PFAS Toxicology: A focus on metabolics

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Liver Weight? What does it tell you?

- ✓ Increased cellular proliferation
- ✓ Increased hypertrophy (increased organelle content, protein content
- ✓ Increased lipid infiltration

Species differences?
PPAR-alpha contribution
What about other receptors?

Hall et al., *Toxicol Pathol.* 2012 40:971-94.

Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop.

Metabolic Outcomes

Obesity (BMI, adiposity)

Insulin Resistance → **Diabetes (↑ blood glucose)**
NAFLD (↑ fat in liver)

Dyslipidemia → **Inflammation**
CVD

THINK BIG WE DO

Among Rhode Island's adults age 18 and over:

•62.9% of adults were overweight, with a Body Mass Index of 25 or greater

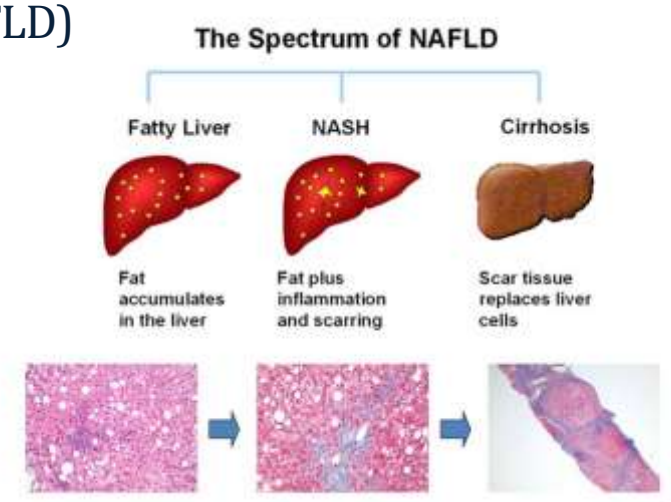
•25.5% of adults were obese, with a Body Mass Index of 30 or greater

Among Rhode Island's adolescents in grades 9 through 12:

- 16.7% were overweight (≥85th and < 95th percentiles for BMI by age and sex)
- 10.4% were obese (≥95th percentile for BMI by age and sex)

http://www.cdc.gov/obesity/stateprograms/fundedstates/rhode_island.html

Non-Alcoholic Fatty Liver Disease (NAFLD)

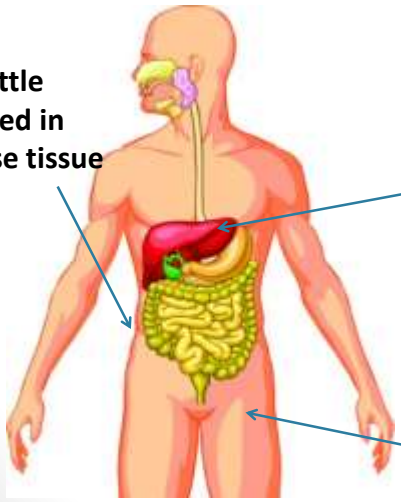


- ✓ **What does increased liver weight mean? What makes a liver weight go up?**
- ✓ **Is NAFLD an innocent disease?**
- ✓ **Discussions about whether fat accumulation in the liver is a adaptive response and are they a concern for adverse health effect to liver?**

DoHAD Hypothesis

- ✓ Developmental Origins of Health and Disease (DOHaD). *Windows of exposure matter.*
- ✓ Undernutrition during gestation reprograms the relationship between glucose and insulin and between growth hormone and IGF [insulin-like growth factor]"
- ✓ Exposure to chemicals/hormones at key times in development may re-program stem cells and change susceptibility to disease.

PFASs – Where do they distribute in the body?



Very little detected in adipose tissue

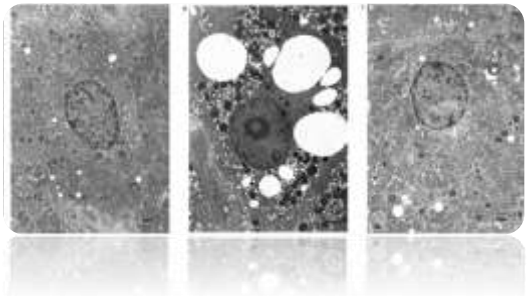
PFOS (1.3:1) Perfluorocarboxylic acids with carbon chain lengths C9, C10, and C11.

