PFOA Induces Liver & Serum Dyslipidemia in a Humanized PPARa Mouse Model Fed an American Diet

How do we design studies in animal models to test associations observed in human epidemiology?

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Perfluorooctanoic acid, PFOA

Perfluorooctane sulfonic acid, PFOS

1) PFAS are large group of chemicals (2000 and counting) have been identified in the environment
   - Exposure typically occurs as a mixture
2) PFAS have an “unusual” chemistry for environmental contaminants
   - Amphipathic, leading to binding to serum proteins, require transport to cross membranes
3) In humans, serum PFAS concentrations are associated with adverse birth outcomes, reduced response to vaccines, reduced bone quality, and increased serum cholesterol.

https://www.atsdr.cdc.gov/pfas/

Atherogenic dyslipidemia (elevated triglycerides, decreased HDL-C) and increased blood LDL-C are major contributors to cardiovascular disease, the leading cause of mortality in the US.

HDL-C = high density lipoprotein cholesterol particles
LDL-C = low density lipoprotein cholesterol particles

Global Burden of Cardiovascular Diseases, C. *et al.* 2018.
PMID: 29641820

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 22 March 2018

doi: 10.2903/j.efsa.2018.5194

Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food

PFOA

- **"a considerable proportion of the population exceeds the TWI of 6 ng/kg bw per week"**

PFOS

- **"a considerable proportion of the population exceeds the TWI of 13 ng/kg bw per week"**

Point of departure determined based on increased serum total cholesterol (& reduced vaccine response for PFOS) based on human epidemiology

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 9 July 2020

doi: 10.2903/j.efsa.2020.6223

Risk to human health related to the presence of perfluoroalkyl substances in food

"Based on available studies, in animals and humans, effects on the immune system were considered most critical for risk assessment."

Why the change in the endpoint for determining the point of departure?

Rodent toxicology

Do rodent studies imply the epidemiology studies are incorrect?

e.g., Pouwer et al 2019 found *decreased* serum cholesterol and triglycerides in mice (APOE*3-Leiden.CETP) given high doses of PFOA

<u>Some considerations:</u>

- **Dose (and serum levels) in mice vs humans**
- **Rodent chow (normally low in fats/cholesterol)**
- **Strain differences**
- **Potentially important species differences**
  - **Rodents do not express CETP***
  - **PPARα (a target of PFAS)**

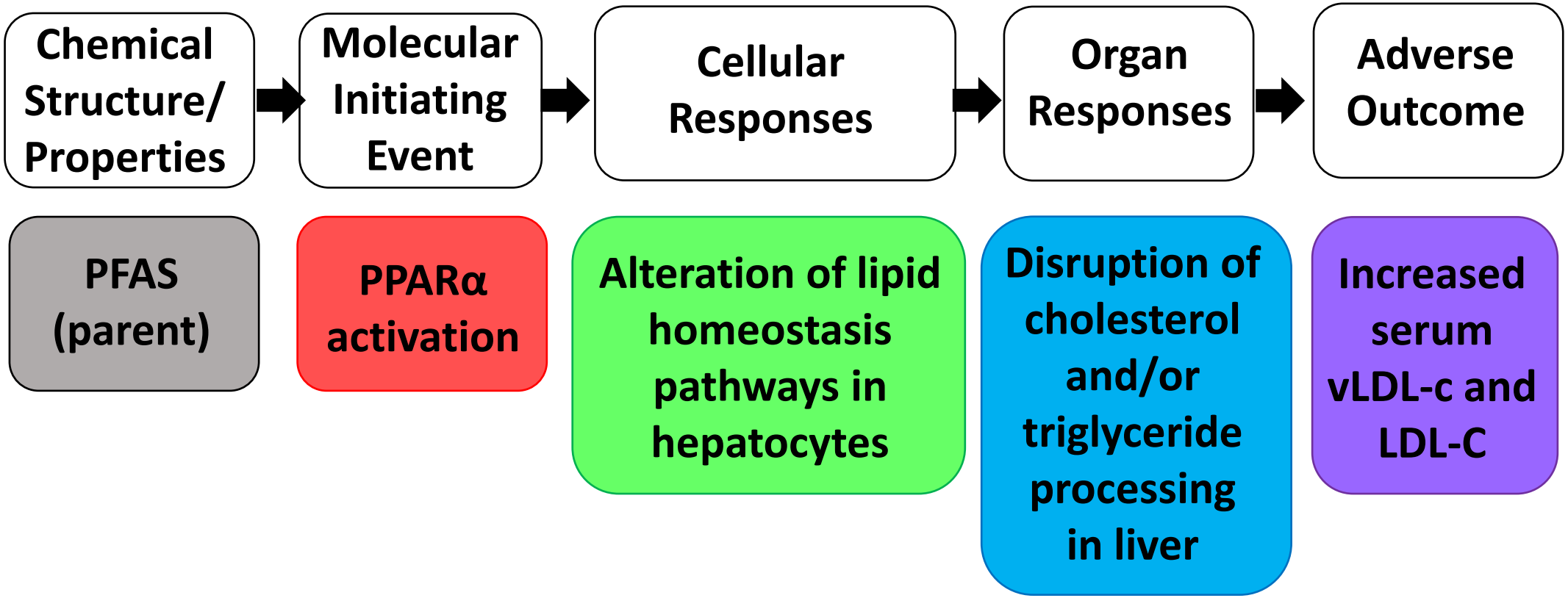
*** transfer cholesterol esters from HDL to LDL and triglycerides from LDL to HDL**

Pouwer et al., 2019. PMID: 30657992
Rebholz et al., 2016. PMID: 26942110

Hypothesis: PFAS induce dyslipidemia* through human PPARα activation.

