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Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: A meet-in-the-middle approach

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ABSTRACT

Background: Prenatal exposures to per- and polyfluoroalkyl substances (PFAS) have been linked to reduced fetal growth. However, the detailed molecular mechanisms remain largely unknown. This study aims to investigate biological pathways and intermediate biomarkers underlying the association between serum PFAS and fetal growth using high-resolution metabolomics in a cohort of pregnant African American women in the Atlanta area, Georgia.

Methods: Serum perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) measurements and untargeted serum metabolomics profiling were conducted in 313 pregnant African American women at 8–14 weeks gestation. Multiple linear regression models were applied to assess the associations of PFAS with birth weight and small-for-gestational age (SGA) birth. A high-resolution metabolomics workflow including metabolome-wide association study, pathway enrichment analysis, and chemical annotation and confirmation with *a meet-in-the-middle* approach was performed to characterize the biological pathways and intermediate biomarkers of the PFAS-fetal growth relationship.

Results: Each $\log_2$-unit increase in serum PFNA concentration was significantly associated with higher odds of SGA birth (OR = 1.32, 95% CI 1.07, 1.63); similar but borderline significant associations were found in PFOA (OR = 1.20, 95% CI 0.94, 1.49) with SGA. Among 25,516 metabolic features extracted from the serum samples, we successfully annotated and confirmed 10 overlapping metabolites associated with both PFAS and fetal growth endpoints, including glycine, taurine, uric acid, ferulic acid, 2-hexyl-3-phenyl-2-propenal, unsaturated fatty acid C18:1, androgenic hormone conjugate, parent bile acid, and bile acid-glycine conjugate. Also, we identified 21 overlapping metabolic pathways from pathway enrichment analyses. These overlapping metabolites and pathways were closely related to amino acid, lipid and fatty acid, bile acid, and androgenic hormone metabolism perturbations.

Conclusion: In this cohort of pregnant African American women, higher serum concentrations of PFOA and PFNA were associated with reduced fetal growth. Perturbations of biological pathways involved in amino acid, lipid and fatty acid, bile acid, and androgenic hormone metabolism were associated with PFAS exposures and reduced fetal growth, and uric acid was shown to be a potential intermediate biomarker. Our results provide opportunities for future studies to develop early detection and intervention for PFAS-induced fetal growth restriction.

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1. Introduction

Reduced fetal growth is an indicator of adverse *in utero* environment conditions and has been associated with both short- and long-term health outcomes (Mayer & Joseph, 2013). Numerous studies have shown that fetal growth using either birth weight or small-for-gestational age (SGA) as endpoints can predict perinatal health risks such as morbidity and mortality (Madden et al., 2018; Wilcox, 2010), and even adult health risks such as metabolic syndrome, type II diabetes, and cardiovascular diseases (Barker, 2006; Risnes et al., 2011), supporting the concept of the developmental origins of health and disease. Therefore, exposure to environmental chemicals at developmental periods which may influence fetal growth are particularly of concern (Heindel et al., 2015).

Per- and polyfluorinated alkyl substances (PFAS), a group of industrial compounds, have been frequently detected in both environmental and biological samples due to their wide range of applications and long biological half-lives (Houde et al., 2006; Lau et al., 2007). Prenatal exposures to some PFAS, in particular perfluorooctanoic acid (PFOA), have been associated with lower birth weight and small-for-gestational age (SGA) birth in both animal and human studies (Bach et al., 2015; Johnson et al., 2014; Koustas et al., 2014; Lam et al., 2014; Souza et al., 2020). Several potential biological mechanisms have been suggested, including the disruption of sex and thyroid hormones, changes in lipid metabolism, oxidative stress, and impaired placental functions (Abbott et al., 2007; Du, et al., 2013; Herrera & Ortega-Senovilla, 2010; Szilagyi et al., 2020). However, the exact biological mechanisms linking PFAS exposure to fetal growth have not yet been fully established.

High-resolution metabolomics has served as a powerful tool to characterize immediate cellular responses to different stressors from detected endogenous and exogenous metabolites in biological samples (Lankadurai et al., 2013; Miller & Jones, 2014). Therefore, metabolomics has been applied to improve the understanding of mode of action to certain exposures, to detect biomarkers for preclinical outcomes, and to support the diagnosis and clinical decisions (Deng et al., 2019; Fearnley & Inouye, 2016). Additionally, recent studies suggest using the *meet-in-the-middle* (MITM) approach to search for early biological effects and intermediate biomarkers in prospective cohort studies can inform the causal links or serve as markers for early responses (Chadeau-Hyam et al., 2011). Some epidemiology studies have successfully utilized the MITM approach to examine metabolic signals linking air pollutant exposure to asthma, cardio- and cerebro-vascular diseases, and reproductive outcomes (Fiorito et al., 2018; Gaskins et al., 2021; Jeong et al., 2018), smoke exposure to adverse birth outcomes (Tan et al., 2021), serum PFAS to impaired glucose metabolism or the severity of nonalcoholic fatty liver disease (Alderete et al., 2019; Chen et al., 2020; Jin et al., 2020), and lifestyle factors to hepatocellular carcinoma (Assi et al., 2015).

Previous human observational studies have used metabolomics to examine metabolic perturbations associated with serum PFAS (Alderete et al., 2019; Chen et al., 2020; Hu et al., 2020; Jin et al., 2020; Kingsley et al., 2019; Koshy et al., 2017; Lu et al., 2019; Mitro et al., 2021; Salihovic et al., 2019; Yu et al., 2016) or fetal growth (Clinton et al., 2020; Heazell et al., 2012; Horgan et al., 2011). However, no study has utilized the MITM approach to aid in the understanding of possible biological pathways and intermediate biomarkers for PFAS-related fetal growth restriction. We hypothesized that PFAS would be associated with common pathways and metabolites that were also associated with fetal growth endpoints. Ultimately, our work can strengthen the inference of PFAS-fetal growth causality by validating previously proposed biological processes in mechanistic studies. Moreover, the results provide opportunities for early detection and intervention to mitigate the health burden associated with PFAS exposures.

2. Materials and methods

2.1. Study population

This study examined participants from the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, which is a prospective birth cohort study that enrolled African American pregnant women. The participants were recruited during prenatal visits from the Emory Healthcare and Grady Health systems in metropolitan Atlanta, Georgia, in order to include a wider range of demographics. Inclusion criteria included self-reported U.S.-born African American, 18–40 years old, 8–14 weeks gestation, singleton pregnancy, ability to communicate in English, and no chronic medical conditions. The details of the cohort were previously published (Brennan et al., 2019; Corwin et al., 2017).

In this study, we retrieved information on 448 participants enrolled in the cohort between March 2014 and May 2018 with available PFAS measurements and information on birth outcomes of their offspring. We excluded 22 participants whose pregnancy ended with abortion ($n = 6$), stillbirth ($n = 4$), or delivered babies with congenital abnormalities ($n = 12$) in the analysis, resulting in 426 participants remaining in the analyses of PFAS and fetal growth. Additionally, 313 of these 426 participants (73.5%) had data on serum metabolomics measurements. All participants provided informed consent at enrollment. This study was reviewed and approved by the Institutional Review Board of Emory University (approval reference number 68441).

2.2. Sample and data collection

The collection of blood samples, clinical data, and questionnaire data has been described in detail previously (Corwin et al, 2017). Items relevant to this study are summarized below:

Blood Samples Collection. Blood samples were collected from routine blood drawn via venipuncture. We only used the blood samples collected at 8–14 weeks gestation for serum PFAS and serum metabolomics measurements in this study. After sample collection, the samples were transported to the laboratory, processed to obtain the serum, and stored at −80 °C for future analyses.

Clinical Data. The data collection was completed by the research team using a standardized chart abstraction tool to ascertain the following characteristics, conditions, and birth outcomes: (1) Parity; (2) First prenatal body mass index (BMI), calculated from measured height and weight at the first prenatal visit between 8 and 14 weeks gestation and categorized according to accepted definitions (obesity ≥ 30 kg/m^2, overweight 25–<30 kg/m^2, healthy weight 18.5–<25 kg/m^2, and underweight < 18.5 kg/m^2); (3) Gestational age at delivery was determined from the delivery record using the best obstetrical estimate (American College of Obstetricians and Gynecologists, 2014) based upon the date of delivery in relation to the estimated date of conception established by last menstrual period (LMP) and/or early ultrasound; (4) Birth weight was determined from the first weight measured in the delivery room. Birth weight percentiles based on gestational age at delivery and infant's sex were derived using the population information from the U.S. natality files for singleton births in 2017 (Aris et al., 2019). Infants whose birth weight was < 10th percentile in the reference population were defined as having an SGA birth.

