Environmental Science & Technology

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Article

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

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Cite This: *Environ. Sci. Technol.* 2023, 57, 21627−21636

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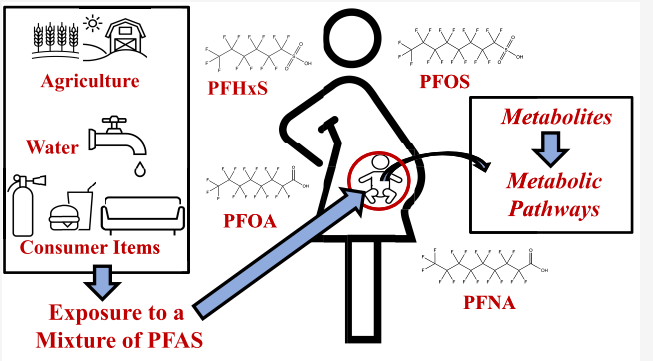
ABSTRACT: Per- and polyfluoroalkyl substances (PFAS) are ubiquitous and persistent chemicals associated with multiple adverse health outcomes; however, the biological pathways affected by these chemicals are unknown. To address this knowledge gap, we used data from 264 mother−infant dyads in the Health Outcomes and Measures of the Environment (HOME) Study and employed quantile-based g-computation to estimate covariate-adjusted associations between a prenatal (∼16 weeks' gestation) serum PFAS mixture [perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), and perfluorononanoic acid (PFNA)] and 14,402 features measured in cord serum. The PFAS mixture was associated with four features: PFOS, PFHxS, a putatively identified metabolite (3-monoiodo-L-thyronine 4-*O*-sulfate), and an unidentified feature (590.0020 *m*/*z* and 441.4 s retention time; false discovery rate <0.20). Using pathway enrichment analysis coupled with quantile-based g-computation, the PFAS mixture was associated with 49 metabolic pathways, most notably amino acid, carbohydrate, lipid and cofactor and vitamin metabolism, as well as glycan biosynthesis and metabolism (P(Gamma) <0.05). Future studies should assess if these pathways mediate associations of prenatal PFAS exposure with infant or child health outcomes, such as birthweight or vaccine response.



KEYWORDS: *epidemiology, PFAS, prenatal, untargeted metabolomics, mixture*

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous synthetic chemicals used in consumer products, such as cookware, food packaging, textiles, and firefighting foams for their oil-, water-, and heat-resistant properties.[1−4] PFAS do not readily degrade in the environment and contaminate drinking water, ground water, and surface water, as well as food, air, biosolids, and agricultural products.[5] As a result of their ubiquitous distribution and persistence in the environment, people are continuously exposed to PFAS via ingestion of contaminated drinking water, food, indoor dust, or air.[1,6,7] Many PFAS bioaccumulate in humans, including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorohexanesulfonic acid (PFHxS), with half-lives ranging from 3.8 to 8.2 years.[8,9]

Prenatal exposure to individuals and mixtures of PFAS has been associated with reduced birthweight, preterm birth, decreased vaccine response in children, and unfavorable cardiometabolic outcomes.[1,10−15] Yet, the mechanisms by which PFAS exposure leads to these conditions are unclear. Thus, studies are needed to investigate the range of biological pathways with which PFAS interact to provide insight into the array of adverse health effects associated with PFAS exposures.

Untargeted metabolomics is a discovery-based technique that comprehensively assesses metabolic profiles and pathways.[16] In tandem with statistical tools like metabolome-wide association studies (MWAS) and pathway enrichment analysis, relations between exposures to PFAS and hundreds to thousands of metabolites and their corresponding biological pathways can be investigated.[16−25] As such, untargeted metabolomics is being increasingly used to identify metabolites and pathways associated with human exposure or disease.[16]

While numerous statistical methods have been developed to quantify the potential effects of chemical mixtures, few studies have evaluated the impact of PFAS mixtures on the fetal

Received: September 12, 2023
Revised: November 22, 2023
Accepted: November 27, 2023
Published: December 13, 2023

https://doi.org/10.1021/acs.est.3c07515
Environ. Sci. Technol. 2023, 57, 21627−21636

Table 1. Maternal Serum Per- and Polyfluoroalkyl Substance (PFAS) Concentrations in the HOME Study (2003 to 2006, N = 264) and NHANES (2003 to 2008, N = 180)[a]

	NHANES	HOME Study							
PFAS (ng/mL)	median (IQR)[b]	geometric mean (GSD)[c]	min[d]	25%	50%	75%	max	LOD	N (%) ≥ LOD
carboxylic acids									
PFOA	2.2 (1.2, 3.3)	5.5 (1.8)	0.5	3.9	5.6	8.0	26	0.1	100
PFNA	0.6 (0.4, 0.9)	0.9 (1.5)	0.1	0.7	0.9	1.2	2.9	0.1	100
sulfonic acids									
PFHxS	1.0 (0.5, 1.9)	1.5 (2.1)	<LOD	0.9	1.5	2.4	31	0.1	99
PFOS	10 (5.3, 14)	13.24 (1.7)	0.4	9.9	14	18	57	0.2	100
sulfonamides									
MeFOSAA		0.5 (1.9)	<LOD	0.3	0.4	0.8	2.8	0.09	100