PFOA, PFHxS, and PFOSA (<LOD) in liver; PFOA and PFHxS >blood than liver

Excretion to urine

NAFLD and PFOS

- In a study of monkeys, PFOS decreased body weights and increased liver weights
  - Hepatocellular hypertrophy and lipid vacuolation



(Seacat et al, 2002)

PFAS tissue distribution in post mortem human tissue

"Human donor liver and serum concentrations of perfluorooctanesulfonate and other perfluorochemicals"

Cohort: Thirty-one donors (16 male and 15 female, age range 5-74) provided serum and/or liver samples for analysis of PFOS and three other fluorochemicals: perfluorosulfonamide (PFOSA, C8F17SO2NH2), perfluorooctanoate (PFOA, C7F15CO2-), and perfluorohexanesulfonate (PFHxS, C6F13SO3-).

Findings:

- ✓ Liver PFOS concentrations ranged from <4.5 ng/g (LOQ) to 57.0 ng/g.
- ✓ Serum PFOS concentrations ranged from <6.1 ng/mL (LOQ) to 58.3 ng/mL. Among the 23 paired samples
- ✓ liver to serum ratio was 1.3:1
- ✓ This liver to serum ratio is comparable to that reported in a toxicological study of cynomolgus monkeys, which had liver and serum concentrations 2-3 orders of magnitude higher than observed in these human donors

Environ Sci Technol. 2003 Mar 1;37(5):888-91.

PFAS tissue distribution in post mortem human tissue

"Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples."

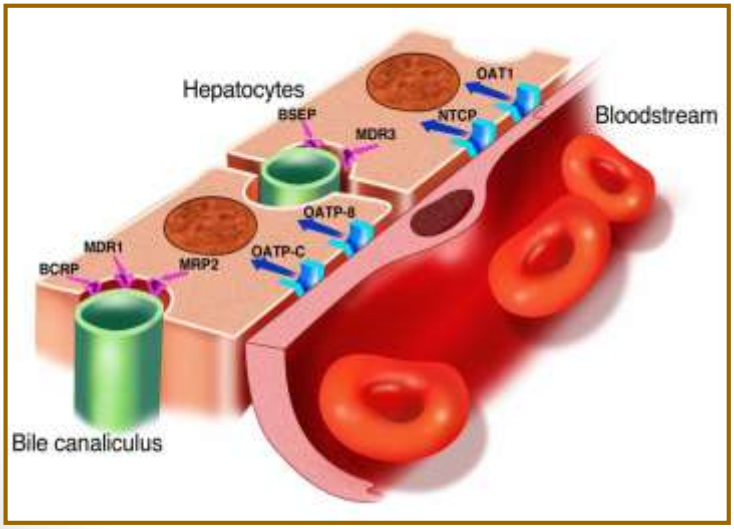
Cohort: Human liver (n = 12) and milk (n = 10) samples were collected in 2007 and 2008 in Catalonia, Spain. Liver samples were taken postmortem from six males and six females aged 27-79 years. Milk samples were from healthy primipara women (30-39 years old).

Findings:

- ✓ Six PFCs were detected in liver
- ✓ PFOS highest in liver (26.6 ng/g wet weight) being the chemical with the highest mean concentration.
- ✓ Perfluorohexanesulfonate (PFHxS), perfluorooctanoic acid (PFOA), and acids with chain lengths up to C11 were also detected, (0.50 and 1.45 ng/g wet weight).
- ✓ For PFOA and PFHxS, - fivefold and 14-fold higher concentrations, respectively, were seen in serum as compared to liver.
- ✓ Breast MilK: On the other hand, PFOS and PFHxS were the only PFCs detected in human milk (0.12 and 0.04 ng/mL), respectively. **Liver – 200x higher concentration!!**

Environ Sci Pollut Res Int. 2010 Mar;17(3):750-8. doi: 10.1007/s11356-009-0178-5.

Why do PFASs distribute to liver?



PFAS and Liver Function – Recent Findings

- ✓ **Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010.**
- ✓ **Goal: Assess PFHxS, PFOS, PFOA, and PFNA association with uric acid, alanine transferase (ALT), gamma-glutamyl transferase (GGT), asparate aminotransferase (AST), alkaline phosphate (ALP), and total bilirubin**
- ✓ **Sample set: 2007-2008 and 2009-2010 combined National Health and Nutrition Examination Survey (NHANES).**
- ✓ **Findings:**
  - ✓ PFHxS was associated with ALT.
  - ✓ PFOS was statistically associated with total bilirubin [(Q2: OR=1.44, 95% CI 1.12-1.84), (Q3: OR=1.65, 95% CI 1.25-2.18), and (Q4: OR=1.51, 95% CI 1.06-2.15)], with evidence of an increasing trend (p-value=0.028).
  - ✓ PFOA was associated with uric acid, ALT, GGT, and total bilirubin. PFNA was linearly associated with ALT (p-value <0.001), and there was statistically significant increasing trend (p-value=0.042).

