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Biological Mechanisms for Per and Poly-fluoroalkyl Substances (PFAS) Exposure Effects on Fetal Growth among Pregnant African American Women in Atlanta, Georgia

By

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Doctor of Philosophy

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Abstract

Biological Mechanisms for Per and Poly-fluoroalkyl Substances (PFAS) Exposure Effects on Fetal Growth among Pregnant African American Women in Atlanta, Georgia

By Che-Jung Chang

Exposure to per and poly-fluoroalkyl substance (PFAS) has been linked to many adverse health outcomes including reduced fetal growth. However, limited studies have characterized the exposures and related outcomes among pregnant African American women, who are particularly at-risk for increased environmental exposures and adverse birth outcomes. Furthermore, the biological mechanisms linking PFAS exposure to fetal growth have not yet been elucidated. Thus, four study aims were developed to fill these research gaps in an African American birth cohort in Atlanta, Georgia. **Aim 1** quantified 14 serum PFAS concentrations and evaluated their predictors. **Aim 2** investigated the associations of PFAS with fetal growth outcomes and preterm delivery. To explore the biological mechanisms, **Aim 3** examined the associations between serum PFAS and vitamin D biomarker concentrations and **Aim 4** utilized high-resolution metabolomics coupling with a *meet-in-a-middle* approach to identify biological pathways and intermediate biomarkers associated with both PFAS concentrations and fetal growth outcomes.

In **Aim 1**, we found that PFAS exposures are ubiquitous among participants with > 95% detection frequencies in perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA). PFHxS concentrations in this cohort were higher when compared to the matched U.S. population. Several predictors, including education, sampling year, parity, BMI, tobacco and marijuana use, age of house, drinking water source, and cosmetic use, were significantly associated with PFAS levels. In **Aim 2**, PFNA and PFOA levels were significantly associated with odds of small-for-gestational age with the same directionality across most fetal growth outcomes. **Aim 3** suggested that individual PFAS and PFAS mixtures may affect vitamin D biomarker concentrations, and some associations were modified by fetal sex. Furthermore, **Aim 4** identified 10 metabolites and 21 metabolic pathways associated with both PFAS levels and fetal growth endpoints, and the results were closely related to the perturbations of amino acid, lipid and fatty acid, bile acid, uric acid, and androgenic hormone metabolisms.

This dissertation demonstrated that PFAS exposures are ubiquitous and may contribute to health disparities in fetal growth in this cohort. The identified biological pathways show potentials for future studies to develop early detection and intervention for PFAS-induced fetal growth restriction.

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Chapter 1. Introduction

Per and poly-fluoroalkyl substances (PFAS), a broad range of man-made chemicals with more than 4,700 identified substances, have been manufactured since the 1940s (The Organisation for Economic Co-operation and Development, 2018; US Environmental Protection Agency, 2018). PFAS contains a hydrophobic and thermally resistant fluorinated carbon chain and a hydrophilic functional group such as carboxylic or sulfonic acid (Figure 1-1). Due to their unique chemical properties, PFAS have been applied in a variety of consumer products and industrial process streams. For example, PFAS have been used as water- and stain-resistant coating for clothing, textiles, leather, furniture, and carpets, oil-repellent coating for food wrappers and packages, cardboards, firefighting foams, paints, and waxes, and surfactants, emulsifiers, and additives in the production of other chemicals. Some perfluoroalkyls such as perfluorooctanoic acid (PFOA) are used as processing aids in producing nonstick coating for cookware (Kissa, 2001; Schultz et al., 2003; The Agency for Toxic Substances and Disease Registry, 2018).

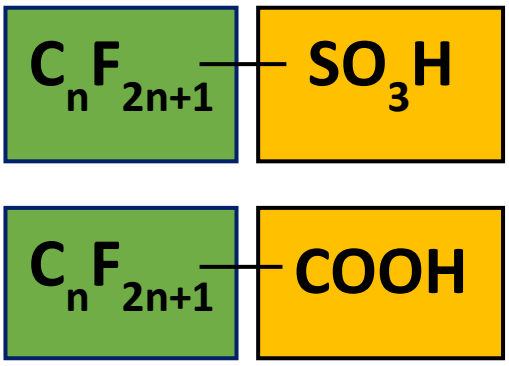

Figure 1-1. General chemical structure of PFAS.