[a]HOME, Health Outcomes and Measures of the Environment; NHANES, National Health and Nutrition Examination Survey; IQR, interquartile range; SD, standard deviation; Min, minimum; Max, maximum; LOD, level of detection; N, frequency; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDEA, perfluorodecanoic acid; PFHxS, perfluorohexanesulfonic acid; PFOS, perfluorooctanesulfonic acid; PFOSA, perfluorooctanesulfonamide; MeFOSAA, N-methylperfluorooctane sulfonamidoacetic acid; EtFOSAA, N-ethyl perfluorooctane sulfonamidoacetic acid. [b]Data reported in ref 43. [c]PFAS below the level of detection were imputed using LOD/$\sqrt{2}$ for geometric means and SDs. [d]Some minimums may be below the level of detection for values with 100% above the LOD due to rounding.

metabolome.[16−24] Understanding the impact of PFAS exposures on the fetal metabolome is important because PFAS can cross the placenta, and the fetus may be more sensitive to environmental exposures compared to later life stages.[1] Furthermore, the investigation of chemical mixtures is critical, given that PFAS exposure does not occur in isolation.

To address these research gaps, we evaluated the impact of a mixture of PFAS [PFOS, PFOA, PFHxS, and perfluorononanoic acid (PFNA)] concentrations on the fetal metabolome. We used PFAS levels measured in prenatal serum samples and untargeted metabolic profiling of newborn cord serum collected from participants enrolled in a prospective pregnancy cohort study in Cincinnati, Ohio. Additionally, we examined each PFAS individually and included N-methylperfluorooctane sulfonamidoacetic acid (MeFOSAA) in the mixture analysis to gain insight into their potential influence on the mixture in secondary and sensitivity analyses, respectively.

MATERIALS AND METHODS

Study Participants. This study was based on participants from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort that recruited participants between March 2003 and January 2006. Details for this study, including inclusion criteria, have been described previously by Braun et al. (Figure S1).[26] Briefly, 8878 pregnant people were identified from obstetric practices in Cincinnati, Ohio, and the surrounding area, with 1263 (14%) eligible for participation.[26] From these participants, 468 pregnant people enrolled in the study (37%), with 389 having live singleton births.[26] In this study, we included pregnant people with at least one prenatal serum PFAS measurement and untargeted cord serum metabolomics data, resulting in a final sample size of 264 mother−infant dyads.

Serum PFAS Measurements. Methods and procedures have been previously validated and described.[27−29] In brief, after the serum was separated from whole blood, samples were stored at −80 °C in 2.0 mL polypropylene vials.[27−29] Samples were then shipped on dry ice to the Centers for Disease Control and Prevention (CDC) laboratory for analysis.[27−29] PFAS was quantified at the CDC using solid-phase extraction coupled with high-performance liquid chromatography-isotope dilution with tandem mass spectrometry (HPLC-MS/MS), following a modified analytical method.[27−29] Details on instrument settings can be found in Kato et al.[27] Quality control (QC) and blank samples were included in each batch and coefficients of variation (CVs) for QC materials were ∼6%.[27] Levels of detection were 0.1 ng/mL for all PFAS, except for MeFOSAA and PFOS, which were 0.09 and 0.2 ng/mL, respectively. Descriptive statistics are discussed in detail in "Statistical Analysis" Section and reported in Table 1 and in the "Results and Discussion" Section.

We selected four PFAS of interest *a priori*; these were PFOA, PFNA, PFHxS, and PFOS. These four PFAS were identified *a priori* for investigation as these particular PFAS have been recognized by the Environmental Protection Agency as likely to cause adverse health effects and are subject to proposed regulation under the Clean Drinking Water Act.[30] Moreover, these four PFAS have been detected in 97% of the US population, observed to have longer half-lives compared to many other PFAS, such as MeFOSAA, and are often identified at higher levels in the serum compared to other PFAS.[31,32] PFAS samples from 16 weeks' gestation (86%) were preferred to reduce the potential impact of pregnancy-related hemodynamic changes on serum concentrations; however, samples from 26 weeks' gestation (11%) or delivery (3%) were used when a 16-week sample was unavailable. We imputed PFAS values below the limit of detection (LOD) using the LOD divided by the square root of 2 for all analyses and $\log_2$-transformed PFAS to reduce the influence of outliers.

Untargeted Metabolomics. We performed untargeted metabolomics using previously established methods for liquid chromatography high-resolution mass spectrometry (LC-HRMS).[17,33] We collected venous cord blood at delivery and stored isolated serum aliquots at −80 °C before thawing them on ice prior to extraction. We then performed sample extraction by adding 130 μL of acetonitrile containing a mixture of stable isotope standards to 65 μL of serum, vortexing the solution, equilibrating the sample for 30 min, and centrifuging to remove proteins. The resulting supernatant was transferred to an LC-HRMS analysis vial and analyzed on a Q-Exactive HF equipped with a Vanquish ultrahigh-performance liquid chromatograph (Thermo Scientific). For each sample, we analyzed 10 μL aliquots in triplicate using hydrophilic interaction chromatography (HILIC) with positive electrospray ionization and reversed-phase chromatography (C_{18}) with negative electrospray ionization. Further details of the analytical methods, including chemicals and reagents, the chromatography scheme,

https://doi.org/10.1021/acs.est.3c07515
Environ. Sci. Technol. 2023, 57, 21627−21636

QA/QC samples and frequency, and sample batching, are provided in the Supporting Information (Tables S1 and S2).