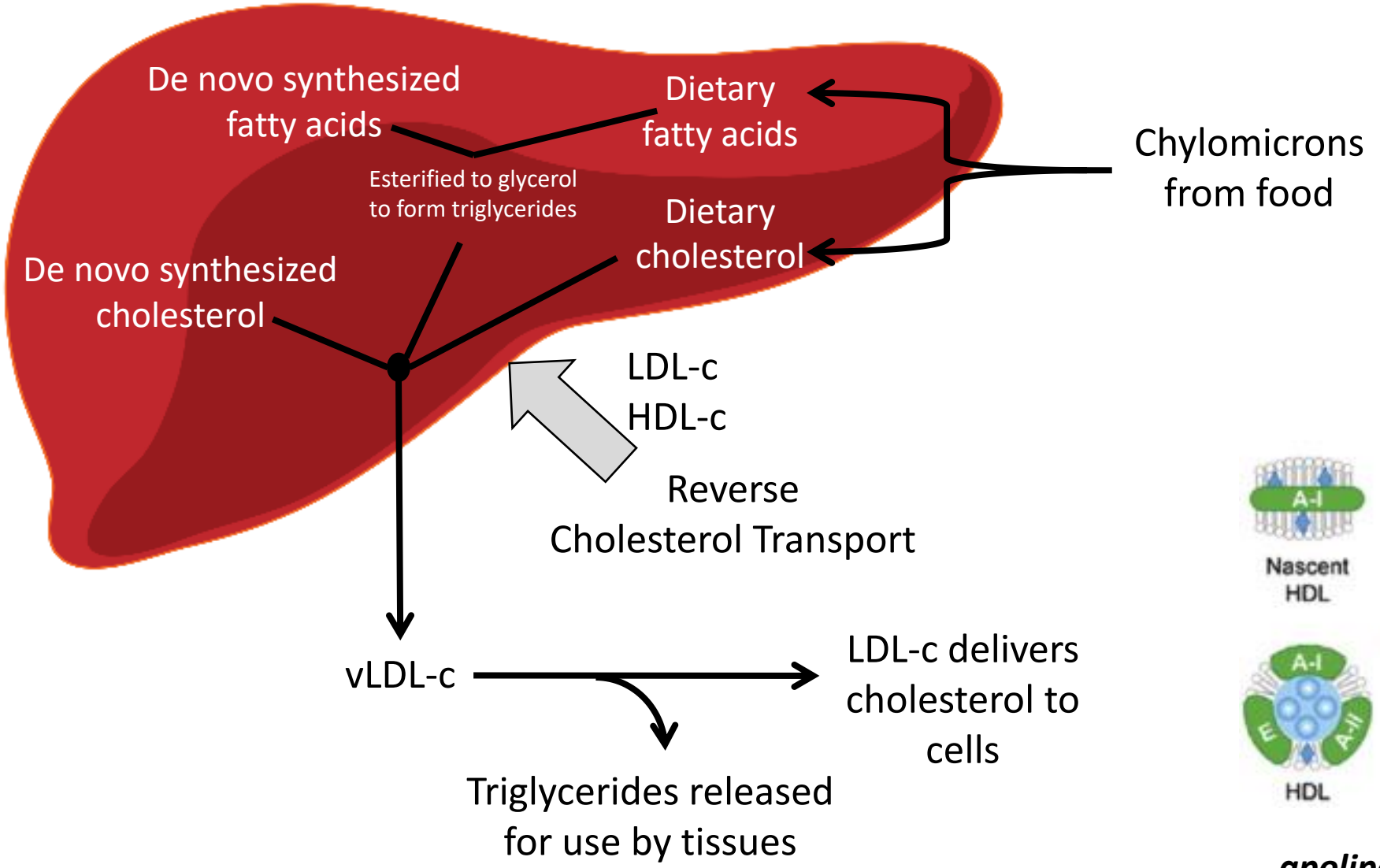
*Increased serum triglycerides (vLDL-C) and LDL-C, major contributors to CVD

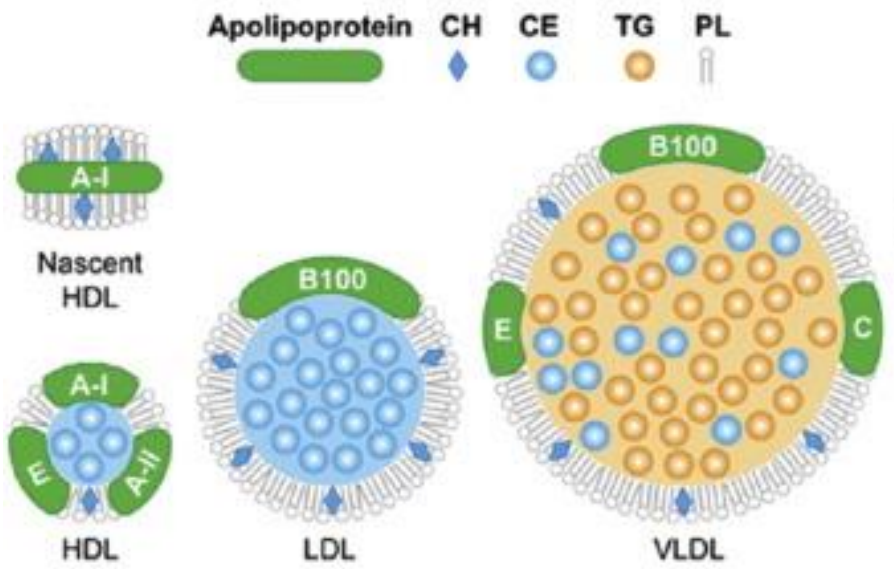
Adverse Outcome Pathway (AOP)



https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-aop/aop.html

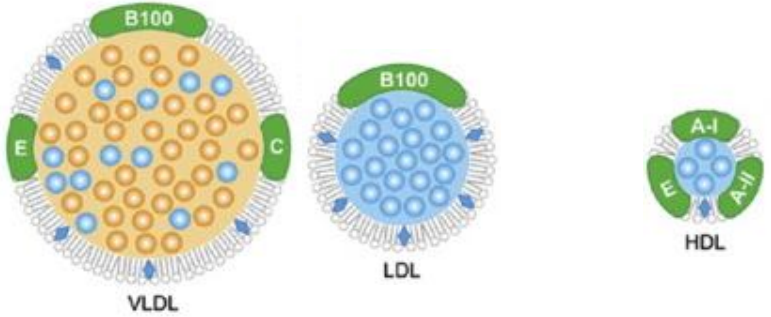
Cholesterol is packaged in particles with triglycerides in the liver.