Despite the ubiquity, PFAS are extremely persistent and resistant to environmental degradation due to their strong carbon-fluorine bonds. Perfluorooctane sulfonic acid (PFOS) and PFOA, along with theirs salts, and related compounds were listed as persistent organic pollutants (POPs) by the Stockholm Convention for either a global restriction or elimination on production and use in 2009 and 2019, respectively (United Nations environment programmes Stockholm Convention, 2009, 2019). Accordingly, perfluorohexane sulfonic acid (PFHxS) is currently under review and was suggested to be listed as POP and eliminated

from production (United Nations environment programmes Stockholm Convention, 2020). Widespread occurrence of PFAS has been reported in various environment media and biota worldwide, including aquatic (Ahrens, 2011; Hu et al., 2016; Kim & Kannan, 2007; Krafft & Riess, 2015; Post et al., 2012; Zareitalabad et al., 2013) and atmospheric environment (Rauert et al., 2018), as well as wildlife (Houde et al., 2011; Reiner & Place, 2015; Sedlak et al., 2017). Additionally, previous studies found detectable PFAS in off-source areas such as Arctic regions and open ocean waters, suggesting that PFAS could be distributed through long-range atmospheric transport and/or oceanic currents (Ahrens, 2011; Kwok et al., 2013; Muir et al., 2019; Zhao et al., 2012).

Human can be exposed to PFAS from various pathways such as food, water, air, soil, indoor dust, and other pathways (Figure 1-2) (DeLuca et al., 2021; Muir et al., 2019; Silva et al., 2021; Sunderland et al., 2019). Among these exposure pathways, food has been considered as the primary PFAS exposure pathway to humans. Due to contamination and bioaccumulation in the food chain, detectable PFAS have been reported in vegetables, fruits, egg, milk, meat, and seafood (Gebbink et al., 2015; Ghisi et al., 2019; Muir et al., 2019; Schrenk et al., 2020). PFAS concentrations in human blood have been linked to certain dietary patterns such as freshwater fish and seafood consumption (Augustsson et al., 2021; Berger et al., 2009; Denys et al., 2014; Domingo & Nadal, 2017; Gebbink et al., 2015; Noorlander et al., 2011; Sjogren et al., 2016; Vestergren et al., 2012; Yamada et al., 2014). In a recent report from European Food Safety Authority, fish and other seafood contribute to approximately 86% of dietary PFAS exposure in adults (Schrenk et al., 2020). Moreover, PFAS can also contaminate food during food processing, migrating from food-contact materials such as fast-food packaging and microwave popcorn bags to food (Begley et al., 2005; Susmann et al., 2019; Yuan et al., 2016). Drinking water has also been an important source for PFAS exposure, especially among the populations living close to contaminated communities (Domingo & Nadal, 2019; Herrick et al., 2017; Hoffman et al., 2010; Hu et al., 2016; Seals et al., 2011). Additionally, the U.S. Environmental Protection Agency (EPA) conducted PFAS measurements in public water from 2013 to 2015 under the third Unregulated Contaminant Monitoring Rule (UMCR3), which indicated that PFOS and

PFOA in large public water supplies serving about six million peoples have exceeded the U.S. EPA's lifetime health advisory levels from drinking water (Hu et al., 2016). Lastly, indoor environment has also been an important exposure scenario for PFAS despite relatively little attention. Because PFAS were used extensively in a variety of consumer products in the indoor environment, previous studies have reported that human PFAS exposure was associated with dermal absorption of furniture, cleaning products, and personal care products, inhalation of dust and airborne volatiles, and ingestion of indoor dust (DeLuca et al., 2021; Harrad et al., 2010; Nadal & L. Domingo, 2014; Schultes et al., 2018; Sunderland et al., 2019). It is noted that contribution of each exposure predictors often varies by different population characteristics such as age, sex, behaviors, regions, and different time periods due to the changing chemical landscape where legacy PFAS might be gradually eliminated from the market. Surprisingly, relatively limited information of the exposure predictors has found in the U.S., especially among underrepresented and underserved populations (Domingo & Nadal, 2017; Park et al., 2019).