Data-dependent acquisition (DDA) data were kept and analyzed in the .RAW file. The full scan MS1 data were saved in the .RAW file format and converted to cdf format using Xcalibur File Converter. The converted files were extracted and aligned using apLCMS.[34] Uniquely detected ions, termed features, consisted of mass to charge (m/z), retention time, and ion abundance. We detected 19,032 and 36,243 negative and positive features, respectively. Since we analyzed our samples in triplicate, we limited our analysis to features with coefficients of variation <30% to improve the reliability of our measures. We summarized triplicate injections using the mean and removed features with >20% nondetection (i.e., ion abundance = 0), which resulted in 5863 negative and 8539 positive features. For features with ≤20% nondetect, values with ion abundance = 0 were imputed using the minimum area within a feature divided by the square root of 2. We performed batch correction on feature data using the *WaveICA_2.0* package in R.[35] Finally, metabolic features were $\log_2$-transformed to satisfy the normality assumptions of our statistical models and stabilize the mean−variance relationship.

Covariates. We identified covariates of interest from a literature review and explored relations between these covariates using a directed acyclic graph (DAG) (Figure S2).[36] From this DAG, we obtained a minimal sufficient adjustment set that included maternal age at birth (continuous), self-reported maternal race/ethnicity (White non-Hispanic, Black non-Hispanic, Asian/Pacific Islander, Native American, Hispanic, other race), parity (nulliparous, parous), household income (continuous), and serum cotinine concentrations at 16 weeks gestation (continuous); race was used in this study as a proxy for structural racism. For all analyses, we categorized maternal race/ethnicity as White non-Hispanic vs other race to address data sparsity. All covariates in the minimal sufficient adjustment set were determined via self-reported questionnaire except for cotinine concentrations, which were quantified in maternal serum collected at 16 weeks' gestation using HPLC-MS/MS. Cotinine concentrations were $\log_{10}$-transformed to reduce the influence of outliers.

Statistical Analysis. *Descriptive Statistics.* We calculated the median [interquartile range (IQR)] for continuous variables and the frequency (percentage) for categorical variables included as covariates in our models. We also calculated the median, IQR, minimum value, maximum value, and percentage below the LOD for all measured PFAS. Finally, we calculated Spearman correlation coefficients among PFOA, PFNA, PFHxS, PFOS, and MeFOSAA to evaluate correlations between PFAS.

Multipollutant MWAS. We employed quantile-based g-computation (QGComp) to examine associations between prenatal concentrations of a PFAS mixture and the neonatal metabolome.[37] QGComp calculates the parameters of a marginal structural model; these parameters characterize the difference in feature intensity, resulting from a simultaneous, one-quantile increase of all PFAS in the mixture.[37] Four PFAS of interest were identified *a priori* for inclusion in our PFAS mixture: PFOA, PFNA, PFHxS, and PFOS. These PFAS were identified for inclusion due to their increased likelihood to cause adverse health effects, widespread detection, and longer half-lives compared to other PFAS, such as MeFOSAA and higher serum levels.[30−32] We categorized all PFAS into quartiles for this analysis. To control for the risk of false discoveries due to the large number of statistical tests, we employed the Benjamini−Hochberg method at a false discovery rate (FDR) < 0.2.[38]

As PFOSA and EtFOSAA had low levels of detection (1% and 32% ≥ LOD, respectively), these PFAS were not explored further. Additionally, PFDEA was not further explored due to the lack of variability as 41% of values had concentrations of 0.2 ng/mL (N = 108) and 19% of 0.3 ng/mL (N = 51). However, we included MeFOSAA in a sensitivity analysis given its high level of detection (100% > LOD) and absence of variability concerns. For this sensitivity analysis, we reran analyses using a 5-PFAS mixture (the 4-PFAS mixture plus MeFOSAA), additionally using an FDR < 0.2 to denote statistical significance.[38] These analyses were conducted using the *qgcomp* package in R with default settings.[37]

Single-Pollutant MWAS. To conduct the MWAS of the association of each PFAS included in our PFAS mixture individually with the fetal metabolome, we used multivariable linear regression, adjusting for maternal race, maternal age at delivery, parity, serum cotinine, and household income. Similar to the multipollutant MWAS, we calculated FDR values based on the Benjamini−Hochberg method and utilized an FDR < 0.2.[38]

Annotation of Features. We assigned identities to features by matching commonly detected adducts formed in positive and negative ESI at ±5 ppm using the Human Metabolome Database via xMSannotator.[39] Annotation confidence was reported using the Metabolomics Standards Initiative (MSI) recommendations.[40] In this scale, level 1 is a compound identified by comparison to an authentic reference standard (in this study, we examined MS^2 data in comparison to a verified reference standard), level 2 is a compound annotated by matching isotopic patterns and matching m/z, level 3 is a putatively annotated compound class, and level 4 is an unknown feature.