Questionnaire Data. Sociodemographic survey based on maternal self-report and prenatal administrative record review was used to ascertain maternal age upon entry into the study, education, income-to-poverty ratio, prenatal health insurance type (categorized as Medicaid or private insurance), marital and cohabiting status, and substance use (tobacco and marijuana).

2.3. PFAS measurement

Serum PFAS were analyzed at two laboratories. These two laboratories are part of the Children's Health Exposure Analysis Resource

(CHEAR) laboratories, including Wadsworth Center/New York University Laboratory Hub (Wadsworth/NYU) and the Laboratory of Exposure Assessment and Development for Environmental Research (LEADER) at Emory University. In total, 342 and 84 samples were analyzed in Wadsworth/NTU and LEADER, respectively. Laboratories in CHEAR have participated in activities to produce harmonized measurements among them (Balshaw et al., 2017). Each serum sample was spiked with internal standards, treated by solid phase extraction, and quantified by liquid chromatography interfaced with tandem mass spectrometry (LC-MS/MS) for four PFAS – perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), PFOA, and perfluorononanoic acid (PFNA). Quantification of PFAS was performed using isotope dilution calibration. The details of analytical methods used in Wadsworth/NYU (Honda et al., 2018) and LEADER (Chang et al., 2021b) were described previously. Both laboratories have participated in and been certified by the German External Quality Assessment Scheme (http://g-equas.de/) twice annually for serum PFAS quantification. Good agreement of the measurements was obtained from 11 overlapped samples – Pearson correlation coefficients between 0.88 and 0.93 and relative percent differences (RPD) ranging from 0.12% to 20.2% (median 4.8%) (Table S1).

2.4. High-resolution metabolomics

Untargeted high-resolution metabolomics profiling was conducted at Emory Clinical Biomarker Laboratory using established protocol. Serum samples were first added with two sample volumes of ice-cold acetonitrile to precipitate proteins. The samples were then incubated on ice for 30 mins, centrifuged (at 14,000 g for 10 mins) to separate supernatant from precipitated proteins, and stored at 4 °C until analysis (Johnson et al., 2010). Extractants were then analyzed in triplicate by liquid-chromatography and Fourier-transform high-resolution mass spectrometry (LC-HRMS) (Dionex Ultimate 3000, Thermo Scientific Q-Exactive HF).

Two chromatography types were applied the hydrophilic interaction liquid chromatography (HILIC) (Waters XBridge BEH Amide XP HILIC column; 2.1 × 50 mm2, 2.6 μm particle size) with positive electrospray ionization (ESI) and reverse phase (C18) chromatography (Higgins Targa C18 2.1 × 50 mm2, 3 μm particle size) with negative ESI. Analyte separation for HILIC was performed using water, acetonitrile, and 2% formic acid mobile phases under the following gradient elution: initial 1.5 min period consisted of 22.5% water, 75% acetonitrile, and 2.5% formic acid, followed by a linear increase to 75% water, 22.5% acetonitrile, and 2.5% formic acid at 4 min and a final hold of 1 min. Analyte separation for C18 was performed using water, acetonitrile, and 10 mM ammonium acetate mobile phases under the following gradient elution: initial 1 min period consisted of 60% water, 35% acetonitrile, and 5% ammonium acetate followed by a linear increase to 0% water, 95% acetonitrile, and 5% ammonium acetate at 3 min and held for the remaining 2 min. For both types of chromatography, mobile phase flow rate was 0.35 mL/min for the first min and increased to 0.4 mL/min for the final 4 min. Although the gradient elution starting at 60% aqueous condition in C18 column might miss some metabolites, which could be separated between 100% and 60% aqueous, these metabolites are likely to be better detected in the HILIC column. Thus, applying two chromatography types in this study can enhance the coverage of metabolic feature for each sample. The void volume ends at approximately 15 seconds after injecting samples. LC-HRMS was operated in full scan mode at 120 k resolution and cover the range of mass-to-charge ratio (m/z) from 85 to 1,275. Tune parameters for sheath gas were 45 (arbitrary units) for positive ESI and 30 for negative ESI. Auxiliary gas was set at 25 (arbitrary units) for positive ESI and 5 for negative ESI. Spray voltage was set at 3.5 kV for positive ESI and − 3.0 kV for negative ESI. Two internal standards which include pooled serum and standard reference material for human metabolites in plasma (NIST SRM 1950) were added at the beginning and the end of each batch of 20 samples for quality control and standardization (Liu et al., 2020; Johnson et al., 2007).

After instrument analysis, raw instrument files were converted to .mzML and metabolic signals were extracted and aligned by apLCMS with modification of xMSanalyzer, which enhanced data quality control and reduced batch effects (Uppal et al., 2013; Yu et al., 2009). To filter out the noise signals and optimize the metabolomics data quality, we excluded the metabolic features which were detected in < 15% of the samples, with coefficient of variation among technical replicates > 30%, and with Pearson correlation coefficient < 0.7. The intensities of the extracted metabolic features were then averaged across triplicates for future statistical analyses (Liang et al., 2018; Liang et al., 2019; Li et al., 2021).

2.5. Statistical analysis

Descriptive analyses were performed for the serum PFAS concentrations including detection frequencies, geometric means (GMs), geometric standard deviations (GSDs), and distribution percentiles. Serum PFAS concentrations below the limit of detections (LODs) were imputed with LOD/ $\sqrt{2}$ (Hornung & Reed, 1990). All the PFAS concentrations were $\log_2$-transformed to reduce the potential effects from outliers in the analyses. Additionally, Pearson correlations were calculated among $\log_2$-transformed PFAS concentrations.

We investigated the associations of serum PFAS concentrations with birth weight (continuous; gram) and SGA birth (categorical; yes/no) by fitting multivariable linear regressions and logistic regressions, respectively. Continuous birth weight was regressed on serum PFAS concentrations adjusting for maternal age (continuous; years), education (categorical; less than high school, high school, some college, college and above), parity (categorical; 0, 1, ≥2), BMI (categorical; <18.5, 18.5–<25, 25–<30, ≥30 kg/m^2), tobacco use (categorical; during pregnancy, not during pregnancy), marijuana use (categorical; during pregnancy, not during pregnancy), and infant's sex (categorical; male, female). This analysis using birth weight as the dependent variable was restricted to the population of term births to remove the effect of length of gestation. The log odds of SGA birth were regressed on serum PFAS concentrations controlling for the same covariates except for infant's sex because sex was already accounted for when defining SGA birth. To evaluate dose–response relationships, we used categorical PFAS concentration groups divided by quartiles to model birth weight and log odds of SGA birth. Test for trend across quartile groups were examined using the median serum PFAS concentrations of each group as a continuous variable. The covariates were selected by the guidance of directed acyclic graph to identify the potential confounders (Figure S1).

The metabolome-wide association study (MWAS) was conducted to investigate the associations of global metabolomics with PFAS and fetal growth endpoints. The metabolic features in MWAS were analyzed without *a priori* knowledge of the actual chemical identities. Since intensities of the metabolic features were right-skewed, $\log_2$-transformation was conducted to normalize the data. We used the following models to evaluate the effects of PFAS exposure and the potential predictors of fetal growth endpoints:

$$log_2(Intensity) = \beta_0 + \beta_1 log_2(PFAS) + \beta_2 Age + \beta_3 Education + \beta_4 Parity + \beta_5 BMI + \beta_6 Tobaccouse + \beta_7 Marijuanause + \beta_8 Sex + \epsilon \quad (1)$$

$$Birthweight = \beta_0 + \beta_1 log_2(Intensity) + \beta_2 Age + \beta_3 Education + \beta_4 Parity + \beta_5 BMI + \beta_6 Tobaccouse + \beta_7 Marijuanause + \beta_8 Sex + \epsilon \quad (2)$$

$$ln\left(\frac{P(SGA)}{1-P(SGA)}\right) = \beta_0 + \beta_1 log_2(Intensity) + \beta_2 Age + \beta_3 Education + \beta_4 Parity + \beta_5 BMI + \beta_6 Tobaccouse + \beta_7 Marijuanause \quad (3)$$

where *Intensity* denotes the intensity of each metabolic feature. β_0 represents the intercept and β_{1-8} are the coefficients corresponding to each covariate. The covariates having potential to alter metabolic homeostasis and associate with either serum PFAS or fetal growth in this population were controlled in the models, including maternal age, education, parity, BMI, tobacco use, marijuana use, and infant's sex. Infant's sex was not included in the model (3) because sex was considered in defining the birth size. These three models were performed for each metabolic feature detected by two different analytical columns. We implemented the Benjamini-Hochberg procedure to correct for multiple comparison (Benjamini & Hochberg, 1995). All the analyses were performed in R (version 3.6.1).