Environ Res. 2015 Jan;136:8-14. doi: 10.1016/j.envres.2014.10.004. Epub 2014 Nov 19.

PFAS and Liver Function – Key Findings

Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure

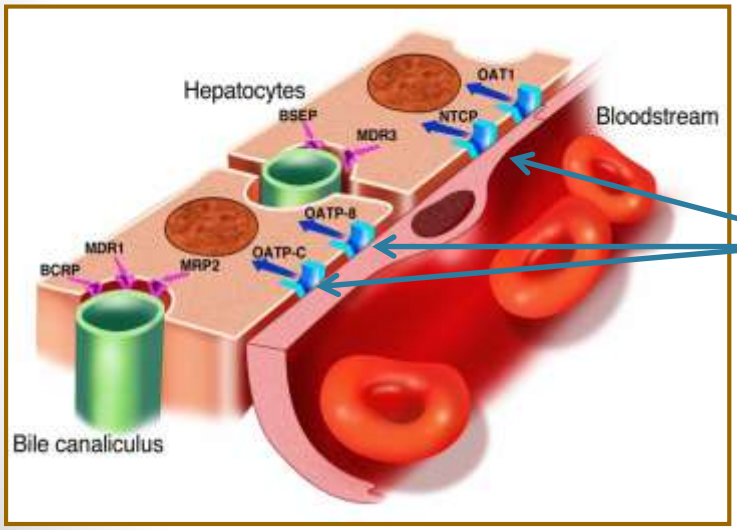
- ✓ **Methods:**
  - ✓ The C8 Health Project collected data on 69,030 persons; of these, a total of 47,092 adults were included in the present analysis. Linear regression models were fitted for natural log (ln)-transformed values of alanine transaminase (ALT), γ-glutamyltransferase (GGT), and direct bilirubin on PFOA, PFOS, and potential confounders.
- ✓ **Findings:**
  - ✓ These results show a positive association between PFOA and PFOS concentrations and serum ALT level, a marker of hepatocellular damage.
  - ✓ ALT enzyme released by the liver when liver cells are damaged

Environ Res. 2015 Jan;136:8-14. doi: 10.1016/j.envres.2014.10.004. Epub 2014 Nov 19.

What are the limitation of these studies?

- AST, ALT are good biomarkers for necrotic liver injury
- Poor biomarkers for NAFLD
- Best diagnosis is via thin-needle biopsy
- Do we know whether Homer's PFAS ADME is the same?

Can disease impact PFAS levels in liver?



Liver disease can affect these processes

Liver function may affect serum PFAS levels

Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia

Methods: Cross-sectional study investigated 12 perfluoroalkyl substances (PFASs) in serum (n=79) and liver (n=66) samples from patients who had undergone liver transplantation for a range of conditions, such as hepatocellular carcinoma (HCC), cirrhosis due to chronic hepatitis C viral infection (HCV), both HCC and HCV, amyloidosis or acute liver failure.

PFAS data from patients were compared to those in control serum (n=25) samples from liver donors with no known liver disease and to those in control liver (n=9) tissues collected during liver resection surgery.

Results: All samples showed detectable PFOS (serum: 0.621-126ng/mL; liver: 0.375-42.5ng/g wet wt) and PFOA (serum: 0.437-45.5ng/mL; liver: 0.101-2.25ng/g wet wt) concentrations. In general, in paired serum and liver samples, serum had higher PFOS, PFHxS, PFDA, PFNA, and PFOA concentrations than those in explanted livers from patients.

Conclusion: These findings also suggest that pathological changes in diseased livers alter the distribution of PFASs between liver and serum.

Yeung et al., 2013, Ecotoxicol Environ Saf. 96:139-46.

PFASs and Cholesterol: C8 Panel Findings

At least 14 human studies, most of them cross-sectional in design, have linked PFOA exposure with heart disease risk factors (including higher levels of uric acid and homocysteine in serum) and higher serum cholesterol

Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Winquist and Steenland, EHP, 2014.