Metabolic Pathway Enrichment Analysis. We applied *mummichog* pathway enrichment analyses to identify enriched pathways using MetaboAnalystR 3.2 and data from both positive and negative modes.[25] We restricted the adducts used to those that could potentially form based on our mobile phases and internal standards. For the negative mode, we used the adducts M + FA − H [1−], M − H [1−], 2M − H [1−], M − H_2O − H [1−], M − H + O [1−], M(C13) − H [1−], 2M + FA − H [1−], M − 3H [3−], M − 2H [2−], M + ACN − H [1−], M + HCOO [1−], and M + CH_3COO [1−]. For the positive mode, we used the adducts M [1+], M + H [1+], M + 2H [2+], M + 3H [3+], M + H_2O + H [1+], M − H_2O + H [1+], M(C13) + H [1+], M(C13) + 2H [2+], M(C13) + 3H [3+], M − NH_3 + H [1+], M + ACN + H [1+], M + ACN + 2H [2+], M + 2ACN + 2H [2+], M + 3ACN + 2H [2+], M + NH_4 [1+], M + H + NH_4 [2+], 2M + H [1+], and 2M + ACN + H [1+]. We conducted our analyses with the human MFN network, which is curated from multiple libraries, including KEGG, BiGG, and the Edinburgh model. We utilized a mass tolerance of 5 ppm, 10,000 permutations, and a p-value cutoff <0.05 to delineate between significantly enriched and nonsignificantly enriched pathways. We restricted analyses to pathway-specific metabolite data sets containing at least 3 entries. A p(Gamma) < 0.05 was considered statistically significant. A detailed description of our *mummichog* settings can be found in the Supporting (Table S3). All analyses were conducted using R (version 4.2.1).[41] R code for this analysis is available on Github.[42]

https://doi.org/10.1021/acs.est.3c07515
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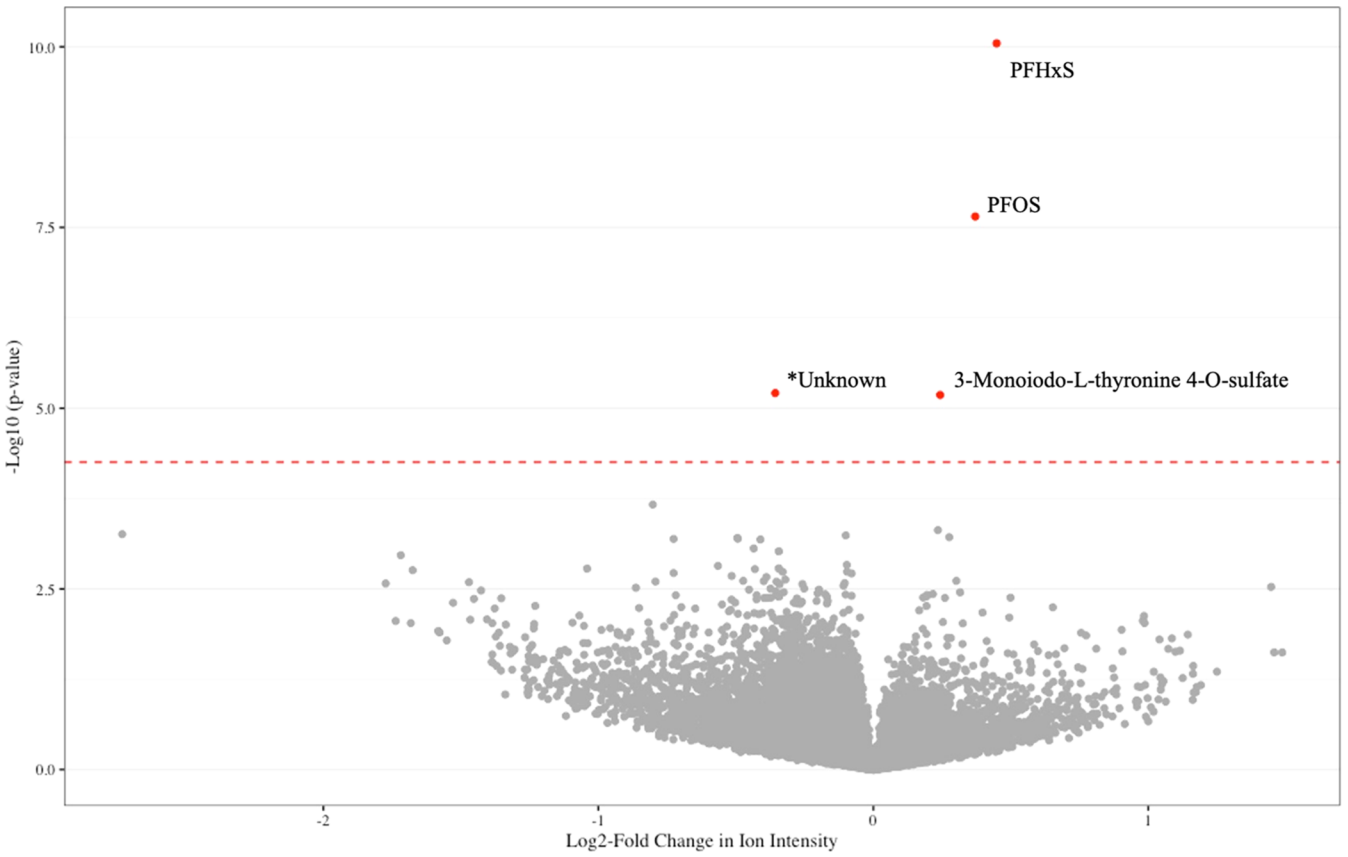