2.6. Pathway enrichment analysis

We used *Mummichog* (v1.0.10), a statistical application leveraging the organization of metabolic pathways and networks to predict the functional activity without upfront chemical identification. Briefly, *Mummichog* matches all the possible metabolites to the significant metabolic features (*m/z*), and searches for the pathways that can be constructed by these tentative chemicals. For HILIC column, the adducts $M^{[1+]}$, $M + H^{[1+]}$, $M-H2O + H^{[1+]}$, $M + Na^{[1+]}$, $M + K^{[1+]}$, $M + 2H^{[2+]}$, and $M(C_{13}) + 2H^{[2+]}$ were considered. For C18 column, the adducts $M-H^{[1-]}$, $M + Cl^{[1-]}$, $M + ACN\text{-}H^{[1-]}$, $M + HCOO^{[1-]}$, $M(C_{13})\text{-}H^{[1-]}$, $M-H2O-H^{[1-]}$, and $M + Na\text{-}2H^{[1-]}$ were evaluated. The significance of pathways can then be calculated by Fisher's exact test on the null distribution, which is estimated by permutation where the features were randomly drawn from the list of all the extracted metabolic features (Li et al., 2013).

Although multiple-testing correction may provide stringent criteria to avoid false-positive candidates, it can also exclude weaker yet relevant features, especially given the intercorrelated nature of metabolomics. Because we found a limited number of significant features at either 5% or 20% false discovery rate (FDR) thresholds, the cut-off for the significance was set as unadjusted p-value < 0.05 to include a sufficient number of features in the pathway enrichment analyses (Table S2). The analyses were separately conducted for four PFAS, birth weight, and SGA birth by two different analytical columns. We created heat maps to show the enriched metabolic pathways associated with more than two PFAS and fetal growth endpoints, and shaded each cell based on the strength of the associations.

2.7. Chemical annotation and confirmation

To minimize the false positive discovery, we visually examined the extracted ion chromatographs (EICs) of each significant metabolic feature to differentiate true peak from noise (exhibiting clear gaussian peak shapes and signal-to-noise ratio above 3:1) (Yu & Jones, 2014). The features passing the examination were annotated and confirmed using the Metabolomics Standards Initiative criteria described below (Sumner et al., 2007). First, the features whose *m/z* (±10 ppm difference) and retention time (±10 seconds) matched the authentic compounds analyzed under identical experimental conditions were assigned with level 1 confidence. Second, additional metabolic features not assigned with level 1 confidence were annotated by *xMSannotator*. *xMSannotator* is a R package utilizing multicriteria clustering, retention time characteristics, mass defect, and isotope/adduct patterns to assign identities to metabolic features based on multiple chemical databases (i.e., Human Metabolome Database (HMDB), Kyoto Encyclopedia of Genes and Genomes (KEGG), the Toxin and Toxin Target Database (T3DB), and LipidMaps). The adducts considered were the same as pathway enrichment analysis (refer to section 2.6) and the mass error range was set at 10 ppm (Uppal et al., 2017). We presented the features with level 2 confidence when the features were annotated with "high confidence" by xMSannotator and further confirmed by in-source fragmentation patterns from previous literature or library spectra at the retention time corresponding to the predominant fragment (Domingo-Almenara et al., 2019; Xue et al., 2020).

2.8. Meet-in-the-middle (MITM) approach

Fig. 1 shows the workflow of the MITM approach. We conducted MWAS and pathway enrichment analyses separately for serum PFAS concentrations and fetal growth endpoints and identified the overlapping pathways and metabolic features. The overlapping features were annotated and confirmed following the steps described in section 2.7. These overlapping pathways and metabolites were then used to explore the potential biological mechanisms and intermediate biomarkers linking PFAS concentrations to fetal growth endpoints. Compared with traditional mediation analysis, which requires strong underlying assumptions for the counterfactual framework, MITM was only used to detect the intersecting signals associated with exposure and outcome (Chadeau-Hyam et al., 2011; Pearl, 2014).

2.9. Sensitivity analysis

Although birth weight is an accepted proxy measurement of fetal growth and is strongly related to neonatal morbidity and mortality, the adjustment for length of gestation is suggested (Wilcox, 2010). Moreover, a previous study in the U.S found that shorter length of gestation was shown to be the strongest predictors of birth weight differences among African American infants (Morisaki et al., 2017). Thus, different methods separating the effect of length of gestation from fetal growth were examined in this study. We restricted the analyses to term births in the main analysis, whereas in sensitivity analysis, we included all births but additionally adjusted for gestational week at delivery as a covariate. Additionally, to evaluate the impact of using different cut-offs of p-value for the significance in the pathway enrichment analyses, we performed