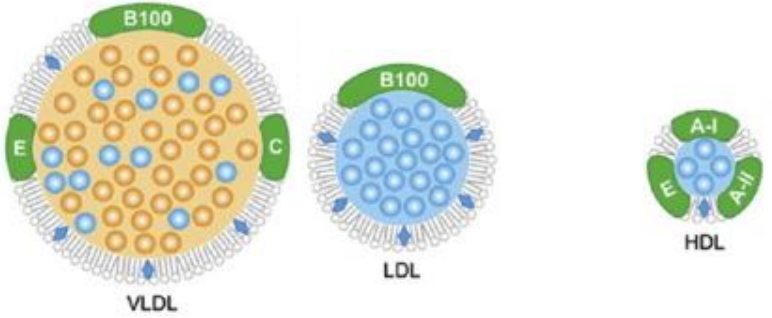


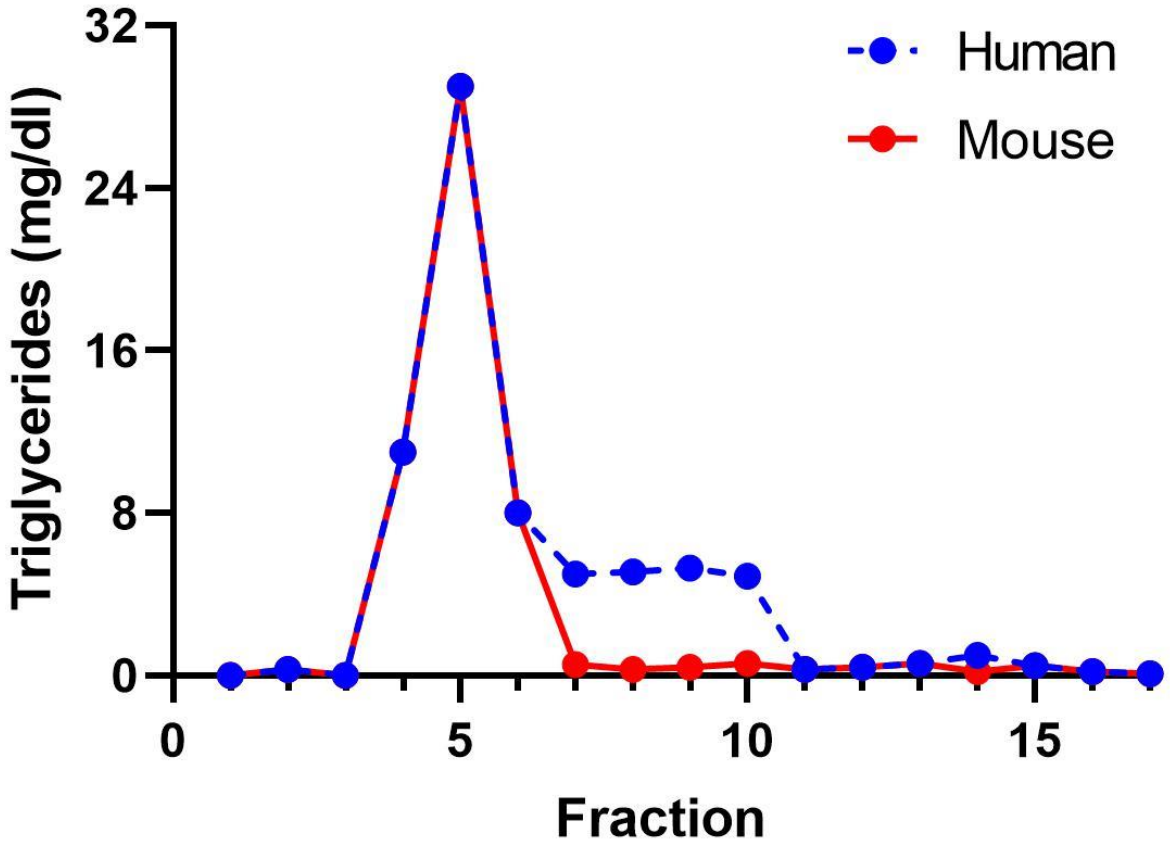


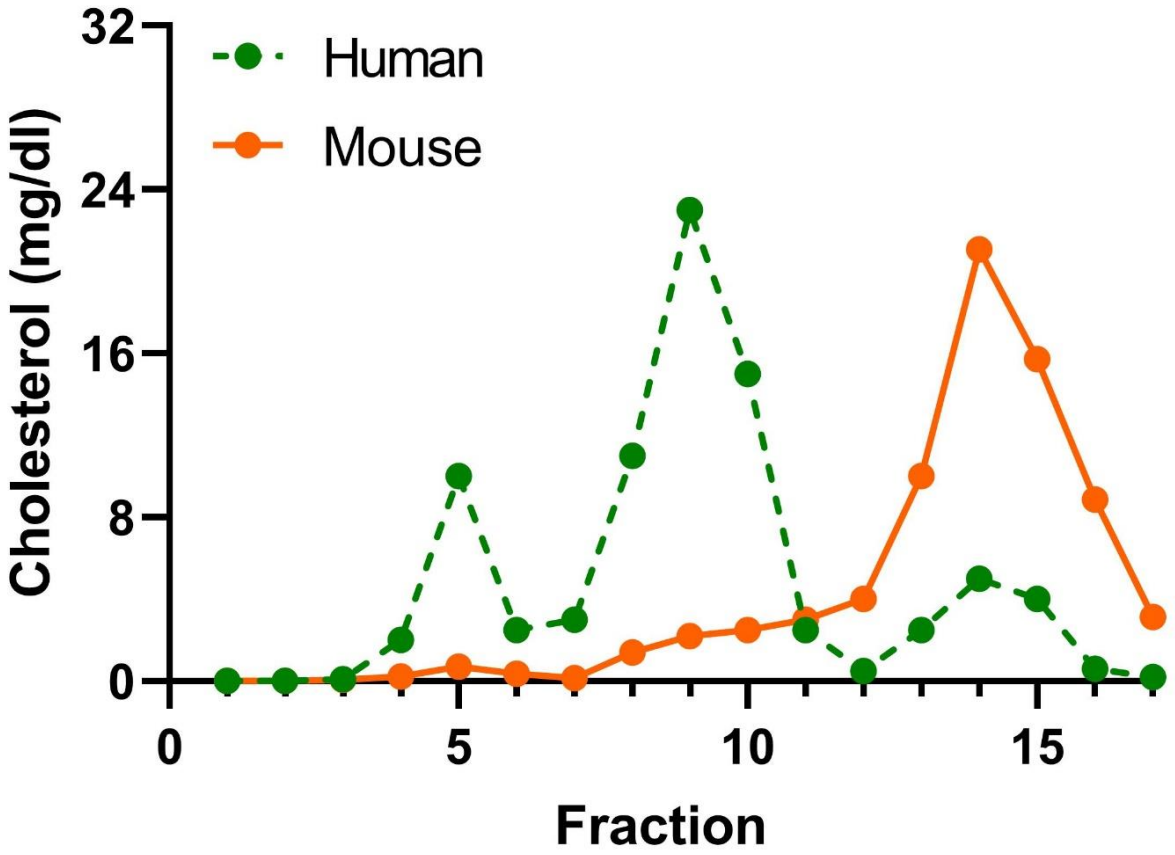
apolipoprotein A-I and ***apolipoprotein B***