Figure 1. Identified and putatively identified cord serum metabolic features associated with a prenatal (~16 weeks' gestation) mixture of four PFAS in the HOME Study (2003 to 2006, $N = 264$). This analysis used a quantile-based g-computation model of a mixture of four serum PFAS concentrations (PFOA, PFNA, PFHxS, and PFOS) with metabolic features adjusted for household income, maternal race, parity, maternal age at delivery, and tobacco exposure at 16 weeks' gestation; a false discovery rate (FDR) < 0.2 was employed (red dashed line). Annotation was conducted using the Human Metabolome Database (HMBD) and manually inspecting MS2 mass spectra for confirmation and confidence was determined using the Metabolomics Standards Initiative (MSI); PFOS and PFHxS were confirmed at a level 1 confidence, 3-monoiodo-L-thyronine 4-*O*-sulfate was putatively identified at level 3, and the unknown features was level 4. *Unknown feature has a mass-to-charge ratio of 590.0020 and a retention time of 441.4 s.

RESULTS AND DISCUSSION

Mothers in the present study were similar to the full HOME Study cohort in terms of race/ethnicity, household income, parity, age at delivery, and tobacco use (Table S4).[26] Median serum PFNA, PFHxS, and PFOS concentrations in our study were generally similar to medians in pregnant people in NHANES between 2003 and 2008 (Table 1).[43] However, median PFOA concentrations were over 2-fold higher in our study compared to pregnant people in NHANES between 2003 and 2008, possibly due to increased drinking water exposure emanating from a fluoropolymer manufacturing plant upstream of Cincinnati, OH (Table 1).[43,44] MeFOSAA had considerably lower levels than any of the 4-PFAS included in our mixture, with a median of 0.4 ng/mL (IQR: 0.3−0.8). All PFAS analyzed in our study had 99% or more of their values ≥ the LOD (Table 1). Additionally, these PFAS had low-to-moderate pairwise correlations, with Spearman correlation coefficients between 0.02 and 0.51 (Figure S3).

The mixture of PFOA, PFOS, PFNA, and PFHxS was associated with four metabolic features after covariate adjustment (FDR < 0.20; Figure 1). These features include PFOS, PFHxS, 3-monoiodo-L-thyronine 4-*O*-sulfate, and a metabolite with an m/z of 590.0020 and a retention time of 441.4 s. PFOS and PFHxS were identified at a level 1 confidence (confirmed structures; Figure S4), 3-monoiodo-L-thyronine 4-*O*-sulfate was putatively identified at level 3, and the metabolite with an m/z of 590.0020 and a retention time of 441.4 s was level 4 (unknown feature) using the MSI scale. The identification of PFOS and PFHxS (at a level 1 confidence) as significantly associated with the targeted/quantified PFAS mixture containing PFOS, PFHxS, PFNA, and PFOA was expected, despite their detection on two different analytical platforms and further validates this laboratory and statistical methodology. Although PFNA was not identified using this method (either due to the lack of identification by LC-HRMS or removal due to high CVs), the PFAS mixture was also significantly associated with PFOA before FDR correction (non-FDR corrected p-value <0.001).

When examining the relationship between individual PFAS to the cord serum metabolome, PFOA, PFOS, PFNA, and PFHxS were associated with 5, 14, 4, and 3 features, respectively (Figure S5); no significant associations were found between MeFOSAA and any features.

The mixture of PFOA, PFOS, PFNA, and PFHxS was significantly associated with 49 enriched pathways p(Gamma) < 0.05] in our pathway enrichment analysis (Figures 2 and S7). The pathways identified most frequently were associated with amino acid metabolism ($N = 11$), glycan biosynthesis and metabolism ($N = 11$), carbohydrate metabolism ($N = 10$), lipid metabolism ($N = 8$), and metabolism of cofactors and vitamins ($N = 5$); four pathways not grouped into a larger group (referred to as "other" pathways) were also identified. The results for the

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Environ. Sci. Technol. 2023, 57, 21627−21636