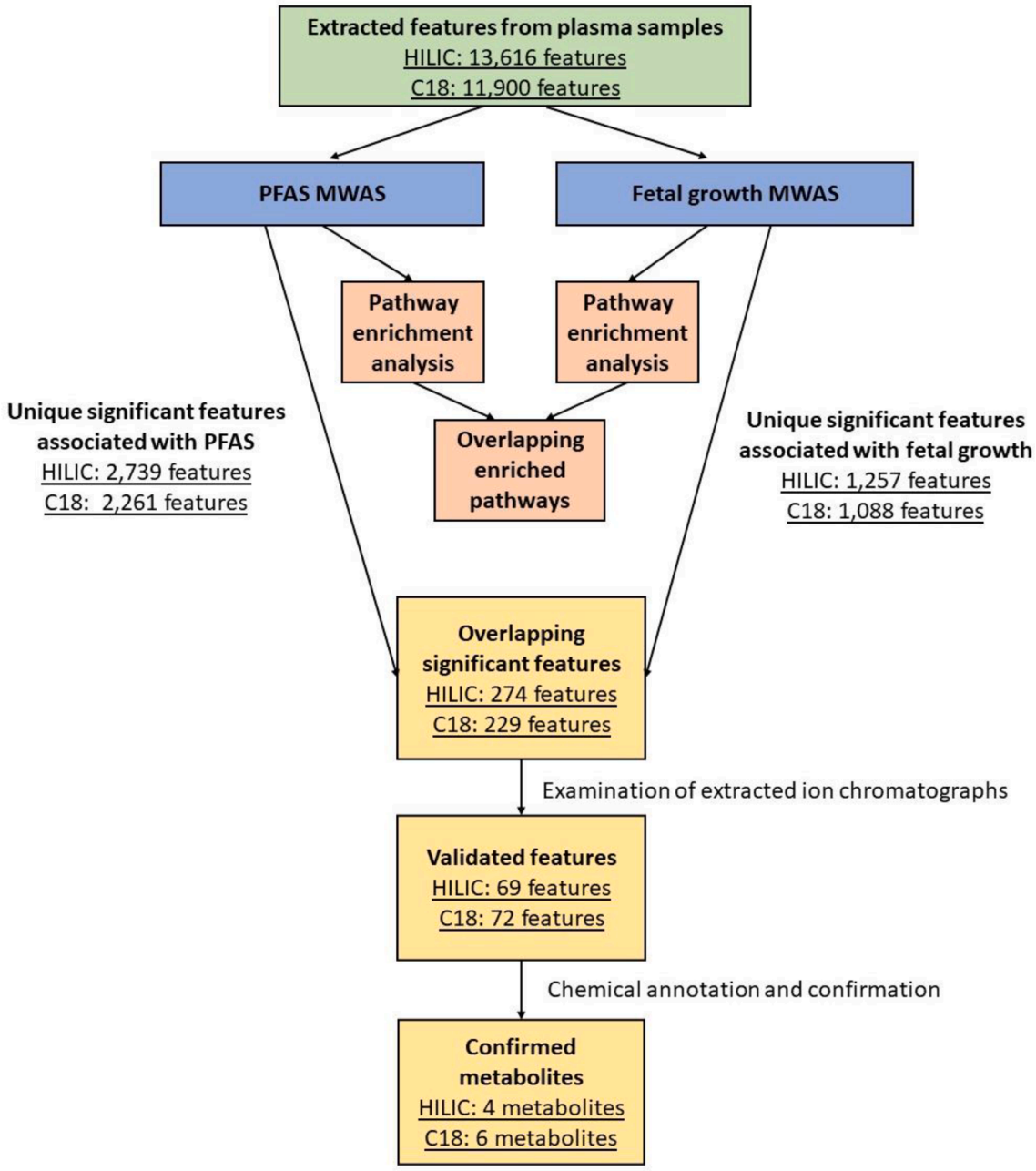


Fig. 1. Workflow of the meet-in-the-middle (MITM) approach and the number of extracted or significant metabolic features in each analytical step. Overlapping significant features are the metabolic features associated with both PFAS and fetal growth endpoints. Validated features are the overlapping features exhibiting clear gaussian peak shapes and signal-to-noise ratio above 3:1 from their extracted ion chromatographs. Confirmed metabolites are the validated features successfully annotated and confirmed with chemical identities. (HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column; PFAS = per- and polyfluorinated alkyl substance; MWAS = metabolome-wide association study)

sensitivity analyses using p-value < 0.005, < 0.01, and < 0.05.

3. Results

3.1. Population characteristics

Among 313 healthy African American women, the average age was 24.9 years (standard deviation [SD] = 4.73), and the majority had high school education or less (n = 167; 54%), had income-to-poverty ratio < 100% (n = 134; 43%), and were supported by Medicaid (n = 248; 79%) instead of private medical insurance. The participants had lower education and income levels compared to a similarly matched population (African American women aged 18–40 years) in U.S Census Bureau's 2014–2018 American Community Survey (45% of them had high school education or less, and 29% of them had income-to-poverty ratio < 100%) (Table 1) (Ruggles et al., 2021). In total, 56 participants (18%) delivered their infants preterm, and the average birth weight was 3,050 g (SD = 611). There are 39 (13%) infants born with low birth weight (birth weight < 2,500 g), and 77 (25%) infants defined as SGA. Detailed information on the population characteristics is presented in Table 1. The characteristics among these 313 participants with metabolomics data were similar to the larger population (n = 426) in this study (Table S3). The four PFAS were detected with high frequencies (97–98%) among the subsets with metabolomics data. The GMs are 1.00

Table 1
Selected population characteristics in pregnant African American women in the Atlanta area, 2014–2018 (n = 313).

Characteristics	n (%)	Characteristics	n (%)
Age (years)		**BMI (kg/m^2)**	
Mean ± SD	24.9 ± 4.73	< 18.5	9 (3%)
18–25	166 (53%)	18.5–<25	121 (39%)
25–30	81 (26%)	25–<30	70 (22%)
30–35	54 (17%)	≥ 30	113 (36%)
≥ 35	12 (4%)	**Infant's sex**	
Education[a]		Male	157 (50%)
Less than high school	49 (16%)	Female	156 (50%)
High school	118 (38%)	**Marijuana use**	
Some college	97 (31%)	Not during pregnancy	240 (77%)
College and above	49 (16%)	During pregnancy	73 (23%)
Income-to-poverty ratio (%)[a,b]		**Tobacco use**	
< 100	134 (43%)	Not during pregnancy	267 (85%)
100–150	48 (15%)	During pregnancy	46 (15%)
150–300	48 (15%)	**Birth weight (grams)**	
≥ 300	38 (12%)	Mean ± SD	3050 (611)
Married or cohabiting		**Low birth weight (LBW) (<2,500 g)**	
Yes	147 (47%)	No	274 (88%)
No	166 (53%)	Yes	39 (13%)
Insurance		**Birth weight percentile for gestational age**	
Private	65 (21%)	Mean ± SD	35.9 ± 27.3
Medicaid	248 (79%)	**Small-for-gestational age (SGA) (<10th percentiles)**	
Hospital		No	236 (75%)
Private (Emory)	123 (39%)	Yes	77 (25%)
Public (Grady)	190 (61%)	**Gestational week at delivery**	
Parity (#)		Mean ± SD	38.5 ± 2.64
0	137 (44%)	**Preterm birth (<37 gestational weeks)**	
1	88 (28%)	No	257 (82%)
≥ 2	88 (28%)	Yes	56 (18%)

Note: SD = standard deviation
[a] Information of a similarly matched population (female, African American/Black, and age 18–40 in U.S Census Bureau's 2014–2018 American Community Survey: education (less than high school 8%, high school 37%, some college 34%, college and above 20%); income-to-poverty ratio (%) (<100 29%, 100–150 13%, 150–300 28%, ≥ 300 30%) (Ruggles et al., 2021)
[b] The sample numbers do not be summed up to the total sample size due to missingness in some cases.

(GSD = 1.91), 1.97 (GSD = 2.13), 0.62 (GSD = 2.35), and 0.23 ng/mL (GSD = 2.34) for PFHxS, PFOS, PFOA, and PFNA, respectively (Table S4). Pearson correlation coefficients between these four PFAS ranged from 0.35 to 0.75 (all p-values < 0.05) (Table S5).

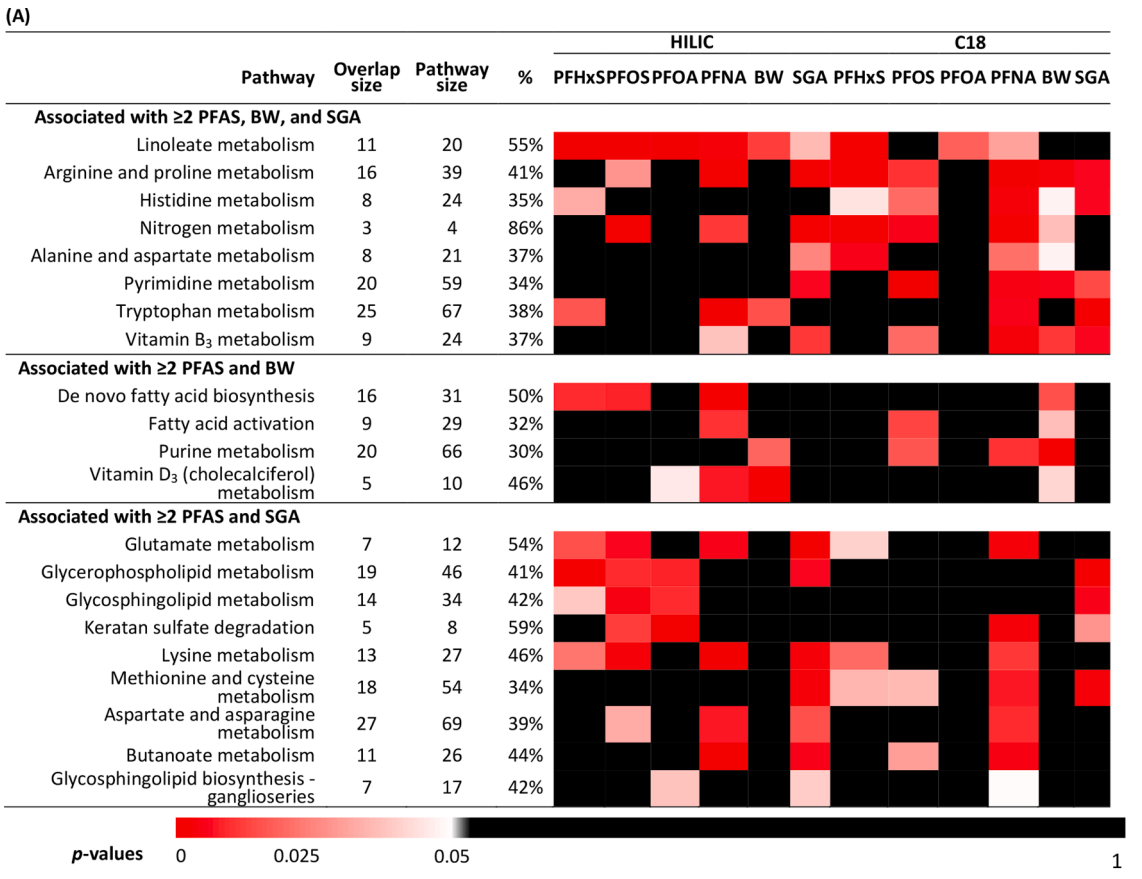
Table 2
Associations of serum PFAS with birth weight and small-for-gestational age (SGA) in pregnant African American women in the Atlanta area, 2014–2018.