https://peterattiamd.com/the-straight-dope-on-cholesterol-part-ii/





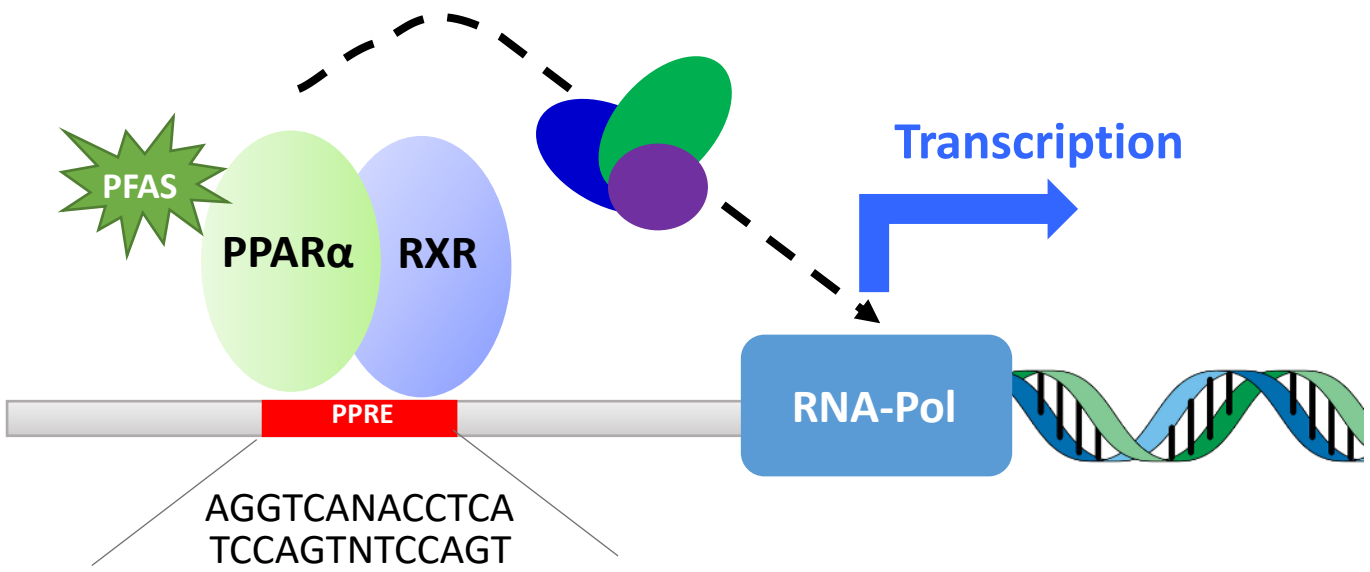


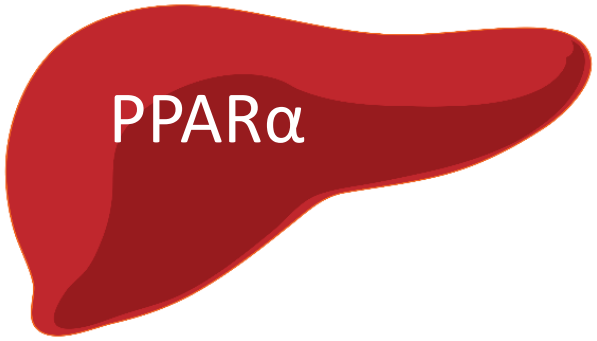


Adapted from: Weisner et al., 2009. PMID: 18832345

Activation of PPARα as a molecular initiating event.

PPARα is a nuclear receptor.





Lipid Use

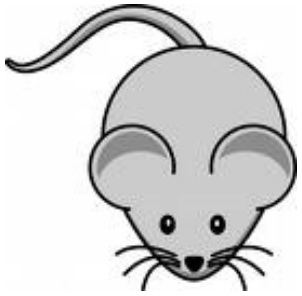
Control of genes involved in lipid uptake, synthesis, transport, storage, oxidation, and excretion

What is the Big Stink about PPARα?

Experimental design.

Hypothesis: PFAS induce dyslipidemia through human PPARα activation.

PPARα null
Humanized PPARα
SV129 strain

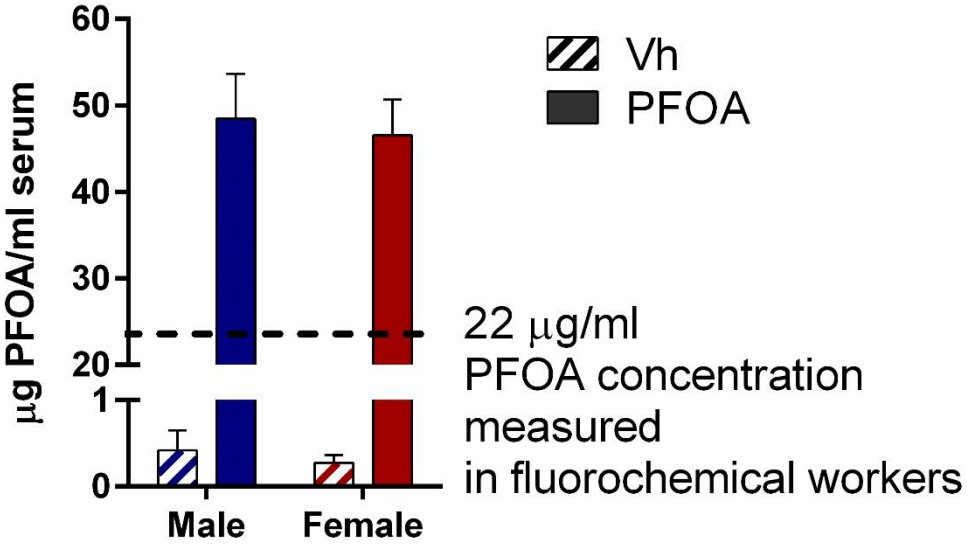


♀♂

Control Water
PFOA Water

Treated 3-9 weeks of age

“What we eat in America” diet
NHANES 2013/14
2-19 year olds



[PFOA] in drinking water:

3509 ± 138 µg/l

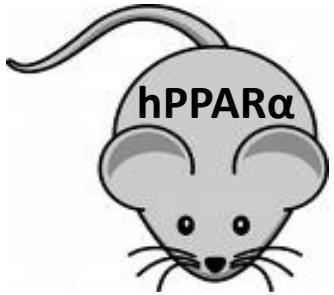
Mouse $C_s/C_w \approx 19$

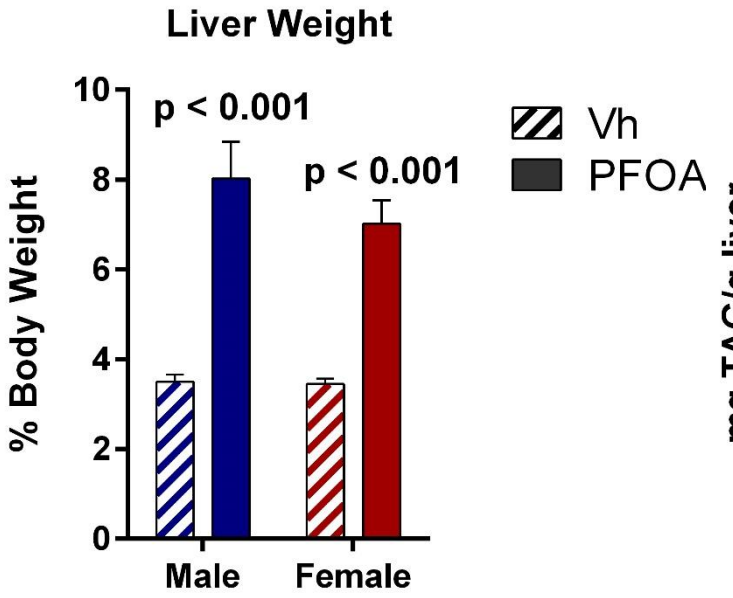
(assuming a 20 day half life and that 75% of steady state was reached)