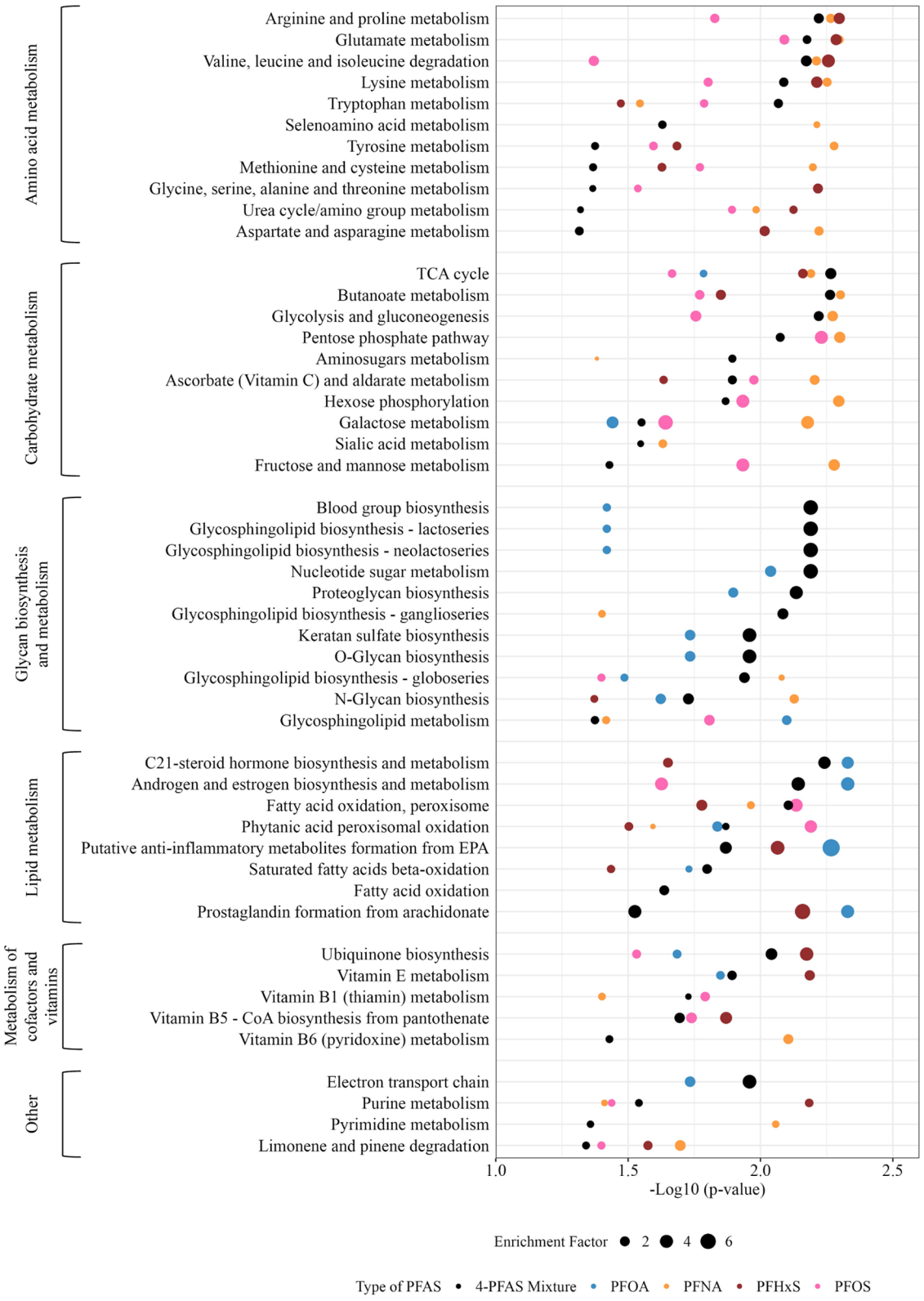

Figure 2. Cord serum metabolic pathways significantly associated with a mixture of four PFAS and individual PFAS concentrations during gestation in the HOME Study (2003 to 2006, $N = 264$). For the 4-PFAS mixture model (PFOA, PFNA, PFHxS, and PFOS) and the single-pollutant models, PEA employed results from a quantile-based g-computation model and four linear regression models, respectively, adjusting for annual household income, maternal race, maternal age at delivery, parity, and tobacco exposure at 16 weeks' gestation. $P(\text{Gamma}) < 0.05$ was considered statistically significant for all mummichog pathway enrichment analyses.

two most enriched pathways for amino acid metabolism (arginine and proline metabolism; valine, leucine, and isoleucine degradation) appeared to be driven by PFHxS, and, to a lesser extent, PFOS, based on the magnitude of the significant p-values observed from single-pollutant models (Figure 2). In contrast, associations between the PFAS mixture and carbohydrate

https://doi.org/10.1021/acs.est.3c07515
Environ. Sci. Technol. 2023, 57, 21627−21636