	Birth weight (continuous; grams)[a,b] β (95 %CI) (n = 370)	SGA[c] OR (95 %CI) (n = 426)
PFHxS (ng/mL)		
Q1: < LOD-0.75	0 (Ref)	1.00 (Ref)
Q2: 0.75–1.10	−36 (-154, 83)	1.36 (0.71, 2.61)
Q3: 1.10–1.53	5 (-112, 123)	1.35 (0.70, 2.61)
Q4: 1.53–4.80	−54 (-173, 66)	1.11 (0.57, 2.17)
p for trend[d]	0.50	0.84
Per $\log_2$-unit	−14 (-58, 31)	1.10 (0.85, 1.42)
PFOS (ng/mL)		
Q1: < LOD-1.44	0 (Ref)	1.00 (Ref)
Q2: 1.44–2.19	78 (-40, 196)	0.92 (0.47, 1.78)
Q3: 2.19–3.24	20 (-98, 138)	1.32 (0.69, 2.53)
Q4: 3.24–12.40	−16 (-136, 105)	1.09 (0.56, 2.13)
p for trend[d]	0.48	0.65
Per $\log_2$-unit	−7 (-48, 34)	1.12 (0.88, 1.42)
PFOA (ng/mL)		
Q1: < LOD-0.45	0 (Ref)	1.00 (Ref)
Q2: 0.45–0.71	**−126 (-241, −10)***	**2.22 (1.10, 4.50)***
Q3: 0.71–1.07	−44 (-162, 73)	**2.44 (1.21, 4.92)***
Q4: 1.07–4.42	−107 (-227, 13)	**2.23 (1.10, 4.54)***
p for trend[d]	0.23	0.06
Per $\log_2$-unit	−14 (-49, 21)	1.20 (0.97, 1.49)
PFNA (ng/mL)		
Q1: < LOD-0.16	0 (Ref)	1.00 (Ref)
Q2: 0.16–0.27	−41 (-159, 77)	1.73 (0.87, 3.43)
Q3: 0.27–0.42	−48 (-165, 69)	1.72 (0.87, 3.40)
Q4: 0.42–2.27	−106 (-227, 14)	**2.22 (1.12, 4.38)***
p for trend[d]	0.09	**0.04***
Per $\log_2$-unit	–32 (-67, 3)	**1.32 (1.07, 1.63)***

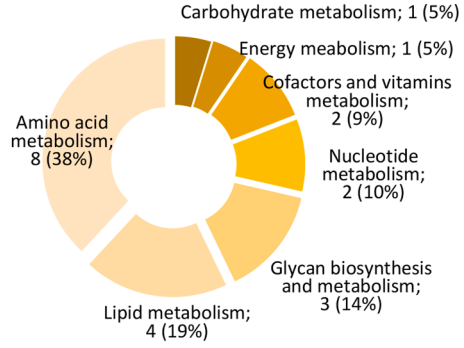
Note: SGA = small-for-gestational age; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid.
[a] Adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex.
[b] Restricted to only term births (>37 gestational weeks and 0 day).
[c] Adjusted for maternal age, education, BMI, parity, tobacco use, and marijuana use.
[d] Median serum PFAS concentrations of each quartile group were used as a continuous exposure variable.
* p-value < 0.05.

3.2. Associations between serum PFAS concentrations and fetal growth endpoints

Table 2 shows the associations of $\log_2$-transformed PFAS concentrations with birth weight and SGA birth. We found each $\log_2$-unit increase in PFNA concentration was associated with higher odds for SGA birth (odds ratio [OR] = 1.32 [95 %CI 1.07, 1.63]), and the OR for the 4th quartile (Q4) of PFNA (OR = 2.22 [95 %CI 1.12, 4.38]) was significantly higher than the reference group (Q1). We also observed increased odds of SGA birth per $\log_2$-unit increase in PFHxS, PFOA, and PFOS, but the results were not statistically significant. The ORs of the Q2, Q3, and Q4 of PFOA concentrations were significantly higher than Q1. Non-significant inverse associations were observed between $\log_2$-transformed serum PFAS concentrations and birth weight among term births. Lower birth weight was found in Q2 of PFOA (β = -126 g [95 %CI −241, −10]) than Q1. Additionally, dose–response relationships were observed in the associations of serum PFNA concentrations with SGA birth (p for trend = 0.04). The results of sensitivity analyses using different approaches to control for length of gestation for the birth weight models are presented in Table S6; the effect estimates did not materially change.



(B)



(C)

Class of KEGG	Metabolic pathways
Amino acid metabolism (8)	Arginine and proline, histidine, alanine and aspartate, tryptophan, glutamate, lysine, methionine and cysteine, and aspartate and asparagine metabolism
Lipid and fatty acid metabolism (4)	Linoleate, *de novo* fatty acid biosynthesis, fatty acid activation, and glycerophospholipid metabolism
Glycan biosynthesis and metabolism (3)	Glycosphingolipid metabolism , keratan sulfate degradation, glycosphingolipid biosynthesis - ganglioseries
Nucleotide metabolism (2)	Pyrimidine and purine
Cofactors and vitamins metabolism (2)	Vitamin B_3, Vitamin D_3 (cholecalciferol) metabolism
Energy metabolism (1)	Nitrogen metabolism
Carbohydrate metabolism (1)	Butanoate metabolism

Fig. 2. The enriched metabolic pathways significantly associated with ≥ 2 PFAS serum concentrations and fetal growth in pregnant African American women in the Atlanta area, 2014–2018 (n = 313).(a) Heat map of *p*-values. Each cell was colored by the *p*-value of the association of each metabolic pathway and with either serum PFAS or fetal growth endpoints. Overlap size represents the average number of significant putative metabolites (*p*-value < 0.05) that were associated with either serum PFAS or fetal growth endpoints among each metabolic pathway. Pathway size represents the number of metabolites within each metabolic pathway. % is the percentage of overlap size to pathway size. These pathways were ordered by the number of significance results. (b) The percentages of each class among all enriched metabolic pathways. (c) The class of enriched metabolic pathways. (Note: HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; BW = birth weight [the analyses were restricted to term births] ; SGA = small-for-gestational age; KEGG = Kyoto Encyclopedia of Genes and Genomes)

3.3. Maternal metabolome-wide association study (MWAS) on serum PFAS and fetal growth endpoints

After data quality assurance, we successfully extracted 13,616 and 11,900 metabolic features in the serum samples from 313 participants using the HILIC and C18 analytical columns, respectively. We conducted 12 sets of MWAS (four PFAS and two fetal growth endpoints for two analytical columns). In total, when using *p*-value < 0.05 as the threshold of significance, we found 816, 974, 922, 1126, 693, and 742 significant features in HILIC column, and 797, 803, 709, 899, 586, and 673 features in C18 column associated with PFHxS, PFOS, PFOA, PFNA, birth weight, and SGA birth, respectively. The numbers of overlapping significant features associated with at least one PFAS and with either birth weight or SGA birth were 274 and 229 in HILIC and C18 column, respectively.