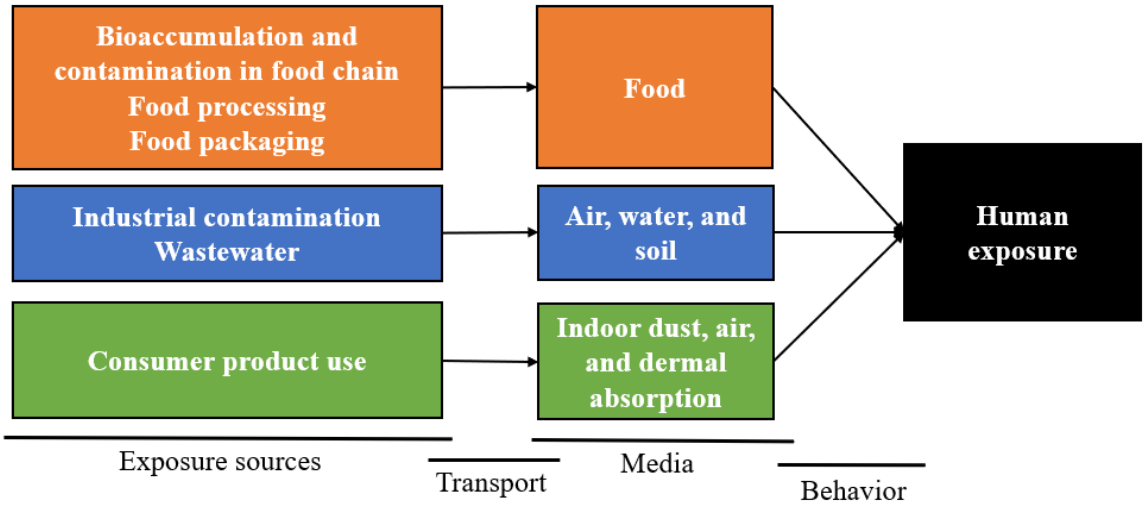

Figure 1-2. Common PFAS exposure pathways.

(adapted from Silva et al., 2021)

Based on the recent biomonitoring data from the National Health and Nutrition Examination Survey (NHANES), four most common PFAS (i.e., PFHxS, PFOS, PFOA, perfluorononanoic acid [PFNA]) were

detected in > 98 % of the samples in the general U.S. population despite the voluntary phase-out of PFOS and PFOA by several major manufactures starting in 2002 (Calafat et al., 2007; US Department of Health and Human Services, 2019). Many adverse health outcomes have been linked to PFAS exposure, including metabolic diseases, thyroid dysfunction, deficits in immune function, hypertensive disorder of pregnancy, reduced birth weight, and cancers (Barry et al., 2013; Darrow et al., 2013; Grandjean et al., 2012; Johnson et al., 2014; Lam et al., 2014; Liu et al., 2018; Lopez-Espinosa et al., 2012; Sunderland et al., 2019; The Agency for Toxic Substances and Disease Registry, 2018). The impacts related to PFAS exposures at critical windows of susceptibility such as prenatal period are particularly concerning because the health burden can not only be observed at the time but later in life. For example, prenatal exposure to PFAS has been linked to fetal growth restriction, and the fetuses and infants who grew restrictively could impart a significant burden of increased perinatal mortality and morbidity (Bach et al., 2015; Lubchenco et al., 1963; Manning, 1995; The Agency for Toxic Substances and Disease Registry, 2018; Williams et al., 1982). More importantly, adult chronic diseases such as cardiovascular diseases, type II diabetes, stoke, and hypertension could originate in developmental plasticity, in response to the environmental exposures during these critical windows (D. J. Barker, 1995; D. J. P. Barker, 2006; D. J. P. Barker et al., 2002; Crispi et al., 2018). However, the associations between PFAS exposure and fetal growth are still lacking among underrepresented and underserved populations, who are more vulnerable to environmental exposure and more frequently at a higher risk of adverse outcomes. Compared with white women, African American women have shown nearly 50% higher risk of adverse birth outcomes such as preterm birth and low birth weight (13.2% vs 8.9% for preterm birth; 13.2% vs 7% for low birth weight [< 2,500 grams]) (Giscombé & Lobel, 2005; Martin et al., 2017), and were exposed to higher environmental pollutants including some PFAS (Calafat, Kuklenyik, et al., 2007; Calafat, Wong, et al., 2007; Jain, 2014; Nelson et al., 2012; Park et al., 2019).

