Associations of a Prenatal Serum Per- and Polyfluoroalkyl Substance Mixture with the Cord Serum Metabolome in the HOME Study

Amber M Hall[1*], Elvira Fleury[1], George D. Papandonatos[2], Jessie P. Buckley[3], Kim M. Cecil[4,5], Aimin Chen[6], Bruce P. Lanphear[7], Kimberly Yolton[4], Douglas I. Walker[8], Kurt D. Pennell[9], Joseph M Braun[1,**] and Katherine E. Manz[9,10**]

[1]Department of Epidemiology, Brown University, Providence, RI, United States; [2]Department of Biostatistics, Brown University, Providence, RI, United States; [3]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States; [4]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, United States; [5]Department of Radiology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH; [6]Department of Biostatistics, Epidemiology and Informatics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States; [7]Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada; [8]Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, United States; [9]School of Engineering, Brown University, Providence, RI, United States; [10]Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, Michigan, United States.

School of Public Health
BROWN UNIVERSITY

Study Aim

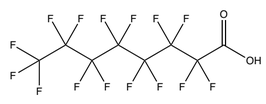
We evaluated the impact of four serum per- and polyfluoroalkyl substances (PFAS) at ~16 weeks' gestation on the cord serum metabolome, finding associations between all PFAS and the cord serum metabolome

Background

- Per- and polyfluoroalkyl substances (PFAS) are ubiquitous and persistent synthetic chemicals used in numerous consumer goods and industrial products for their oil-, water-, and heat- resistant properties.
- The metabolome encompasses millions of small molecules (<1500 Da) derived from endogenous metabolism, environmental exposures, diet, and the microbiome.
- Prenatal exposure to PFAS has been associated with adverse health outcomes; however, the impact of these chemicals on the infant metabolome is largely unknown.

Methods

- 264 mother-infant dyads from the Health Outcomes and Measures of the Environment (HOME) Study were included.
  - Participants enrolled between March 2003 and January 2006.
  - Recruitment occurred at obstetric practices in Cincinnati, Ohio.
- Four PFAS were measured at ~ 16 weeks gestation in maternal serum.
  - These PFAS were
    perfluorooctanoic acid (PFOA)
    perfluorononanoic acid (PFNA)
    perfluorooctanesulfonic acid (PFOS)
    perfluorohexanesulfonic acid (PFHxS)



PFOA

- 14,402 cord serum metabolites were detected.
  - Untargeted metabolomics was performed using liquid chromatography high-resolution mass spectrometry in mixed mode.
    - This analysis was performed in triplicate.
  - Metabolites with coefficients of variation (CVs) >30% were removed.
  - Results were were averaged across triplicate.
  - Metabolites were batch corrected using WaveICA 2.0.

Statistical Analysis

Metabolome Wide Association Study (MWAS)

Purpose: Analyze associations between the PFAS mixture and each PFAS with each metabolite

- A quantile-based *g*-computation model was used to analyze the PFAS mixture and each metabolite.
- Linear regression models were used to analyze individual PFAS (PFOA, PFNA, PFOS, and PFHxS)
- All analyses were adjusted for household income, maternal race, maternal age, cotinine concentrations (i.e., exposure to tobacco), and parity.
- A False Discovery Rate (FDR) <20% was considered statistically significant.
- Metabolites were annotated using the Human Metabolome Database (HMDB).

Pathway Enrichment Analysis (PEA)

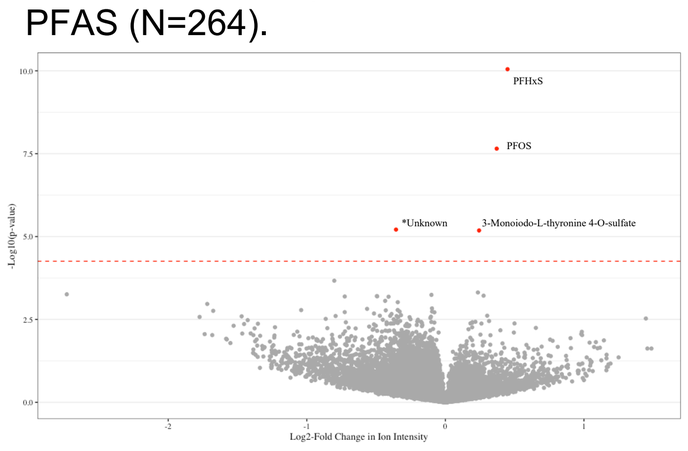
Purpose: Identify biological pathways where endogenous metabolites within the pathway were significantly enriched by prenatal PFAS concentrations

- *Mummichog* PEA was used to identify pathways significantly enriched by the PFAS mixture and each PFAS.
  - These analyses used output from their corresponding MWAS analyses to determine which metabolites were significantly enriched ($p < 0.05$).
- All PEA used a mass tolerance of 5ppm and 10,000 permutations for metabolites in each pathway.
  - These analyses were restricted to pathways with 3 or more metabolites.
- p(Gamma) <0.05 was considered statistically significant.