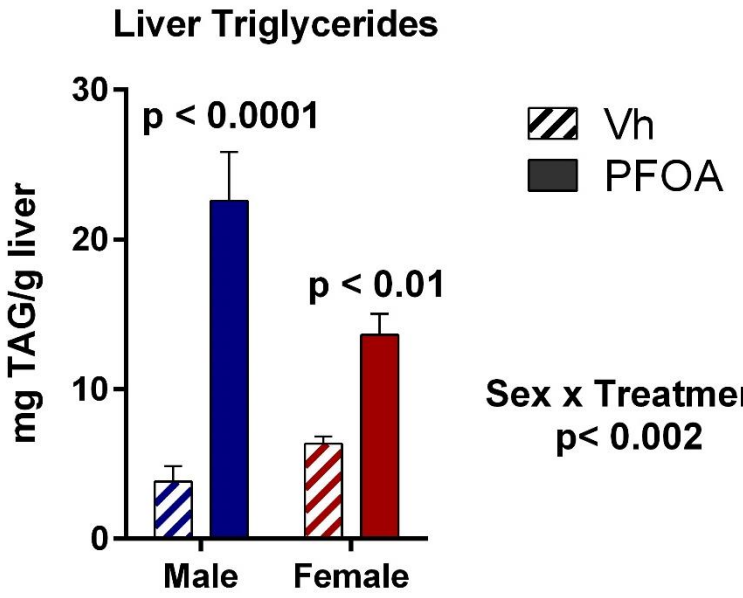
Human $C_s/C_w \approx 141$

Schlezinger et al., 2020. PMID: 32822737
Yang, et al., 2008. PMID: 17690133
Steenland, et al., 2010. PMID: 20423814
Hoffman, et al., 2011. PMID: 20920951

PFOA induces hepatomegaly and increases liver triglycerides.

