 20 um efavirenz	Yes	Yes	Hepatocytes exposed to 20 um efavirenz for 24 hours had increased fatty acid influx as measured by palmitic acid uptake and correlated increased triglycerides and cholesterol to mice exposed to 100 mg/kg/day efavirenz for 1 week.	Gwag *et al.* (2009)

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.

Glatz, J.F.C., Joost, J.F., Luiken, P., and Bonen, A. 2010. Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. Physiological Reviews 90: 367–417.

Gwag, T., Meng, Z., Sui, Y., Helsley, R.N., Park, S.-H., Wang, S., Greenberg, R.N., and Zhou, C. 2019. Non-nucleoside reverse transcriptase inhibitor efavirenz activates PXR to induce hypercholesterolemia and hepatic steatosis Journal of Hepatology 70: 930–940.

Koonen, D.P.Y., Jacobs, R.L., Febbraio, M. Young, M.E., Soltys, C.-L.M., Ong, H., Vance, D.E., and Dyck, J.R.B. 2007. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. Diabetes 56: 2863-2871.

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.

Zhou, J., Zhai, Y., Mu, Y., Gong, H., Uppal, H., Toma, D., Ren, S., Evans, R.M., and Xie, W. 2006. A Novel Pregnane X Receptor-mediated and Sterol Regulatory Element-binding Protein-independent Lipogenic Pathway. The Journal of biological chemistry 281(21): 15013–15020.

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	Antherieu *et al.* (2011)

					tetracycline exposure and after both 1 and 14 days of amiodarone exposure.	
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 membranes, increased mitochondria dysfunction assessed by decreased citrate synthase activity, and correlated increases in triglycerides associated with steatosis, in comparison to wild-type mice.	Liu *et al.* (2016)

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.