Results

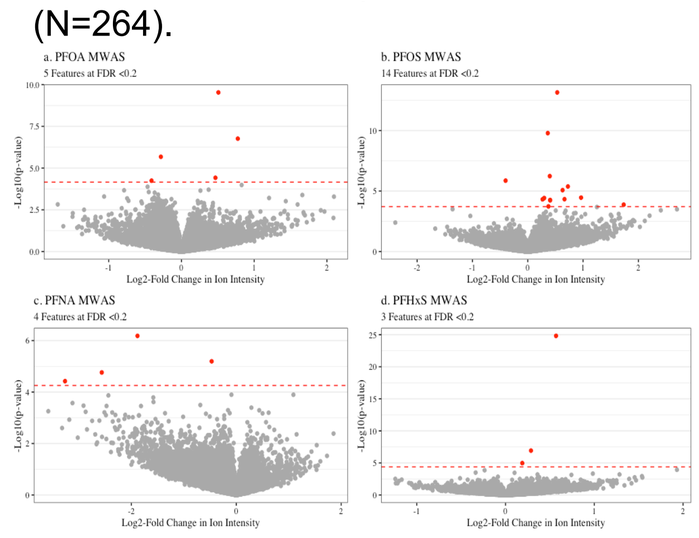
Metabolome Wide Association Study (MWAS)

Figure 1. Identified and putatively identified cord serum metabolic features associated with a prenatal mixture of four PFAS (N=264).



- For the MWAS of the PFAS mixture, four features were significant at FDR <0.2.
  - These features included
    - 2 PFAS: PFOS and PFHxS
    - 3-monoiodo-L-thyronine 4-O- sulfate
    - An unidentified metabolite

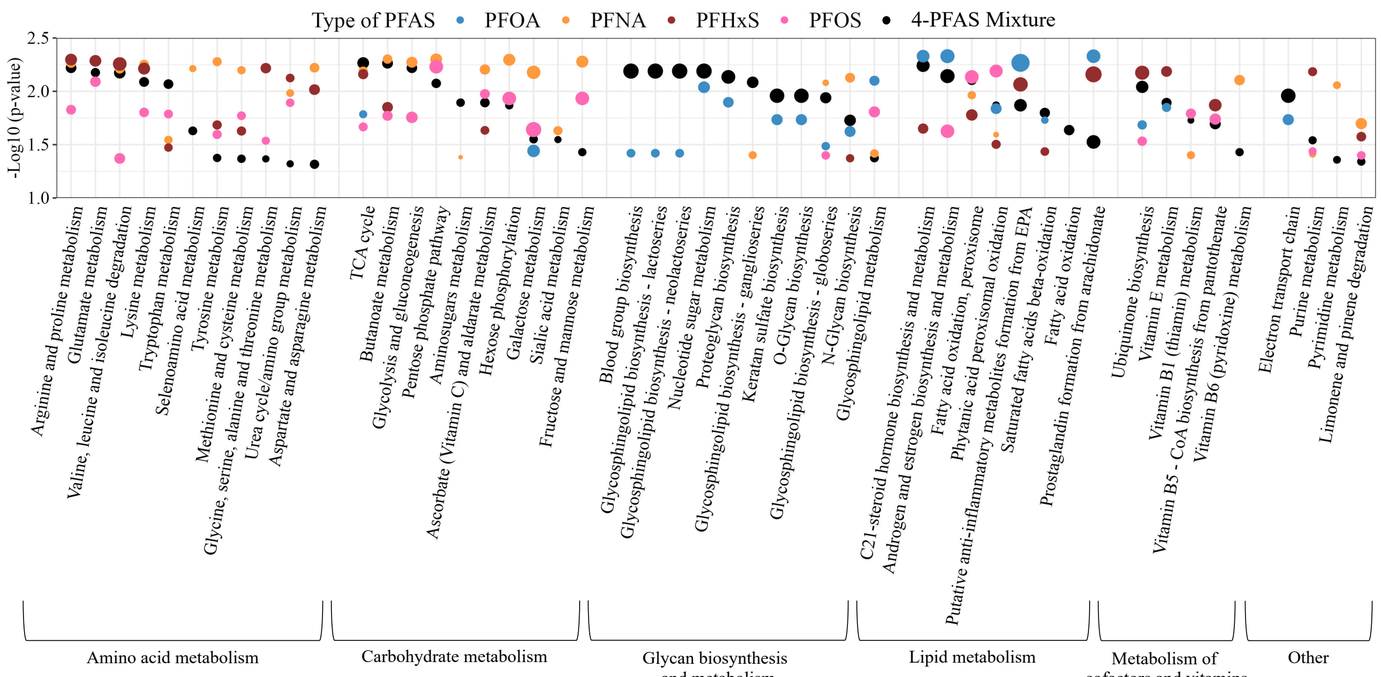
Figure 2. Cord serum metabolic features associated with four prenatal PFAS (N=264).