- ✓ Among 32,254 participants (28,541 community; 3,713 worker), 12,325 reported hypertension with medication, 9,909 reported hypercholesterolemia with medication, and 3,147 reported coronary artery disease (2,550 validated).
- ✓ Hypercholesterolemia incidence increased with increasing cumulative PFOA exposure (sum of yearly serum concentration estimates), most notably among males 40-60 years of age.
- ✓ There was no apparent association between PFOA exposure and hypertension or coronary artery disease incidence.

PFASs and Cholesterol

- Fisher et al., 2013: Canadian Health Measures Survey (CHMS) *cross-sectional* data from the Canadian Health Measures Survey (Cycle 1 2007-2009) found that examined adults (n=2700). No significant evidence to support the association with cholesterol outcomes with PFOS and PFOA. *Did observe several significant associations with the PFHxS and cholesterol outcomes (LDL, TC, NON-HDL, TC/HDL ratio).*

- Eriksen et al., 2013: Cross-sectional study was to investigate the association between plasma PFOA and PFOS and total cholesterol in a general, middle-aged Danish population. The study population comprised 753 individuals (663 men and 90 women), 50-65 years of age, nested within a Danish cohort of 57,053 participants. *Positive associations between plasma PFOA and PFOS levels and total cholesterol .*

Cholesterol Findings: Limitations and Gaps

- Cross-sectional and largely focused on PFOA exposure. Know even less about PFOS or other PFASs.
- Findings - Adults and occupational exposure
- Consistent findings that are prospective, developmental exposures lacking

Early in life exposures: Risk for Obesity

Adiposity and glycemic control in children exposed to perfluorinated compounds

Objective: explore whether childhood exposure to perfluorinated and polyfluorinated compounds (PFCs) and adiposity and markers of glycemic control.

Methods: Body mass index, skinfold thickness, waist circumference, leptin, adiponectin, insulin, glucose, and triglyceride concentrations were assessed in 8- to 10-year-old children in 1997 in a subset of the European Youth Heart Study, Danish component. Plasma PFC concentrations were available from 499 children. Linear regression models were performed to determine the association between PFC exposure and indicators of adiposity and markers of glycemic control.

Results: Increased PFC exposure in overweight 8- to 10-year-old children was associated with higher insulin and triglyceride concentrations

Timmermann et al., *Clin Endocrinol Metab.* 2014, 99(4):E608-14.

Early in life exposures: Risk for Obesity

Early life perfluorooctanoic acid (PFOA) exposure and overweight and obesity risk in adulthood in a community with elevated exposure.

Goal: Examine whether elevated early life PFOA exposure was associated with adult BMI among a group of mid-Ohio valley residents exposed to a wide range of early life PFOA levels due to emissions from a chemical plant.

Methods: 8764 adults aged 20-40 years who reported height and weight on a survey between 2008 and 2011.

Annual retrospective early life PFOA serum concentrations were estimated for each participant based on residential history and nearby chemical plant emissions as well as background exposure not originating from the facility.

Results:

- ✓ Nearly half the participants (45%) had early life PFOA exposure serum concentration estimates above background levels.
- ✓ Odds ratios for adult obesity risk were similar. Regression coefficients from linear models using BMI as a continuous outcome showed no association between early life PFOA exposure and adult BMI.
- ✓ Elevated levels of PFOA exposure in early life were not associated with overweight and obesity risk in adulthood and results did not vary by sex.

Barry *et al*., *Environ. Res.* 2014 132:62-9. 2015

Early in life exposures: HOME Study and Obesity

Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study.

Goal: Prenatal perfluoroalkyl substance (PFAS) exposure versus adiposity in children born to women who lived downstream from a fluoropolymer manufacturing plant.

Methods: Data are from a prospective cohort in Cincinnati, Ohio (HOME Study). Perfluorooctanoic (PFOA), perfluorooctane sulfonic (PFOS), perfluorononanoic (PFNA), and perfluorohexane sulfonic (PFHxS) acids were measured in prenatal serum samples.

BMI, waist circumference, and body fat at 8 years of age (n = 204)
BMI between 2-8 years of age (n = 285) according to PFAS concentrations.