The underlying biological mechanisms linking PFAS exposure to health outcomes remain largely unknown. Although human epidemiological studies often established on biological plausibility, few of them have addressed and focused on specific mechanisms. The studies exploring biological mechanisms are important

in several ways. First, mechanistic research can help strengthen and provide additional understanding to the previously identified exposure-outcome relationships. Second, detailed biological mechanisms provide opportunities for future research to develop early detection and intervention for the PFAS-induced health outcomes. Lastly, the mechanistic findings can contribute to the critical aspects of human risk assessment, which is a major tool to informed decision and policy by the regulatory bodies to protect human health from environmental exposures (Furberg & Ambrosone, 2001; Perera, 1996; US Environmental Protection Agency, 2014).

Dissertation Aims

To address the research gaps, four aims were developed to investigate the participants in a pregnant African American women cohort in Atlanta, Georgia (Figure 1-3).

Aim 1 describes the population-based exposure to PFAS using serum PFAS biomarkers and examines the related sociodemographic and behavioral predictors (Chapter 2) .

Aim 2 investigates the associations between maternal serum PFAS concentrations and fetal growth endpoints including birth weight, sex-specific z-scores for gestational age, birth length, head circumference, ponderal index, small-for-gestational age (SGA), and preterm birth (Chapter 3).

Aim 3 assesses the associations of single and multiple serum PFAS concentrations with vitamin D biomarkers (Chapter 4).

Aim 4 evaluates the interrelationship between maternal serum PFAS concentrations, metabolome perturbation, and fetal growth endpoints (Chapter 5).

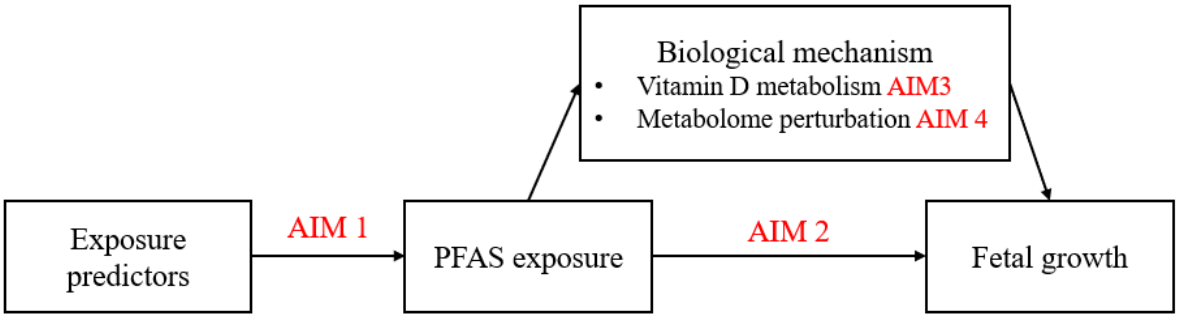

Figure 1-3. The conceptual dissertation research framework.

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Chapter 2. PFAS Exposure Distribution and Predictors

Serum Per- and Polyfluoroalkyl Substance (PFAS) Concentrations and Predictors of Exposure among Pregnant African American Women in the Atlanta Area, Georgia

Chang, C. J., Ryan, P. B., Smarr, M. M., Kannan, K., Panuwet, P., Dunlop, A. L., Corwin, E. J. & Barr, D. B. (2020). Serum per-and polyfluoroalkyl substance (PFAS) concentrations and predictors of exposure among pregnant African American women in the Atlanta area, Georgia. *Environmental Research*, *198*, 110445.