Table 3
Associations of significant confirmed biomarkers with both serum PFAS and fetal growth endpoints in pregnant African American women in the Atlanta area, 2014–2018 (n = 313).

m/z	RT (sec)[a]	Metabolites	HMDB ID	Column	Adduct	Class	β (95% CI)					OR (95 % CI)
							PFHxS[b]	PFOS[b]	PFOA[b]	PFNA[b]	BW[b,c]	SGA[d]
Biomarker with level 1 confidence												
120.0025	60.2	Glycine	HMDB00123	HILIC	M + 2Na-H[e]	Amino acid	0.001 (-0.052, 0.054)	0.02 (-0.01, 0.04)	0.04 (-0.03, 0.11)	**0.15 (0.01, 0.29)***	**−171 (-314, −28)***	2.71 (0.63, 3.79)
126.0220	57.7	Taurine	HMDB00251	HILIC	M + H	Amino acid	−0.01 (-0.09, 0.06)	0.02 (-0.01, 0.05)	0.07 (-0.03, 0.17)	**0.27 (0.06, 0.47)***	**−84 (-188, −3)***	1.76 (0.99, 3.13)
167.0208	18.8	Uric acid	HMDB00289	C18	M−H	Purine derivative	−0.02 (-0.08, 0.05)	0.026 (-0.001, 0.053)	**0.10 (0.02, 0.18)***	**0.18 (0.01, 0.35)***	**−161 (-291, −31)***	1.75 (0.89, 3.43)
195.0661	23.7	Ferulic acid	HMDB00954	C18	M−H	Hydroxycinnamic acids	−0.14 (-0.39, 0.11)	−0.10 (-0.20, 0.01)	**−0.43 (-0.75, −0.11)***	−0.22 (-0.90, 0.46)	−8 (-41, 26)	**1.23 (1.01, 1.50)***
Biomarker with level 2 confidence												
217.1587	21.2	2-Hexyl-3-phenyl-2-propenal	HMDB31736	HILIC	M + H	Cinnamaldehydes	**0.11 (0.03, 0.20)***	**0.05 (0.01, 0.08)***	0.09 (-0.02, 0.20)	0.13 (-0.10, 0.36)	−57 (-142, 27)	**1.78 (1.06, 2.99)***
283.2634	181.6	Elaidic acid	HMDB00573	HILIC	M + H	Long-chain fatty acids	−0.09 (-0.27, 0.09)	−0.06 (-0.14, 0.02)	**−0.29 (-0.53, −0.05)***	**−0.59 (-1.07, −0.10)***	38 (-4, 79)	**0.73 (0.58, 0.92)***
		Oleic acid	HMDB00207	HILIC	M + H	Long-chain fatty acids						
		Vaccenic acid	HMDB03231	HILIC	M + H	Long-chain fatty acids						
367.1585	23.9	Dehydroepiandrosterone sulfate (DHEA-S)	HMDB01032	C18	M−H	Steroid hormone	−0.04 (-0.19, 0.12)	0.01 (-0.06, 0.07)	0.15 (-0.05, 0.35)	**0.52 (0.11, 0.93)***	**−27 (-54, −1)***	1.20 (0.90, 1.61)
		Testosterone sulfate	HMDB02833	C18	M−H	Steroid hormone						
369.1742	23.4	Androsterone sulfate	HMDB02759	C18	M−H	Steroid hormone	−0.13 (-0.31, 0.04)	−0.02 (-0.09, 0.06)	0.14 (-0.08, 0.36)	**0.55 (0.09, 1.01)***	**–22 (-42, −2)***	1.15 (0.88, 1.51)
391.2878	260.9	Chenodeoxycholic acid (CDCA)	HMDB00518	C18	M−H	Bile acid	−0.001 (-0.163, 0.161)	−0.003 (-0.070, 0.065)	**0.23 (0.03, 0.44)***	**0.55 (0.11, 0.98)***	−24 (-77, 29)	**1.44 (1.04, 1.99)***
		Deoxycholic acid (DCA)	HMDB00626	C18	M−H	Bile acid						
		Hyodeoxycholic acid (HDCA)	HMDB00733	C18	M−H	Bile acid						
		Isoursodeoxycholic acid	HMDB00686	C18	M−H	Bile acid						
484.2847	22.3	Chenodeoxycholylglycine	HMDB00637	C18	M + Cl	Bile acid	−0.16 (-0.49, 0.16)	**−0.142 (-0.281, −0.002)***	**−0.45 (-0.85, −0.04)***	−0.64 (-1.49, 0.21)	**50 (12, 87)***	0.88 (0.73, 1.07)
		Deoxycholylglycine	HMDB00631	C18	M + Cl	Bile acid						
		Ursodeoxycholylglycine	HMDB00708	C18	M + Cl	Bile acid						

Note: *m/z* = mass to charge ratio; RT (sec) = retention time (seconds); HMDB ID = Human Metabolome Database ID; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; BW = birth weight; SGA = small-for-gestational age; HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column.

[a] Void volume ends at 15 s.

[b] Adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex.

[c] Restricted to term births (>37 gestational weeks and 0 day).

[d] Adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use.

[e] Glycine [M + H] is outside of our mass range of detection and [M + 2Na-H] was confirmed by authentic glycine standards in the lab; thus, the intensity of [M + 2Na-H] instead of [M + H] is reported.

* *p*-value < 0.05.

3.4. Overlapping enriched pathways associated with serum PFAS and fetal growth endpoints

MWAS results were used to perform pathway enrichment analyses. The enriched metabolic pathways associated with ≥ 2 PFAS and fetal growth endpoints were summarized in Fig. 2. Similar enriched pathways were shown when using different cut-offs for significance (i.e., *p*-values < 0.005, < 0.01, and < 0.05) (data not shown). The results indicated that eight metabolic pathways, including linoleate, arginine and proline, histidine, nitrogen, alanine and aspartate, pyrimidine, tryptophan, and vitamin B_3 metabolism were associated with ≥ 2 PFAS, birth weight, and SGA birth. Four pathways, *de novo* fatty acid biosynthesis, fatty acid activation, purine metabolism, and vitamin D_3 metabolism, were linked to ≥ 2 PFAS and birth weight. Nine pathways, including four amino acid pathways (glutamate, lysine, methionine and cysteine, and aspartate and asparagine), three glycan pathways (keratan sulfate degradation, glycosphingolipid metabolism, and glycosphingolipid biosynthesis – ganglioseries), glycerophospholipid, and butanoate metabolism, were associated with ≥ 2 PFAS and SGA. The percentages of the number of significant putative metabolites (overlap size) to the number of metabolites within each pathway (pathway size) ranged from 30% to 86%. The results of pathway enrichment analyses associated with PFAS or fetal growth endpoints are presented in Figures S2 and S3, respectively. We found more enriched pathways in the birth weight models restricting participants to those with term births than in the models including all births.

3.5. Overlapping metabolites associated with serum PFAS and fetal growth endpoints

We largely decreased the possibility of false positive discovery by excluding ambiguous and noisy peaks after examining EICs. Only 69 and 72 overlapping significant features passed the EIC examination — 75% and 69% of the features detected by HILIC and C18 columns were excluded. As shown in Table 3, the chemical identities of four overlapping metabolites, identified as biomarkers with level 1 confidence, were glycine, taurine, uric acid, and ferulic acid. Glycine, taurine, and uric acid were positively associated with PFNA and inversely associated with birth weight, and uric acid was additionally associated with serum PFOA concentrations. Increased ferulic acid intensities were inversely associated with PFOA concentrations, and positively associated with odds of SGA birth. Glycine [M + H] (*m/z* 76.1) is outside of our mass range of detection (*m/z* 85 to 1,275) and [M + 2Na-H] was confirmed by authentic glycine standards in the lab; thus, the intensity of [M + 2Na-H] instead of [M + H] is reported for glycine.

Six features were annotated as metabolites with level 2 confidence including 2-hexyl-3-phenyl-2-propenal, unsaturated fatty acids C18:1 (i.e., elaidic acid, oleic acid, or vaccenic acid), androgenic hormone sulfate conjugates (i.e., dehydroepiandrosterone sulfate [DHEA-S] or testosterone sulfate, and androsterone sulfate), parent bile acid (i.e., chenodeoxycholic acid [CDCA], deoxycholic acid [DCA], hyodeoxycholic acid [HDCA], or isoursodeoxycholic acid), and bile acid-glycine conjugate (i.e., chenodeoxycholylglycine, deoxycholylglycine, or ursodeoxycholylglycine). The results of sensitivity analyses between term and all births of birth weight models are presented in Table S7, where the directionalities of coefficients were consistent across the two models.