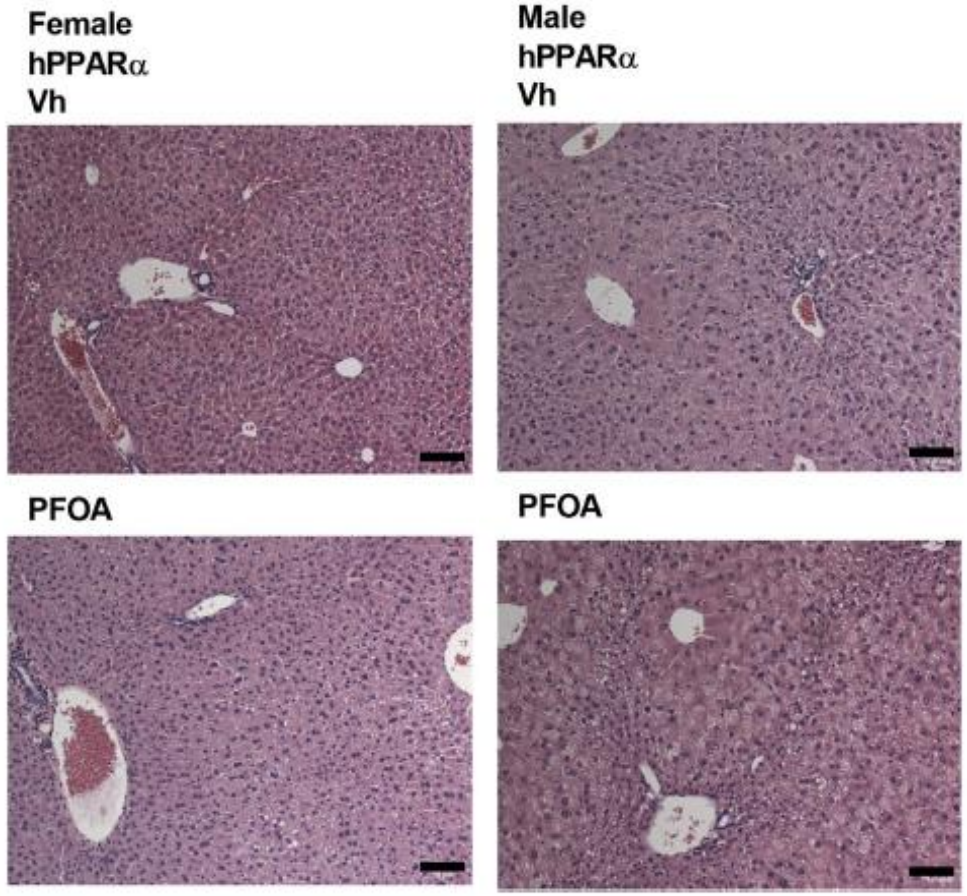






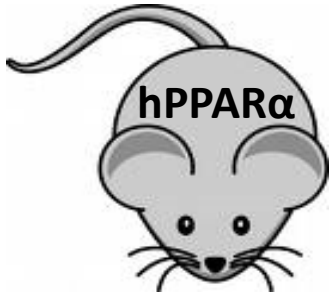
Sex x Treatment
p< 0.002

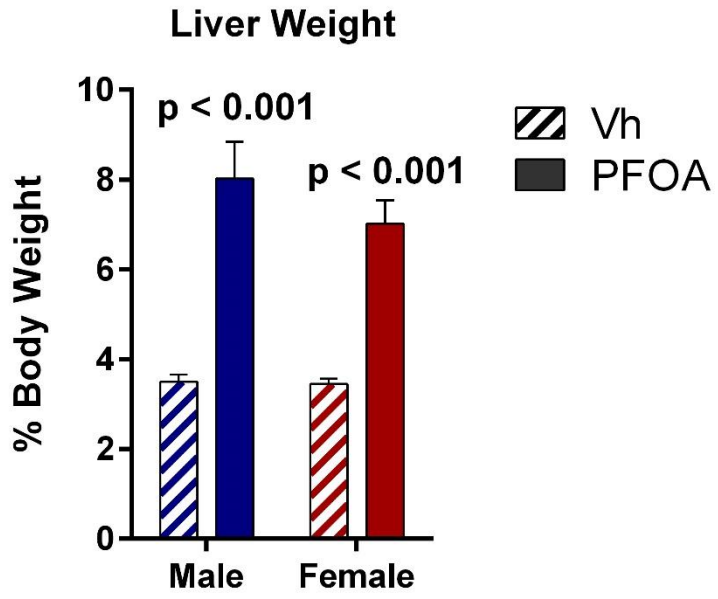
Liver Histology



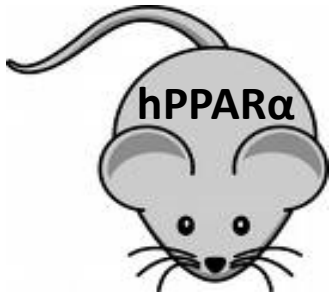
Schlezinger et al., 2020. PMID: 32822737

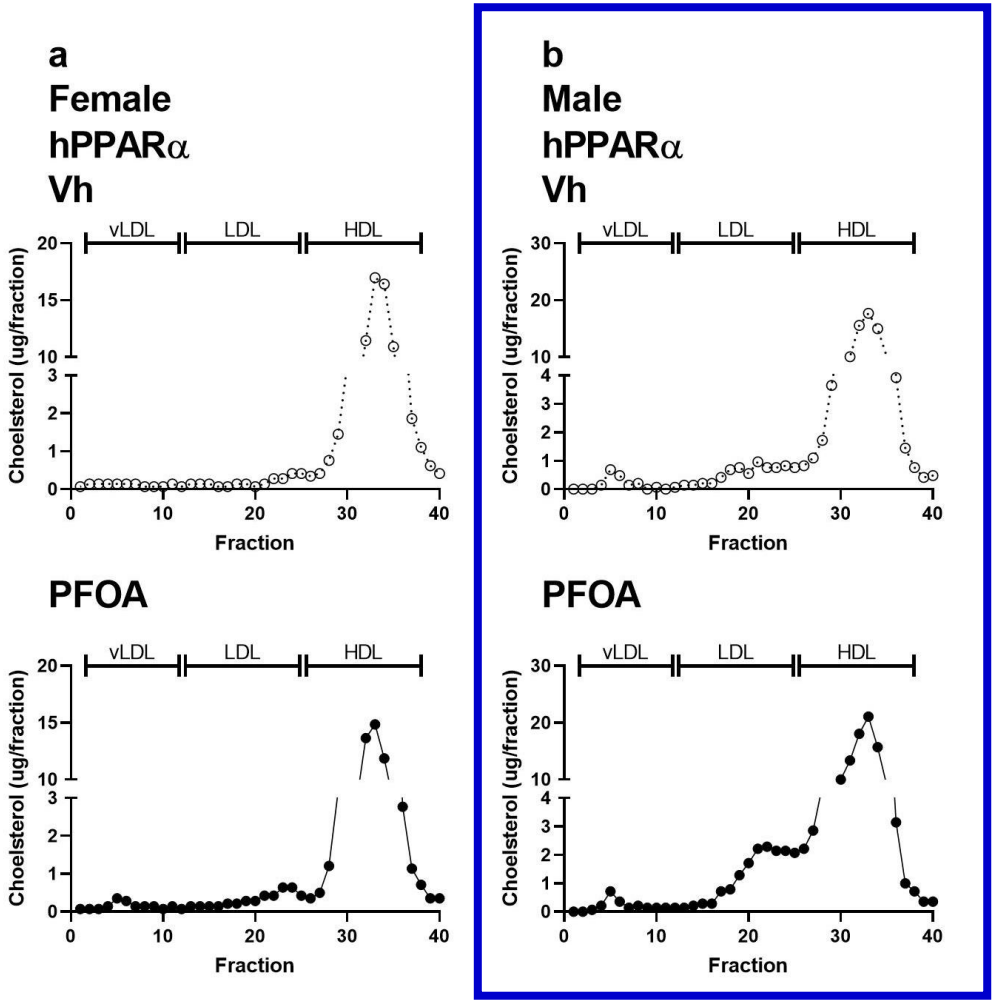
PPARα is an important regulator of liver triglycerides.





PFOA increases serum LDL-C, particularly in male hPPARα mice.



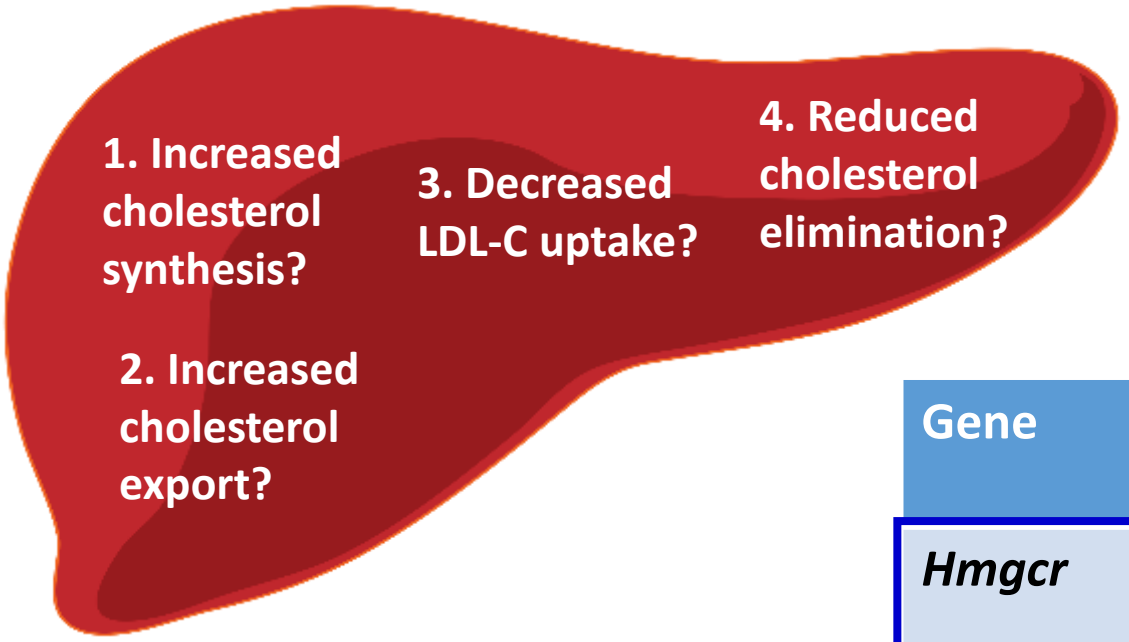


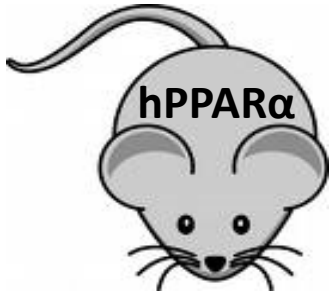
c

	Female		Male	
	Vh	PFOA	Vh	PFOA
Total Cholesterol (mg/dl)	81	78	97	128
	% Distribution			
vLDL-C	1.6	2.2	1.8	1.8
LDL-C	3.5	5.7	8.2	14.5
HDL-C	95.0	92.1	90.0	83.8