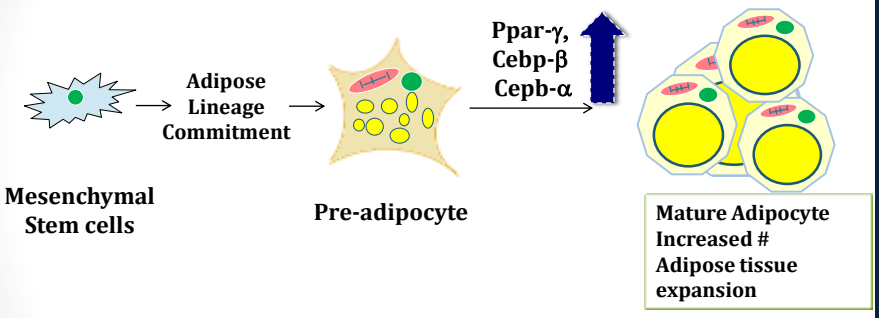
Results: Children born to women in the top two PFOA terciles had greater adiposity at 8 years than children in the 1st tercile.

PFOS, PFNA, and PFHxS were not associated with adiposity.

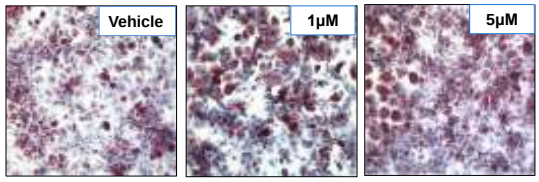
Conclusions: Higher prenatal serum PFOA concentrations were associated with greater adiposity at 8 years and a more rapid increase in BMI between 2-8 year

Obesity (Silver Spring). 2016 Jan;24(1):231-7.

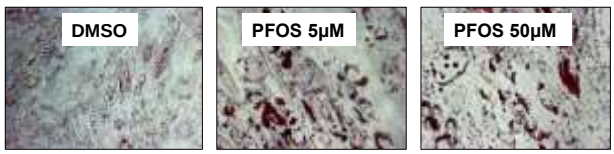
Early life exposure and Adipogenesis



PFOS induces fibroblasts to become adipocytes



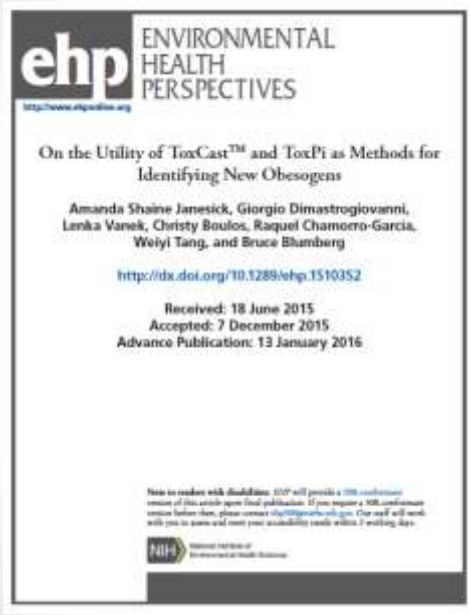
Mouse



Human

Molecular Targets: PPAR-gamma and Nrf2

Xu *et al.,* 2016 (Slitt Lab)



ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

On the Utility of ToxCast™ and ToxPi as Methods for Identifying New Obesogens

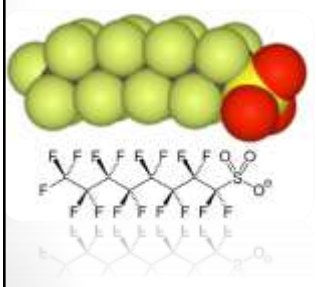
Amanda Shaine Janesick, Giorgio Dimastrogiovanni, Lenka Vanek, Christy Boulos, Raquel Chamorro-Garcia, Weiyi Tang, and Bruce Blumberg

http://dx.doi.org/10.1289/ehp.1510352

Received: 18 June 2015
Accepted: 7 December 2015
Advance Publication: 13 January 2016

Are there additional interactions?

Can PFAS exposure be a risk factor for diet-induced disease or vice versa?



