Exposure of Hyperlipidemic Mice to a Mixture of Legacy, Alternative, and Emerging Per- and Polyfluoroalkyl Substances (PFAS) Modulates Cholesterol Metabolism

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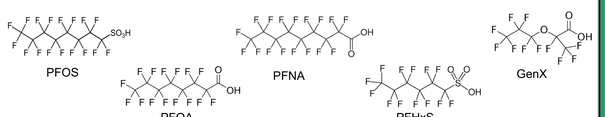
Abstract

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals used in a variety of consumer and industrial products that are ubiquitous in the environment and in humans across the globe. Epidemiological studies have associated PFAS exposure with dyslipidemia and cardiometabolic disorders. Our studies aim to investigate mechanisms linking exposure to a PFAS mixture with alterations in lipid metabolism, notably increased circulating cholesterol, and the development of cardiovascular diseases. To begin, male and female Ldlr KO mice were fed a low-fat atherogenic diet and exposed to drinking water containing a mixture of 5 PFAS representing legacy, alternative, and emerging subtypes (i.e., PFOA, PFOS, PFNA, PFHxS, GenX), each at a concentration of 2mg/L, for 7 weeks. Exposure to PFAS increased circulating cholesterol in both male and female mice after 7 weeks compared to vehicle. LC-MS was then used to determine alterations in bile acid levels in the plasma and feces. These analyses revealed that bile acid levels increased in the plasma of male and female mice exposed to PFAS. However, fecal levels of excreted bile acids were decreased in the PFAS-exposed mice. In the liver, protein levels of the hepatic bile acid uptake transporter NTCP was increased in the PFAS-exposed mice, especially in the males. Also in males, the ileal bile acid uptake transporter ASBT was increased in the PFAS-exposed mice. Finally, we also used 16s sequencing and observed altered diversity and genera associated with the production of secondary bile acids (e.g., *clostridium)* in male PFAS-exposed mice.

Background

- PFAS are chemicals used in a variety of industrial and consumer products including carpets, cookware, food packaging, fire fighting suppressants/foams.
- PFAS are highly resistant to degradation in aquatic environments. They are widely distributed in the environment and detected in the blood of 98% of all adult Americans (NHANES).
- Epidemiological studies supported by animal studies have identified positive associations between PFAS and elevated cholesterol, triglycerides, immune suppression, reproductive/developmental effects and some cancers.
- People living in and around areas of industrial manufacturing, airports, or military bases may be more likely to be highly exposed to PFAS.
- Five commonly found PFAS explored in these studies include: Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Tridecafluorohexane-1-sulfonic acid (PFHxS), and Ammonium perfluoro(2-methyl-3-oxahexanoate (GenX).



7-Week PFAS exposure in Ldlr-/- mice fed pro-atherogenic diet

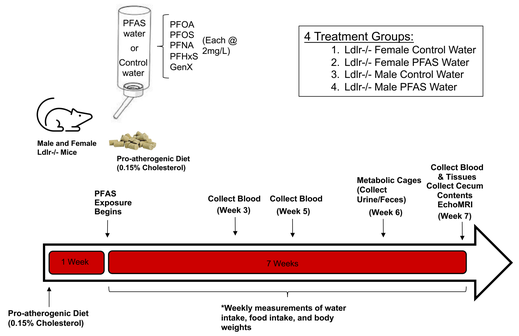
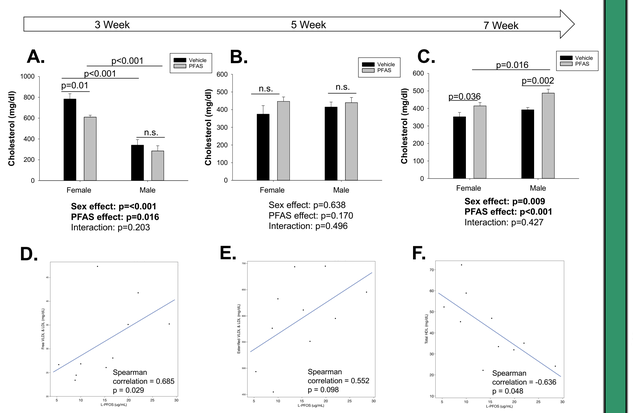


Figure 1: Male and female Ldlr-/- mice were fed an atherogenic diet used in previous studies of pollutant-accelerated atherosclerosis and exposed to water containing a mixture of 5 PFAS representing legacy, replacement, and emerging subtypes (i.e., PFOA, PFOS, PFNA, PFHxS, and GenX), each at a concentration of 2 mg/L, for 7 weeks.