- For the MWAS of PFOA, several significant metabolites were annotated to glucocorticoid metabolites
- For the MWAS of PFNA, several significant metabolites were annotated to lysine metabolites
- For the MWAS of PFOS and PFHxS, a significant metabolite was annotated to be 3-monoiodo-L-thyronine 4-O-sulfate, which was consistent with the PFAS mixture MWAS

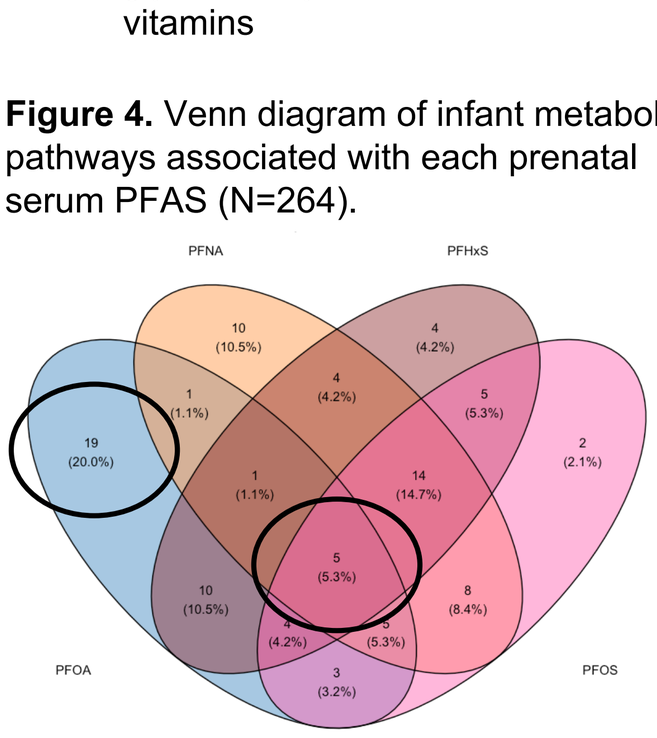
Pathway Enrichment Analysis (PEA)

Figure 3. Cord serum metabolic pathways significantly associated with mixture of four PFAS and individual PFAS concentrations during gestation (N=264).



- 49 biological pathways were associated with the PFAS mixture [p(Gamma)< 0.05]
  - These pathways included amino acid-, carbohydrate-, and lipid- metabolism, glycan biosynthesis and metabolism, and metabolism of cofactors and vitamins

Figure 4. Venn diagram of infant metabolic pathways associated with each prenatal serum PFAS (N=264).



- For the PEA of the four individual PFAS, five pathways overlapped across all PFAS.
  - These pathways were
    - TCA cycle
    - Keratin sulfate degradation
    - Benzoate degradation via CoA ligation
    - Phytanic acid peroxisomal oxidation
    - Alkaloid biosynthesis
- PFOA had most unique pathways
  - Most of these pathways were related to lipid- and glycan- metabolism.

Conclusion

- Prenatal PFAS mixtures can disrupt metabolic processes critical to neonatal and child health.
- Pathways identified in this study have been associated with both PFAS exposure and type 2 diabetes, hepatocellular carcinoma, and low birthweight.
- Given the pervasiveness of PFAS and their known health impacts, future studies should assess if these pathways mediate associations of prenatal PFAS exposure with infant/child health outcomes, such as birthweight or vaccine response.

Research was supported by NIEHS R01 ES032836, P01 ES011261, R01 ES020349, R01ES031621, R01 ES027224, R01 ES025214, and R01 ES030078