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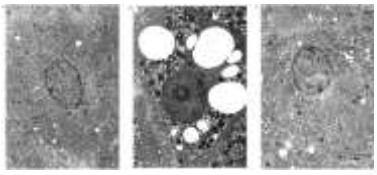
NAFLD

- Difficult to diagnose
  - Liver biopsy
- Often use biomarkers of liver injury
- A study using the National Health and Nutrition Examination Survey (NHANES) found a 30% rate of NAFLD in the United States between 2011 and 2012.
- NAFLD is associated with insulin resistance and metabolic diseases
- Treatment is often diet and exercise
- Mimic NAFLD with diet
  - 60% kcal

(Ruhl and Everhart, 2014)

NAFLD and PFOS

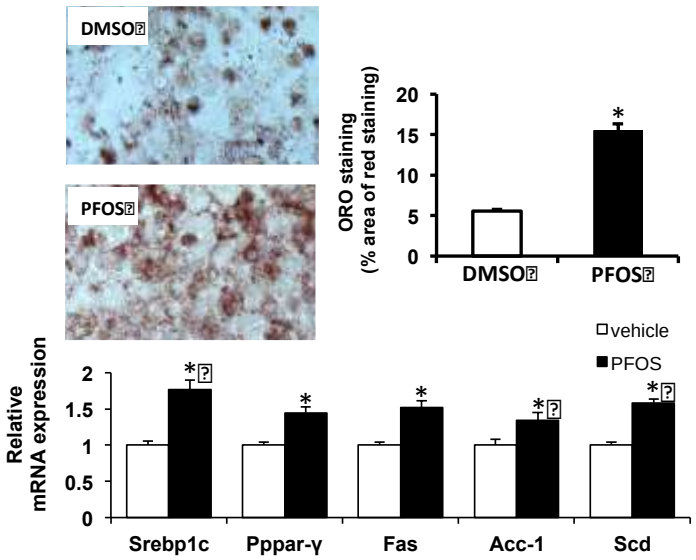
- A human study from the C8 Health Project found a positive association between PFOS concentrations and serum ALT level, a marker of liver damage
- In a study of monkeys, PFOS decreased body weights and increased liver weights
  - Hepatocellular hypertrophy and lipid vacuolation



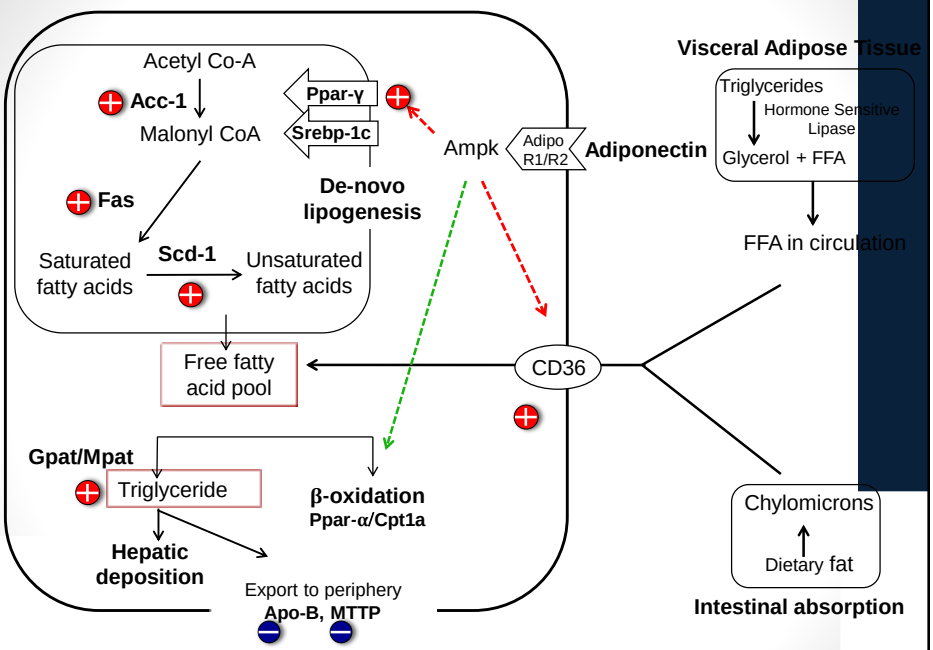
Seacat et al, 2002

(Gallo et al, 2012; Seacat et al, 2002)

PFOS induces lipid deposition in human hepatocytes



Slitt lab, unpublished data generated by Prajakta Shimpi



Goals

- ✓ **Develop a NAFLD Phenotype (4 Wks)**
  - 60% Kcal HFD
- ✓ **Switch to LFD to mimic weight loss improvement of NAFLD**
  - 40% decease in total calorie content
- ✓ **Introduce low dose PFOS exposure in food (~360 µg/kg/day)**
- ✓ **Characterize metabolic effects**
  - Glucose Metabolism
  - Lipid Metabolism