4. Discussion

4.1. Maternal serum PFAS associated to reduced fetal growth

We found that PFNA concentrations were associated with higher odds of SGA birth with a monotonic dose–response relationship. Similar evidence was observed with PFOA despite the borderline significance. However, inconsistent results were observed with serum PFHxS and PFOS. Previous systematic reviews and *meta*-analyses suggest that exposures to PFOA and PFOS may limit fetal growth in both human and animal studies (Bach et al., 2015; Johnson et al., 2014; Koustas et al., 2014; Lam et al., 2014; Souza et al., 2020). Additionally, reduced fetal growth was also observed with higher PFHxS and PFNA concentrations despite their paucity of data and/or consistency in results in the literature (Callan et al., 2016; Kashino et al., 2020; Maisonet et al., 2012). These inconsistent results might be due to heterogeneity of study designs, choice of fetal growth endpoints, study populations, sample sizes, or different exposure ranges.

We recognize that the associations observed in this analysis may differ by fetal growth endpoints, given the difference in interpretation of each endpoint. Specific to birth weight and SGA in the context of fetal growth, there is a distinction between infants who are constitutionally small and those who are growth restricted as the result of extraneous factors. Additionally, it is worth noting that SGA percentiles for this cohort of African American infants were based on a reference population for which there was significant variation, which may limit the interpretation of our findings.

4.2. Amino acid metabolism contributing to PFAS-fetal growth relationship

Several amino acid pathways were associated with PFAS and fetal growth endpoints, including arginine and proline, histidine, alanine and aspartate, tryptophan, glutamate, lysine, methionine and cysteine, and aspartate and asparagine in this study. We also observed that increased glycine and taurine intensities were associated with higher PFNA concentrations and lower birth weights. Previous human studies have shown similar perturbed amino acid pathways associated with PFAS exposure (Alderete et al., 2019; Chen et al., 2020; Hu et al., 2020; Jin et al., 2020; Kingsley et al., 2019; Lu et al., 2019; Mitro et al., 2021; Salihovic et al., 2019). A mouse study indicated that PFOS exposure can reduce the expression levels of amino acid transporter on the placenta, leading to decreased concentrations of amino acids and glucose analogues in the placentas and fetal livers (Wan et al., 2020). A decreased amino acid concentration in the placentas and fetuses may suggest an increased concentration in maternal serum. Accordingly, amino acid concentrations among the pregnant women with an SGA fetus were higher than those carrying an appropriate-for-gestational (AGA) fetus (Cetin et al., 1996; Neerhof & Thaete, 2008). Amino acids are vital nutrients for fetal growth and development; thus, the dysfunction of placental transport function induced by PFAS exposure may, in part, impact fetal growth.

4.3. Lipid and fatty acid metabolism contributing to PFAS-fetal growth relationship

We found that lipid and fatty acid metabolism perturbation, one of the most pronounced effect of PFAS exposure, could mediate the PFAS-fetal growth relationship. We identified several pathways of lipid and fatty acid metabolisms (i.e., linoleate metabolism, *de novo* fatty acid biosynthesis, fatty acid activation, glycerophospholipid metabolism), glycosphingolipid biosynthesis and metabolism, butanoate metabolism (a pathway for short-chain fatty acids and alcohols), and unsaturated fatty acids C18:1 associated with both PFAS concentrations and fetal growth endpoints. These metabolic perturbations were largely consistent with the previous studies focusing on either PFAS concentrations or fetal growth outcomes (Alderete et al., 2019; Bobiński et al., 2013; Chen et al., 2020; Heazell et al., 2012; Herrera & Ortega-Senovilla, 2010; Horgan et al., 2011, 2011; Kingsley et al., 2019; Liu et al., 2017; Salihovic et al., 2019). Lipid and fatty acid metabolism were regulated by nuclear receptors such as peroxisome proliferator-activated receptor subtypes (e.g., PPARα, PPARβ, and PPARγ), which are also substantially involved in physiological processes related to fetal growth including inflammatory responses, oxidative pathways, energy homeostasis, placentation, and trophoblast differentiation (Grygiel-Górniak, 2014;

Szilagyi et al., 2020). PFAS have been shown to interact with these PPAR subtypes as potential ligands, which promote fatty acid accumulation and influence adipocyte differentiation (Bjork et al., 2011; Blake & Fenton, 2020; Jacobsen et al., 2018; Yamamoto et al., 2015). Additionally, PFAS exposure may influence gene expressions of mitochondrial β-oxidation, which breaks down fatty acid and produces acetyl-CoA in the energy generation process (Jacobsen et al., 2018; Wan et al., 2012).

Different maternal lipid and fatty acid profiles were observed between the women with normal and adverse pregnancy and birth outcomes in the previous studies (Heazell et al., 2012; Horgan et al., 2011; Liu et al., 2017; Paules et al., 2020; Starling et al., 2014). Changes in sphingolipid, glycerophospholipids, phospholipids, carnitine, and fatty acid were found among the mother with SGA birth, preterm delivery, or the other adverse birth outcomes (Heazell et al., 2012; Horgan et al., 2011). Several explanations were proposed including placental dysfunction, and oxidative stress and inflammation responses induced by PPARs signaling (Ganss, 2017; Gupta et al., 2005; Herrera & Ortega-Senovilla, 2010; Paules et al., 2020; Szilagyi et al., 2020). Additionally, the alteration of lipid metabolism may subsequently lead to preeclampsia via endothelial damage or oxidative stress (Llurba et al., 2005), and then may impact fetal growth (Ødegård et al., 2000). Collectively, the results from our and the previous studies have shown that lipid metabolism plays a vital role mediating the associations between PFAS exposure and fetal growth.

4.4. Bile acid metabolism contributing to PFAS-fetal growth relationship

Previously, exposure to PFAS was associated with downregulation of 7-alpha-hydroxylase (CYP7A1) expression, resulting in decreased bile acid synthesis but increased reabsorption from the intestine into liver (Beggs et al., 2016; Behr et al., 2020; Salihovic et al., 2019). PFOA and PFOS exposures were associated with altered bile acid profiles and changed bile canalicular morphology, suggesting a potential link to cholestasis. Moreover, bile acid conjugation with glycine and taurine, a process of detoxification before excretion, may be downregulated by PFAS exposure (Behr et al., 2020). These effects could explain the associations of serum PFAS with elevated parent bile acids and decreased bile acid conjugates in our analyses.

Gestational cholestasis has been associated with increased risks of adverse pregnancy and birth outcomes such as preeclampsia (Raz et al., 2015), preterm delivery (Cui et al., 2017), and intrauterine fetal death (Glantz et al., 2004). Even among the women without diagnosed gestational cholestasis, higher serum bile acid concentrations were also linked to higher risk of SGA birth (Li et al., 2020). Since bile acids can stimulate inflammatory response (Li et al., 2017; Shao et al., 2017), induce oxidative stress and apoptosis (Monte et al., 2009), and inhibit miRNA expressions on the placentas (Krattinger et al., 2016), higher circulating levels may result in reduced fetal growth (Amarilyo et al., 2011; Chen et al., 2019). We found that SGA birth was associated with increased parent bile acids and decreased bile acid conjugates (less toxic bile acids), suggesting a negative impact of parent bile acids on fetal growth. Additionally, bile acid metabolism is closely tied to lipid, glucose, and energy metabolism, which may be an important mediating mechanism for PFAS-outcome relationships.

4.5. Androgenic hormones disruption contributing to the PFAS-fetal growth relationship

We observed that PFNA concentrations were associated with higher intensities of androgenic hormone conjugates (i.e., dehydroepiandrosterone sulfate (DHEA-S), testosterone sulfate, and androsterone sulfate), and higher intensities of the conjugates predicted lower birth weights. Dehydroepiandrosterone (DHEA) and DHEA-S are both precursors of sex hormones and can be transformed to androsterone, testosterone, and the other sex hormones. Previous studies have shown that PFAS can disturb endocrine systems via interfering steroidogenesis, expression of endocrine related-genes, androgen receptors, and cholesterol metabolism through PPARα activation (Di Nisio et al., 2019; Du, et al., 2013; Lau et al., 2007). In previous epidemiological studies, no association of testosterone with PFAS was found in female adults in all age groups (Lewis et al., 2015), but positive associations with PFOA and PFHxS were observed among postmenopausal women. (Wang et al., 2021). Inverse associations of testosterone with PFOS were reported among girls at 6–9 years of age from the C8 Health Project (Lopez-Espinosa et al., 2016) and among female adolescents in Taiwan (Tsai et al., 2015). For dehydroepiandrosterone, significant positive and negative associations were found in cord blood with maternal serum PFOS and PFOA concentrations, respectively (Goudarzi et al., 2017). Testosterone and some sex hormones may regulate PFAS levels by interacting with renal transporters (Kudo et al., 2002; Lee et al., 2010); thus, the relationship could be more complicated than the current findings in the epidemiological studies. Further studies are warranted to clarify the mechanisms due to the conflicting results and potential reverse causality.