Schlezinger et al., 2020. PMID: 32822737
Also see: Rebholz et al., 2016. PMID: 26942110

PFOA changes expression of genes involved in multiple aspects of cholesterol homeostasis





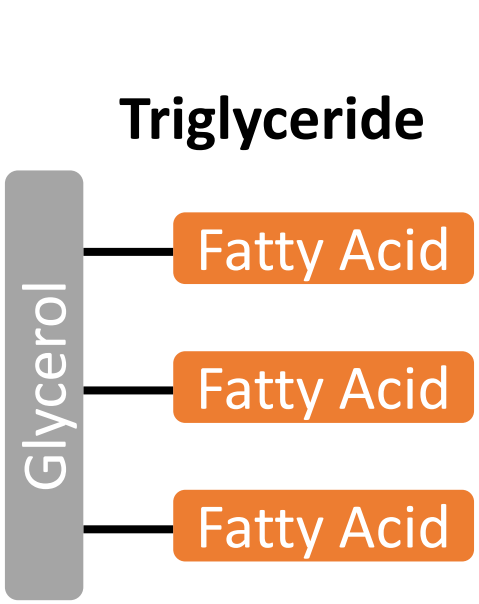
Comparison with PPARα null mice shows that induction is PPARα-dependent

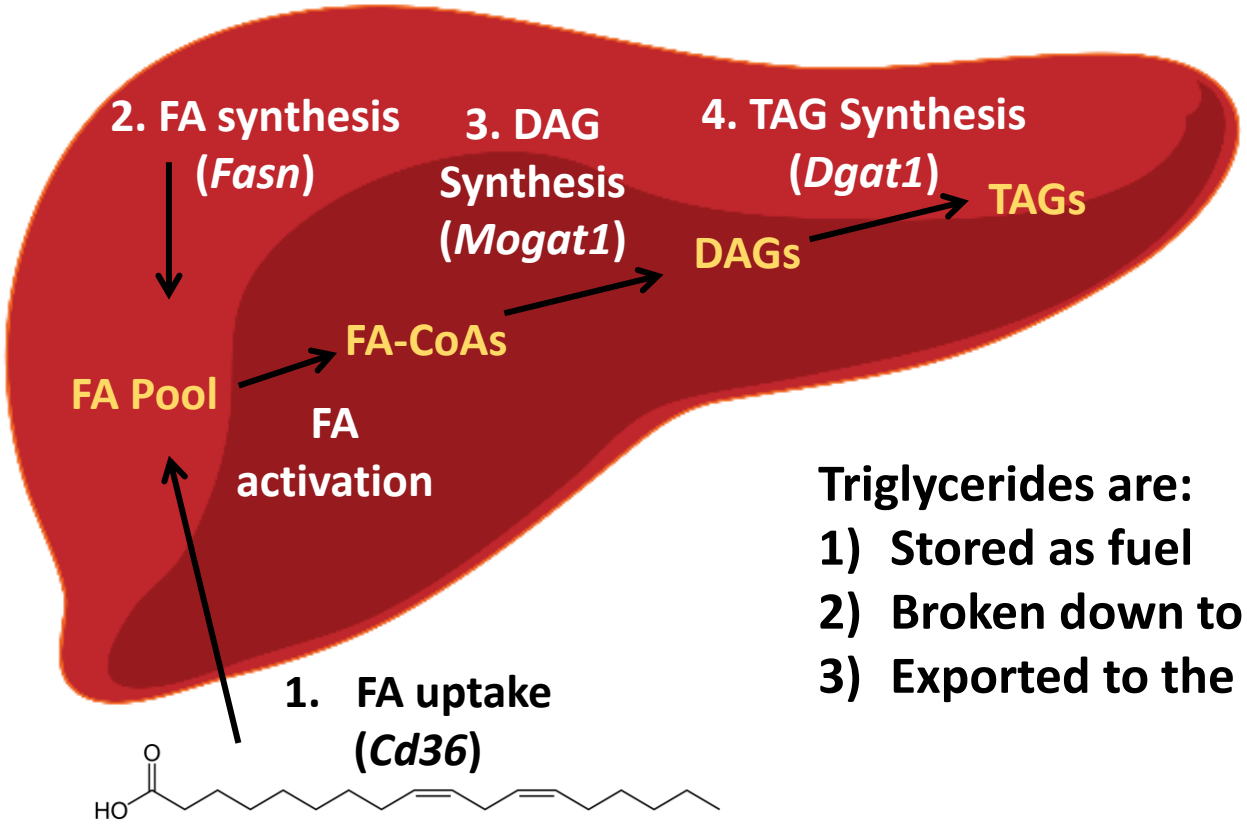
Gene	Protein Function	Female – Fold Change	Male – Fold Change
Hmgcr	1. Rate limiting step in cholesterol synthesis	0.58*	0.77
Apob1	2. Required for VLDL production	1.23	1.05
Ldlr	3. Transports LDL into hepatocytes	0.59**	0.89
Cyp7a1	4. Rate limiting step in synthesis of bile acids	0.30****	0.54

Schlezinger et al., 2020. PMID: 32822737

PFOA increases serum triglycerides, particularly in female PPARα null mice.