Hypothesis

- ✓**Determine if low dose PFOS exposure will also cause exacerbation of NAFLD in high fat diet treated mice**
- ✓**Determine if low dose PFOS exposure in food will cause resistance to weight loss-induced improvement of NAFLD.**

Study design

Start:
6 wk-old male C57BL/6 mice

Introduced PFOS in food (0.0003%)

10 wks

4 wks

Necropsy

Confirm NAFLD phenotype via FBG

2: FBG

4: FBG

5: FBG and GTT

8: FBG and PTT

Diet Switch

Control LFD → LFD

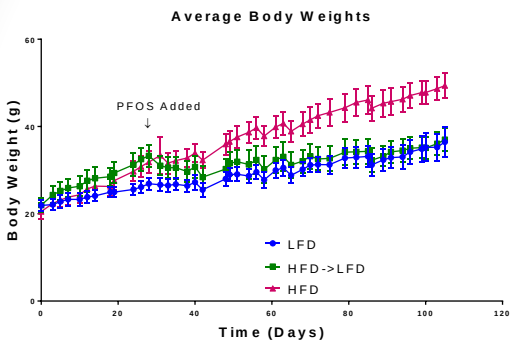
Control LFD → HFD-LFD

60% kCal HFD → HFD-LFD

60% kCal HFD → HFD

Body Weight was measured every Mon, Wed and Fri

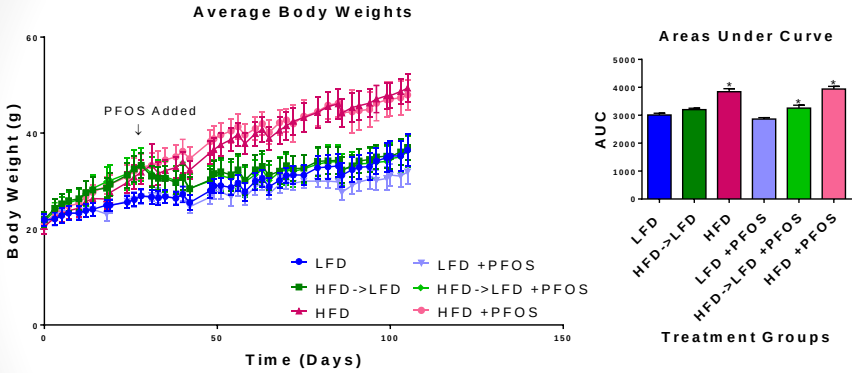
NAFLD Phenotype

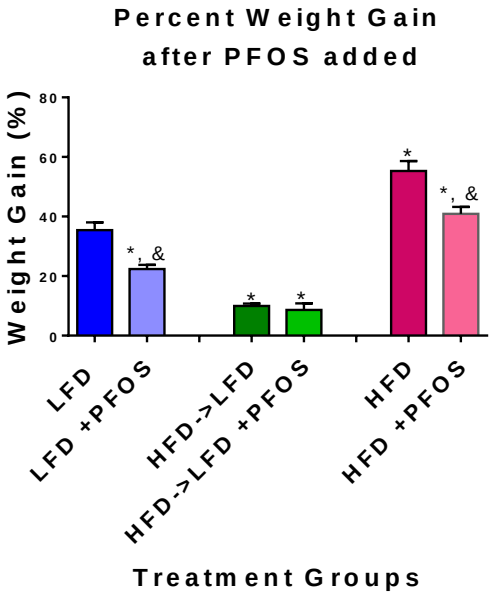


Expect to See:

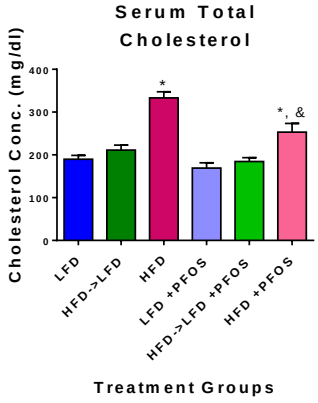
- Increase in body weight
- Increase in liver weight
- Increase in WAT weight
- Increase in insulin insensitivity
- Increase in serum lipids
- Increase in liver lipids