Previous animal models have shown that prenatal exposure to testosterone in early gestation was associated with reduced birth size and catch-up growth during early life (Manikkam et al., 2004; Smith et al., 2010). Several biological mechanisms were reported — maternal testosterone levels can modify maternal energy metabolism (Carlsen et al., 2006), decrease the expression of amino acid transporter on the placentas (Sathishkumar et al., 2011; Wan et al., 2020), and cause vascular dysfunction (Kumar et al., 2018; Vijayakumar et al., 2013), suggesting decreased nutrient supplies from mothers to their fetuses. Alternatively, androgenic hormones can cross the placenta and directly affect fetal energy metabolism and fetal growth (Dell'Acqua et al., 1966). It is worth noting that the previous studies have mainly focused on testosterone but not DHEA, androsterone, or their conjugates. Although the evidence of androgenic hormones disruption was presented, the results of conjugates were less comparable to the existing findings.

4.6. Uric acid as an intermediate biomarker for PFAS-fetal growth relationship

Uric acid is positively associated with PFAS concentrations and inversely associated with birth weight in our analyses. The associations between PFAS exposure and increased uric acid concentrations were published in epidemiological studies (Geiger et al., 2013; Gleason et al., 2015; Mitro et al., 2021; Salihovic et al., 2019; Shankar et al., 2011; Steenland et al., 2010). Two possible mechanisms were discussed. First, PFAS exposure could induce oxidative stress primarily through dysregulation of PPAR and upregulation of NF-E2-related factor 2 (Nrf2), subsequently resulting in increased serum uric acid concentrations (Abbott et al., 2007; Eriksen et al., 2010; Patterson et al., 2003; Stanifer et al., 2018; Wielsøe et al., 2015; Zeng et al., 2019). Second, because PFAS and uric acid share the same proximal renal transporters (Johnson et al., 2018; Stanifer et al., 2018), increased PFAS concentrations may lead to decreased uric acid secretion, and in turn, elevated serum uric acid concentrations. However, this finding should be interpreted with caution due to the possibility of reverse causation (Steenland et al., 2010).

Elevated maternal uric acid has been a predictor and a pathogenic factor for adverse pregnancy and birth outcomes (Akahori et al., 2012; Hawkins et al., 2012; Laughon et al., 2009, 2011; Ryu et al., 2019; Wu et al., 2012). Increased maternal uric acid may cause noninfectious placental inflammation (Wu et al., 2012), oxidative stress (Bainbridge et al., 2009), inhibition of amino acid transport on the placentas (Bainbridge et al., 2009), and dysfunction of endothelial and trophoblast cells (Bainbridge & Roberts, 2008; Gaubert et al., 2018), which could contribute to adverse birth outcomes including reduced fetal growth. Collectively, we found that uric acid may reflect inflammation, oxidative stress, or placental dysfunction partially induced by PFAS exposure and

serve as a predictor for reduced birth weight.

4.7. Other overlapping metabolic pathways and metabolites

We found two cofactor and vitamin metabolic pathways, including vitamin B_3 and D_3, associated with both PFAS concentrations and fetal growth endpoints. PFAS exposure has shown the ability to interfere with vitamin D metabolism previously (Chang et al., 2021a; Di Nisio et al., 2020; Etzel et al., 2019; Khalil et al., 2018). Vitamin D plays an important role of fetal growth on skeletal development, placental function, oxidative stress, inflammatory response, and metabolism of glucose and lipids (Brannon, 2012; Lo et al., 2019), and vitamin D deficiency has been linked to lower birth weight and higher risk of SGA (Leffelaar et al., 2010). Additionally, vitamin B_3 may work as an antioxidant for fetal growth improvement and preeclampsia treatment (Salcedo-Bellido et al., 2017).

We also observed two exogenous metabolites (ferulic acid and 2-hexyl-3-phenyl-2-propenal) that were associated with PFAS concentrations and fetal growth endpoints but are unlikely on the causal pathway of the PFAS-fetal growth relationship. More specifically, the inverse association between PFOA concentration and ferulic acid, a naturally occurring chemical in plants, could be due to dietary preference as PFAS exposure was linked to more fish and meat consumptions (Papadopoulou et al., 2019; Tittlemier et al., 2007). Accordingly, the positive association between ferulic acid and SGA birth could be attributed to the preference of plant-based food consumption during pregnancy (Kesary et al., 2020). 2-hexyl-3-phenyl-2-propenal, a fragrance and flavoring agent in many consumer products (Kim et al., 2018), was positively associated with PFHxS and PFOS concentrations, which might be explained by the use of consumer products (Chang et al., 2021b; Kotthoff et al., 2015). The positive association between 2-hexyl-3-phenyl-2-propenal and SGA birth might be confounded by the factors correlated to the use of consumer products through co-exposure to other chemicals.

4.8. Strength and limitations

The strengths of our study include the use of untargeted metabolomics techniques to explore global metabolic changes and a novel MITM approach to identify potential biological mechanisms and intermediate biomarkers linking exposure to outcome. Second, we assessed different approaches to control for length of gestation in this study. Although most results were similar, the enriched pathways were somewhat distinct (Figure S3), suggesting a possibility of different metabolic pathways associated with fetal growth among women with preterm and term deliveries. Third, we were able to ascertain quality clinical outcomes by using early pregnancy gestational age dating and medical chart abstraction.

We also acknowledge several limitations. First, due to the cross-sectional nature of the associations between PFAS concentrations and metabolomic features, it is difficult to derive causal relationships. Second, dietary and some lifestyle variables were not considered in this study. Since adjusting for more variables might introduce unknown biasing paths and cause issues of overadjustment, we only included a basic set of covariates in the MWAS analyses. Third, only four PFAS were included in the present study. It is possible that other PFAS or co-exposed chemicals may also yield similar results. Fourth, we use raw, but not multiple-testing corrected, *p*-values for pathway enrichment analyses, which could lead to an increased risk of false positive results. However, pathway enrichment analysis by mummichog has been proven to return stable results when varying the cutoffs for significance (Li et al., 2013). Another potential limitation is the use of proxies (i.e., birth weight and SGA) instead of 'gold' standard (i.e., repeated ultrasound measurements of fetal anthropometrics) for fetal growth assessment in this study (Smarr et al., 2013). Still, these commonly utilized proxies of fetal growth allow for comparison of results in our cohort with the other studies. Finally, the findings from this pregnant African American women cohort might reduce generalizability to a broader population. Nevertheless, we observed consistent perturbations in similar metabolic pathways previously reported in other populations (Kingsley et al., 2019; Alderete et al., 2019; Chen et al., 2020).

5. Conclusions

To our knowledge, this is the first study to investigate the interrelationship between serum PFAS concentrations, maternal metabolomic perturbation, and fetal growth. We report associations of maternal serum PFOA and PFNA concentrations with reduced fetal growth in this African American women population. The underlying biological mechanisms of the PFAS-fetal growth relationship were shown to be amino acid, lipid and fatty acid, and bile acid metabolisms, as well as androgenic hormone disruption. Uric acid was identified as a potential intermediate biomarker representing the early responses of PFAS exposure and predicting reduced fetal growth. These biological mechanisms were consistent with previous experimental and observational studies, which strengthen the causal link of the existing associations between PFAS and reduced fetal growth. Additionally, the mechanisms and the potential intermediate biomarker presented in this study are warranted for future investigation in targeted and more controlled studies, which may help to develop early detection and intervention in public health or clinical settings.

Uncited references

CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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