PFAS exposure leads to increased cholesterol LDL-VLDL fractions



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PFAS	Spearman Correlation Coefficient; p-value							
	Total VLDL & LDL Cholesterol		Free VLDL & LDL Cholesterol		Esterified VLDL & LDL Cholesterol		Total HDL	
PFOS	0.515	p=0.128	**0.685**	**p=0.029***	*0.552*	*p=0.098*	**-0.636**	**p=0.048***
PFOA	*0.600*	*0.067*	*0.624*	*0.054*	**0.649**	**0.043***	*-0.624*	*0.054*
PFHxS	0.321	0.366	0.249	0.489	0.406	0.244	-0.321	0.366
PFNA	0.455	0.187	0.467	0.174	0.527	0.117	-0.418	0.229
HFPO-DA	-0.1636	0.652	-0.261	0.467	-0.079	0.829	0.091	0.803

Figure 3: Total cholesterol levels were measured in Ldlr KO mice at A) 3, B) 5, and C) 7 weeks of PFAS exposure. A Spearman's correlation was performed to determine the relationship between serum levels of PFOS and D) free VLDL & LDL cholesterol, E) esterified VLDL & LDL cholesterol, and F) total HDL cholesterol at 7 weeks of PFAS exposure. Spearman's correlation coefficients and corresponding p-values are displayed in G for correlations with all 5 PFAS. For A-C, statistical significance for all (p<0.05) was determined by two-way ANOVA analysis and post-hoc comparisons by Holm-Sidak method. Bars represent mean ± S.E.M. For D-G, * and bolded notation denote significance p<0.05. Italics denote p-values approaching significance.

PFAS exposure increases plasma bile acids

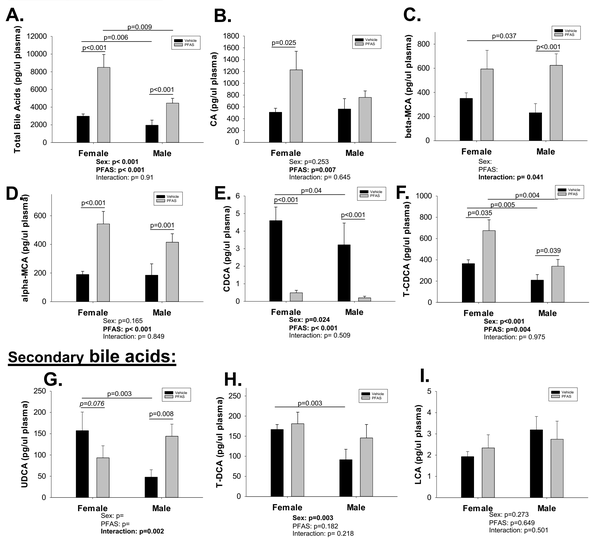


Figure 4: Plasma samples from n=10 Ldlr KO mice from each treatment group were analyzed using LC-MS at 7 weeks PFAS exposure and bile acid concentrations were measured. Statistical significance for all (p<0.05) was determined by two-way ANOVA analysis and post-hoc comparisons by Holm-Sidak method. Bars represent mean ± S.E.M.