metabolism appeared to be driven by PFNA. Moreover, several pathways related to glycan biosynthesis and metabolism were likely driven by PFOA, as PFOA has the highest number of significant *p*-values of the mixture [N = 8/11]. In addition, compared to the p-values derived from the PFAS mixture, PFOA seemed to drive associations with blood group biosynthesis, glycosphingolipid biosynthesis (lactoseries), glycosphingolipid biosynthesis (neolactoseries), nucleotide sugar metabolism, proteoglycan biosynthesis, keratan sulfate biosynthesis, and *O*-glycan biosynthesis given its lower p-values compared to other PFAS. The differences between the magnitude of the PFAS mixture p-values and the PFOA p-values did not appear to be explained by any of the other 3 measured PFAS individually, suggestive of potential synergistic interaction between these four PFAS with relation to some pathways within glycan biosynthesis and metabolism. However, synergism was not directly assessed, and evaluation of synergistic and antagonistic chemical interactions was outside of the scope of this study. It did not appear that any individual PFAS in the mixture was driving associations with lipid metabolism or the metabolism of cofactors and vitamins. Additionally, 40 (82%) of the 49 significantly enriched pathways for the PFAS mixture and infant metabolome assessment remained significantly enriched when MeFOSAA was added to the mixture (Figure S8). In a sensitivity analysis by adding MeFOSAA to our PFAS mixture, 3-monoiodo-L-thyronine 4-*O*-sulfate was no longer significant; all other features remained statistically significant, and no other features were identified as significant (Figure S6).

For the single-pollutant models of the PFAS mixture and the infant metabolome, we identified 95 significantly enriched pathways in total [P(Gamma) < 0.05] (Figures S9−S14). Of these pathways, 5 (5.3%) overlapped across all four PFAS; these pathways were the TCA cycle, keratin sulfate degradation, benzoate degradation via CoA ligation, phytanic acid peroxisomal oxidation, and alkaloid biosynthesis (Figures S9−S15). Of these pathways, the TCA cycle, keratin sulfate degradation, and phytanic acid peroxisomal oxidation were associated with the PFAS mixture (Figure 2). Furthermore, 24 (25%) of the observed pathways overlapped across 3 PFAS, 31 (33%) overlapped across 2 PFAS, and 35 (37%) were associated with a single PFAS (Figure S15). A single PFAS was independently associated with at least one of the 49 significantly enriched pathways associated with the PFAS mixture and infant metabolome (Figures 2 and S9−S14).

Notably, one feature associated with PFOA (FDR <0.20) was annotated to several endogenous glucocorticoids with a level 2 confidence. This is consistent with previous animal studies that have found PFOA may inhibit hydroxysteroid dehydrogenases.[45−47] As such, this pathway could be examined in greater detail as a potential mediator of PFAS toxicity in future studies.

The enriched pathways identified in this study have previously been associated with adverse health outcomes.[19,48,49] Epidemiological studies have established that carbohydrate, lipid, and amino acid metabolism are associated with impaired glucose metabolism, leading to adverse health outcomes, such as type 2 diabetes.[19,48] Additionally, amino acid metabolism and glycan biosynthesis and metabolism have both been associated with a higher risk of hepatocellular carcinoma, the most common primary liver cancer.[49] As our study demonstrates an association between PFAS and these metabolic pathways, this may help explain potential mechanisms between PFAS and these outcomes like liver disease.[19,48,50] Therefore, future studies could investigate these pathways as potential mediators between PFAS and these health outcomes. Prior studies have also observed associations of the prenatal and infant metabolome with adverse birth outcomes, such as birthweight.[51−55] For example, Collicino et al. found 35 prenatal serum metabolites related to lipid metabolism (∼30 weeks' gestation) were associated with lower birthweight z-scores in 410 mother−child dyads from Boston or New York City.[51] Conversely, a study by Yeum et al. did not find that prenatal plasma metabolites (24 to 28 weeks' gestation) were associated with birth anthropometry, including birthweight z-scores.[53] However, when evaluating cord plasma rather than maternal plasma, this study did observe associations between metabolic lipid pathways and birthweight and length z-scores in 413 pregnant people and 787 infants in rural New Hampshire.[53] As PFAS have been consistently associated with adverse birthweight, future studies could examine the prenatal or infant metabolome as a mediator of associations between PFAS and adverse birth outcomes.[56] Additionally, PFAS have also been associated with decreased vaccine response in children; however, we do not know of any studies evaluating the serum or plasma metabolome and decreased vaccine response in children, specifically using untargeted metabolomics.[14] Regardless, future studies could evaluate the metabolome as a mediator for the PFAS−vaccine response association.

In a previous report from the HOME Study, PFOS, PFOA, PFHxS, and PFNA concentrations during pregnancy were associated with leukocyte DNA methylation at birth and during adolescence (∼12 years of age).[57] Many of the CpGs associated with PFAS are related to cancers, cardiovascular disease, and kidney function—all outcomes related to PFAS exposure in adults.[57] This result taken in tandem with our study suggests that PFAS may impact biological pathways at both the epigenetic and metabolomic levels. Future studies could use multiomics approaches to better elucidate these relations.