Body weight

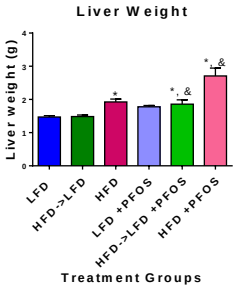

Average Body Weights
Body Weight (g)
PFOS Added
Time (Days)
LFD
HFD->LFD
HFD
LFD +PFOS
HFD->LFD +PFOS
HFD +PFOS
Areas Under Curve
AUC
Treatment Groups

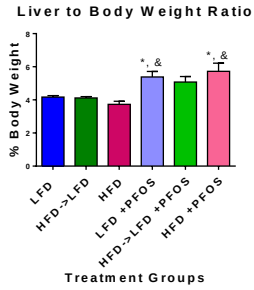

Percent Weight Gain
after PFOS added
Weight Gain (%)
LFD
LFD +PFOS
HFD->LFD
HFD->LFD +PFOS
HFD
HFD +PFOS
Treatment Groups

Serum chemistry at necropsy

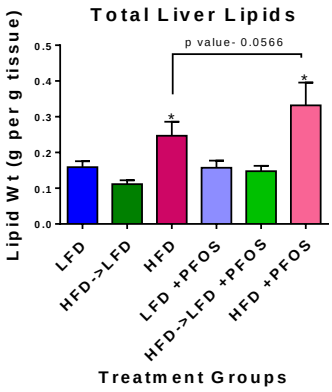


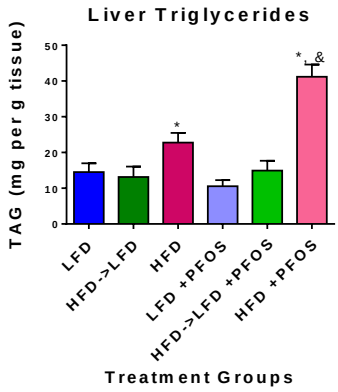
Tissue weights at necropsy

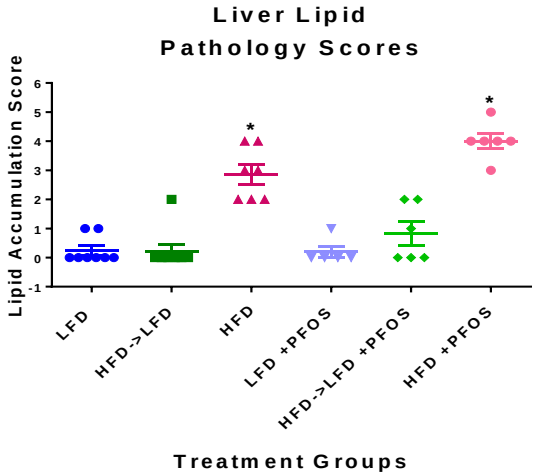




Liver Lipids

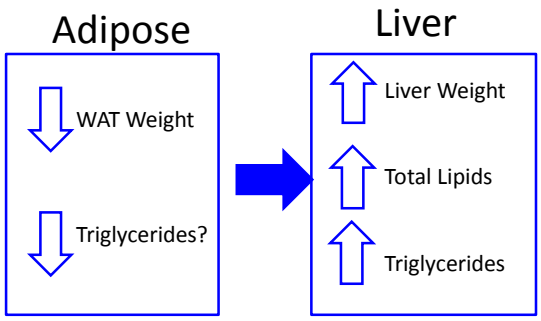






0	No significant lesions (score of 0) = zero percent injury/death
1	Minimal (score of 1)= less than 10 percent injury/death
2	Mild (score of 2)= 10-25 percent injury/death
3	Moderate (score of 3)= 25-40 percent injury/death
4	Marked (score of 4)= 40-50 percent injury/death
5	Severe (score of 5)= 50 percent or greater injury/death

Implications: Lipid Metabolism



Summary and Implications

- PFASs are associated with metabolic and lipid disruption
- Most studies examining cholesterol associations are cross sectional - Not clear whether it is cause or effect
- Some evidence for developmental windows being important – how do we predict this effect?
- Should we be considering “special populations”
- We do need to think about liver, but not with regard to cancer – but perhaps with regard to fatty liver disease

THANK YOU!

URI
- Laura Armstrong
- Deanna Salter
- Prajakta Shimpi
- Emily Martell
- Marisa Pfohl

- **NIEHS (K22, ONES, R15**)
- NCCR (P20 RI-INBRE),
- The Rhode Island foundation
- URI Council For Research