PFAS exposure leads to decreased bile acid excretion

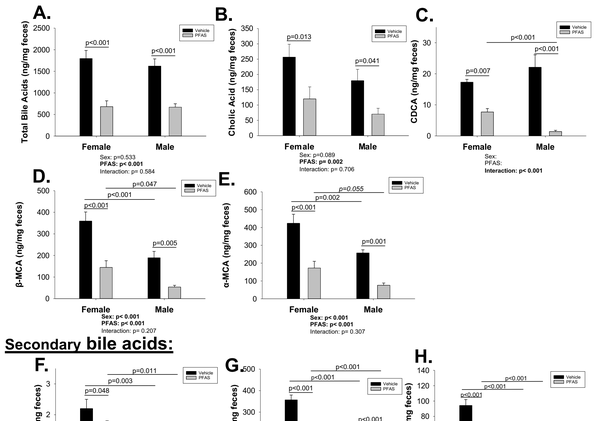
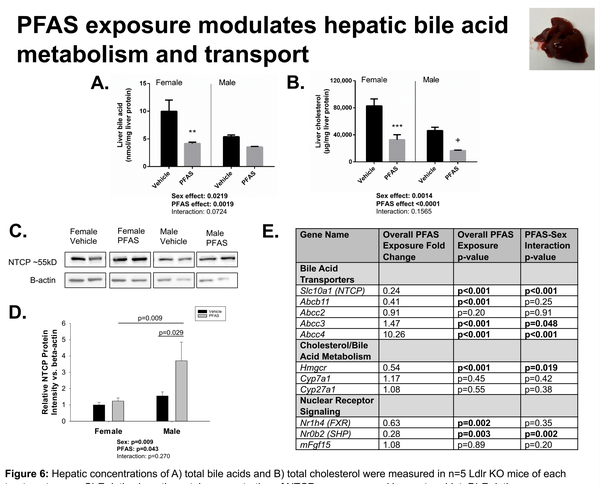


Figure 5: Fecal samples from n=6 mice from each treatment group of Ldlr KO mice were analyzed using LC-MS. Bile acid concentrations were measured at 7 weeks PFAS exposure. Statistical significance for all (p<0.05) was determined by two-way ANOVA analysis and post-hoc comparisons by Holm-Sidak method. Bars represent mean ± S.E.M.

PFAS exposure modulates hepatic bile acid metabolism and transport



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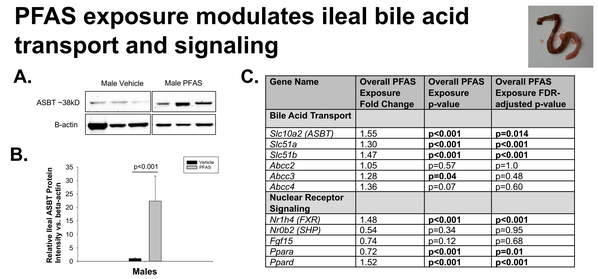
Gene Name	Overall PFAS Exposure Fold Change	Overall PFAS Exposure p-value	PFAS-Sex Interaction p-value
Bile Acid Transporters			
Slc10a1 (NTCP)	0.24	**p<0.001**	**p<0.001**
Abcb11	0.41	**p<0.001**	p=0.25
Abcc2	0.91	p=0.20	p=0.91
Abcc3	1.47	**p<0.001**	**p=0.048**
Abcc4	10.26	**p<0.001**	**p<0.001**
Cholesterol/Bile Acid Metabolism			
Hmgcr	0.54	**p<0.001**	**p=0.019**
Cyp7a1	1.17	p=0.45	p=0.42
Cyp27a1	1.08	p=0.55	p=0.38
Nuclear Receptor Signaling			
Nr1h4 (FXR)	0.63	**p=0.002**	p=0.35
Nr0b2 (SHP)	0.28	**p=0.003**	**p=0.002**
mFgf15	1.08	p=0.89	p=0.20

Figure 6: Hepatic concentrations of A) total bile acids and B) total cholesterol were measured in n=5 Ldlr KO mice of each treatment group. C) Relative hepatic protein concentration of NTCP were measured by western blot. D) Relative concentration of NTCP was measured in n=10 Ldlr KO mice of each treatment group, normalized to beta-actin. E) RNA levels of hepatic genes were measured by RT-PCR. Statistical significance for all (p<0.05) was determined by two-way ANOVA analysis and post-hoc comparisons by Holm-Sidak method. Bars represent mean ± S.E.M.

PFAS exposure modulates ileal bile acid transport and signaling



C.