Results from our study are largely consistent with previous studies examining prenatal PFAS or PFAS mixtures in relation to the maternal or infant metabolome, with the most consistent associations observed for the amino acid and lipid metabolism.[22,24,58,59] Taibl et al. evaluated associations of late pregnancy PFOA, PFNA, PFOS, or PFHxS concentrations with the infant metabolome (measured via blood spots within 48 h of birth) for 267 mother−child pairs in African-American women in Atlanta Georgia between 2016 and 2020.[59] They observed significant associations for mostly amino acid pathways; these pathways included arginine, proline, and lysine metabolism but not glutamine metabolism or valine, leucine, and isoleucine degradation. Additionally, a cross-sectional study evaluating 459 pregnant people in the VDAART Study also found that lipid and amino acid pathways were significantly associated with a mixture of PFOA, PFOS, PFDEA, PFHxS, and PFNA and the maternal metabolome, measured in late trimester blood plasma.[22] Another cross-sectional study evaluating four PFAS individually (PFHxS, PFOS, PFOA, or PFNA) and the maternal metabolome (serum between 8- to 14-weeks' gestation) in 313 African-American people found a considerable number of enriched pathways overlapping the pathways identified in our study (amino acid metabolism, glycan biosynthesis and metabolism, carbohydrate metabolism, lipid metabolism, and metabolism of cofactors and vitamins).[58] Although these studies had similar findings, it is important to note that findings were not identical across studies. Some reasons for observed differences between these studies and ours

https://doi.org/10.1021/acs.est.3c07515
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may be the result of utilizing single vs multipollutant models, inclusion of different PFAS in the PFAS mixture, differences in PFAS concentrations between studies, and/or differences in untargeted metabolomics methods.

The present and prior results regarding PFAS exposure and the human metabolome complement findings from rodent studies as well.[60−68] For example, rodent studies have also observed associations between PFAS and lipid metabolism.[60−64] This association is unsurprising as PFAS are structurally similar to fatty acids and have also been associated with dyslipidemia in adults.[65,69] Additionally, rodent studies have also found associations between PFAS and amino acid metabolism as well as carbohydrate metabolism.[60,64,66−68] However, most of these studies examined only PFOS or PFOA.

Our study has several notable strengths. Using a prospective pregnancy cohort, we were able to establish temporality between our exposure and outcome. In addition, we utilized untargeted metabolomics to identify a wide range of metabolic features and pathways, allowing for an assessment of metabolic features and pathways that may not normally be evaluated using hypothesis-driven targeted approaches. Furthermore, we measured each metabolic feature in triplicate, thus improving the precision of our untargeted metabolomics data. Another strength is the evaluation of the joint effect of a prenatal PFAS mixture on the cord metabolome in addition to the single-pollutant effects. This is important as chemicals may jointly impact the metabolome, even when no individual effects exist or appear minimal.[70] Furthermore, as PFAS are ubiquitous and some PFAS were moderately correlated with each other, participants in this study had simultaneous exposure to multiple PFAS, thus justifying our analysis of joint effects.

This study also had some limitations. Even though we detected a high number of metabolic features in this study using an untargeted approach, not every metabolic feature was reliably detectable as some were nondetectable or had CVs > 30%. Thus, we may have potentially missed some associations, as our MWAS and pathway enrichment analysis were restricted only to those detectable by our instruments with a reasonably reliable signal. Second, with 264 observations, we were underpowered to explore potential modification by child sex.[71] Third, the participants in this study were from a select population, specifically those enrolled in the HOME Study based in Cincinnati, Ohio. As such, results may not be generalizable or transportable.[72] However, we saw a substantial overlap between pathways identified in our study and previous study results, including significant associations between several metabolic pathways associated with amino acid and lipid metabolism.

In this study, we found that a mixture of PFOS, PFOA, PFNA, and PFHxS in maternal serum was significantly associated with four molecular features and 49 enriched pathways related to amino acid metabolism, glycan biosynthesis and metabolism, carbohydrate metabolism, lipid metabolism, and metabolism of cofactors and vitamins in the cord serum metabolome, which was consistent with previous studies. These pathways have been associated with type 2 diabetes, hepatocellular carcinoma, and low birthweight and thus could explain the link between prenatal PFAS exposure and these health outcomes. Future studies could assess if these associations mediate associations between prenatal PFAS exposure and adverse birth or childhood outcomes, such as birthweight or vaccine response.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c07515.

Chemical reagents used; instrument settings for LC-HRMS; mummichog settings; mass spectra of level 1 identified features; and results from the sensitivity analysis of a 5-PFAS mixture with the cord serum metabolome (PDF)

Results of the feature annotation using the Human Metabolome Database (HMBD) and manually inspecting MS2 mass spectra for confirmation (XLSX)

Summary table of untargeted studies on prenatal per- and polyfluoroalkyl substances (PFAS) and the prenatal metabolome (XLSX)

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Notes

The authors declare no competing financial interest.

Dr. Braun was compensated for serving as an expert witness on behalf of plaintiffsin litigation related to PFAS-contaminated drinking water. No other authors have any conflicts of interest to disclose

Informed consent was obtained for all pregnant people and their offspring before enrollment. This study was approved by the institutional review board (IRB) at the Cincinnati Children's Hospital Medical Center (CCHMC).

■ ACKNOWLEDGMENTS

The authors would like to acknowledge the CDC laboratory staff, who measured the PFAS for this study. Additionally, they would also like to thank all of the participants of the HOME Study, who have and who continue to provide their time. This work was supported by grants R01 ES032836, P01 ES011261, R01 ES020349, R01ES031621, R01 ES027224, R01 ES025214, and R01 ES030078 from the National Institute of Environmental Health Sciences.

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Environ. Sci. Technol. 2023, 57, 21627−21636