PFOA changes expression of genes involved in multiple aspects of triglyceride homeostasis





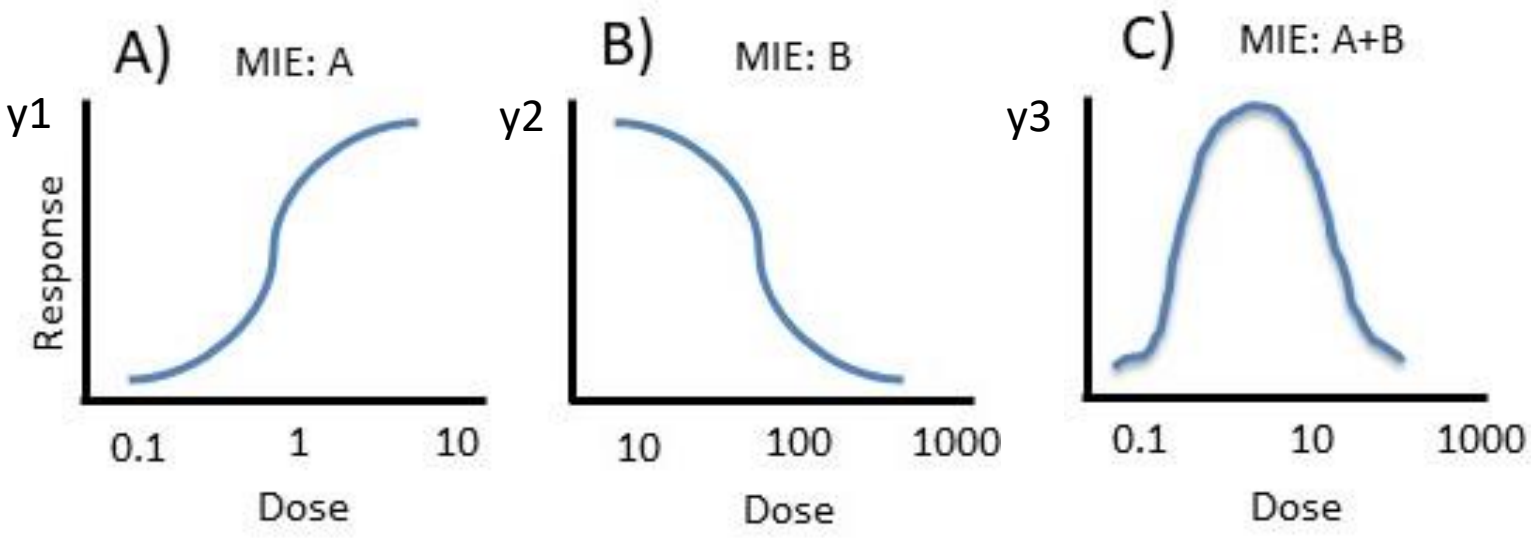
Triglycerides are:

1) **Stored as fuel**
2) **Broken down to release FAs for oxidation**
3) **Exported to the circulation in vLDL**

PFOA changes expression of genes involved in multiple aspects of triglyceride homeostasis

PFOA induces changes in serum triglycerides in a non-monotonic manner.

What can cause a non-monotonic dose response?

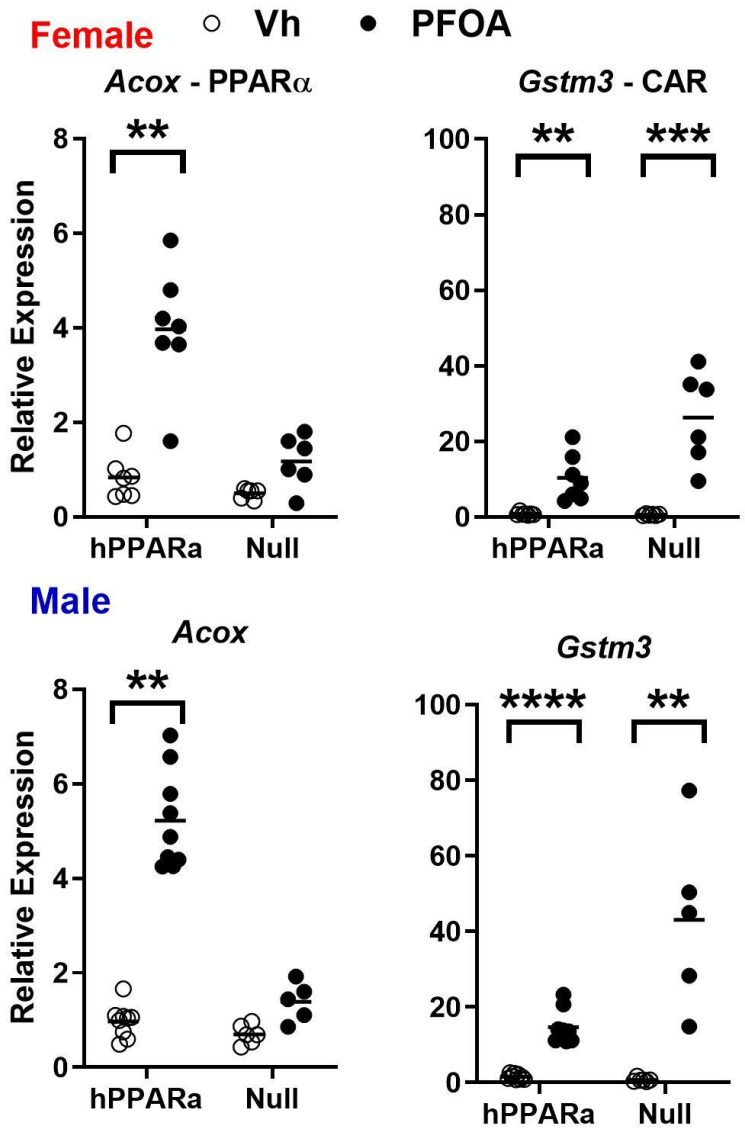


Low Concentrations

High Concentrations

All Concentrations

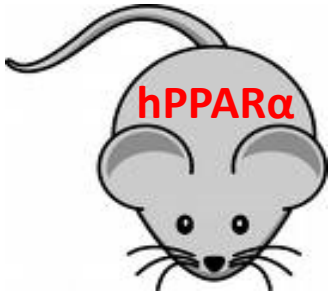
PFOA activates more than one molecular initiating event.



Potential PFOA-related MIEs:

1) PPARα
2) Other nuclear receptors (AR, CAR, ER, PXR, HNF4α)
3) Fatty acid binding proteins
4) Free fatty acid receptors

Schlezinger et al., 2020. PMID: 32822737



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“What we eat in America” diet

Conclusions

- **PFOA at an occupational exposure-related serum concentration:**
  1. Induces liver hypertriglyceridemia
  2. Induces serum hypercholesterolemia, particularly in male hPPARα mice
  3. Does not decrease serum triglycerides in male or female hPPARα mice
  4. Induces serum hypertriglyceridemia, particularly in female PPARα null mice
- **The data are consistent with:**
  1. Human epidemiological data.
  2. A multiple MIE-driven, non-monotonic dose response.
- **Developing human-relevant, *in vivo* models will be essential to understanding mechanisms of action and biological effects of mixtures of PFAS.**

Acknowledgements:

NIEHS: P42ES007381, R01ES027813

Collaborators:

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Tom Webster

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Catherine Boston, Greylin Nielsen, Jennifer Oliver, Hannah Puckett