Gene Name	Overall PFAS Exposure Fold Change	Overall PFAS Exposure p-value	Overall PFAS Exposure FDR-adjusted p-value
Bile Acid Transport			
Slc10a2 (ASBT)	1.55	**p<0.001**	**p=0.014**
Slc51a	1.30	**p<0.001**	**p<0.001**
Slc51b	1.47	**p<0.001**	**p<0.001**
Abcc2	1.05	p=0.57	p=1.0
Abcc3	1.28	**p=0.04**	p=0.48
Abcc4	1.36	p=0.07	p=0.60
Nuclear Receptor Signaling			
Nr1h4 (FXR)	1.48	**p<0.001**	**p<0.001**
Nr0b2 (SHP)	0.54	p=0.34	p=0.95
Fgf15	0.74	p=0.12	p=0.68
Ppara	0.72	**p<0.001**	**p=0.01**
Ppard	1.52	**p<0.001**	**p<0.001**

Figure 7: Relative ileal protein concentration of ASBT were measured by western blot, examples of which are shown in A. B) Relative concentration of ASBT was measured in n=10 male Ldlr KO mice, normalized to beta-actin. C) RNA sequencing was used to analyze relative transcriptional effects on ileal genes. Statistical significance for all (p<0.05) was determined by two-way ANOVA analysis where applicable and post-hoc comparisons by Holm-Sidak method. Bars represent mean ± S.E.M. For B, statistical significance was determined by Mann-Whitney Rank Sum Test.

PFAS decreases microbiota diversity in male mice

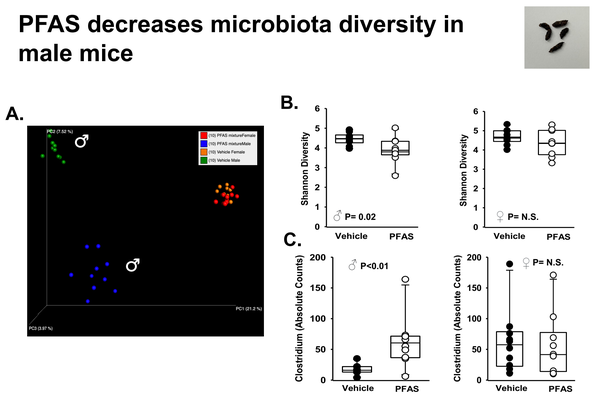


Figure 8: Cecum contents were collected from LDLR KO mice (n=10 per group) exposed to PFAS mixture for 7 weeks. 16s rRNA sequencing was used to determine PFAS-induced changes to bacterial diversity. (A) PCoA plots of beta diversity of Ldlr KO mice. (B) Shannon Diversity index of alpha diversity for Ldlr KO mice (male and female). (C) Clostridium counts in Ldlr KO mice (male and female). Significant difference between vehicle and PFAS-exposed mice (p<0.05).

Synthesis and circulation of cholesterol and bile acids

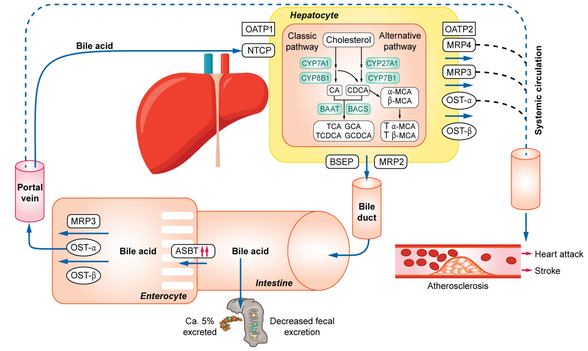


Figure 2: Diagram of transcriptional pathways and transporter proteins involved in the synthesis of bile acids and movement through the enterohepatic circulation.

Conclusions

- Exposure to the PFAS mixture resulted in elevated plasma cholesterol levels and reduced hepatic cholesterol levels in hyperlipidemic mice by 7 Weeks.
- PFOS and PFOA had a positive correlation with LDL-VLDL cholesterol fraction and a negative correlation with HDL cholesterol fraction.
- Exposure to the PFAS mixture resulted in reduced hepatic bile acid levels, increased circulating bile acid levels, and reduced bile acid excretion.
- PFAS exposure modulates bile acid transporters. Hepatic NTCP levels were increased I male mice. Ileal ASBT levels were increased in male mice.
- The gut microbiota demonstrated significantly different effects due to PFAS exposure in the hyperlipidemic Ldlr KO mice that varied depending on sex.

Acknowledgements

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