Abstract

Exposure to per- and polyfluoroalkyl substances (PFAS) has been associated with adverse health outcomes, especially when exposure occurs within sensitive time windows such as the pre- and post-natal periods and early childhood. However, few studies have focused on PFAS exposure distribution and predictors in pregnant women, especially among African American women. We quantified serum concentrations of the four most common PFAS collected in all 453 participants and an additional 10 PFAS in 356 participants who were pregnant African American women enrolled from 2014 to 2018 in Atlanta, Georgia, and investigated the sociodemographic predictors of exposure. Additional home environment and behavior predictors were also examined in 130 participants. Perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were detected in > 95% of the samples with PFOS having the highest concentrations (geometric mean (GM) 2.03 ng/mL). N-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA), perfluoropentanoic acid (PFPeA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) were found in 40-50% of the samples, whereas the detection frequencies for the other six PFAS were below 15%. When compared to the National Health and Nutrition Examination Survey (NHANES) participants matching sex, race, and age with this study, our results showed similar concentrations of most PFAS, but higher concentrations of PFHxS (GM 0.99 ng/mL in this study; 0.63 and 0.4 ng/mL in NHANES 2014-2015 and 2016-2017 cycles). A decline in concentrations over the study period was found for most PFAS but not PFPeA. In adjusted models, education, sampling year, parity, BMI, tobacco and marijuana use, age of house, drinking water source, and cosmetic use were significantly associated with serum PFAS concentrations. Our study reports the first PFAS exposure data among pregnant African American women in the Atlanta area, Georgia. The identified predictors will facilitate the setting of research priorities and enable development of exposure mitigation strategies.

Keywords: biomonitoring, per- and polyfluoroalkyl substance (PFAS), prenatal exposure, exposure predictors, vulnerable population

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic compounds manufactured since the 1940s for use in a range of consumer products because of their ability to repel both water and lipids. Consumer products containing PFAS include food packaging, cookware, fabrics, carpet, upholstery, and personal care products (The Agency for Toxic Substances and Disease Registry, 2018; Paul et al., 2009). Because the carbon-fluorine covalent bond is one of the strongest, PFAS are persistent in the environment and some of them accumulate in wildlife and humans (Lau et al., 2007). Despite their phase-out starting in the early 2000s, PFAS have been widely detected, both in the environment and in human biospecimens over the past two decades (Harris et al., 2017; Kim et al., 2020; Sinclair et al., 2020).

A summary from the Agency for Toxic Substances and Disease Registry (2018) indicated that exposure to PFAS could cause hepatic, immune, reproductive, and developmental effects in animal oral exposure studies, and in a few inhalation and dermal exposure studies. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) exposures were linked to adverse pregnancy and birth outcomes, and neurodevelopmental effects, such as preterm birth, growth restriction, and developmental delays in human studies (Chen et al., 2012; Fei et al., 2007; Hoffman et al., 2010; Jensen et al., 2015; Lau et al., 2007; Lenters et al., 2016; Maisonet et al., 2012; Savitz et al., 2012; Stein et al., 2009, Stein et al, 2014; Souza et al., 2020). More importantly, PFAS have been shown to cross the placenta. Concentrations in maternal peripheral blood highly correlate with infant cord blood concentrations suggesting that placental transfer could contribute to fetal exposure (Beesoon et al., 2011; Chen et al., 2017; Hanssen et al., 2010; Zhang et al., 2013). The positive correlation between duration of breastfeeding and PFAS concentrations in children suggests transfer can occur from mothers to their children during lactation (Mogensen et al., 2015; Tao et al., 2008). PFAS exposure during pregnancy, infancy, and early childhood is of interest because these are susceptible windows of exposure which may result in later health risk (The Agency for Toxic Substances and Disease Registry, 2018).

Racial differences in PFAS exposure have been reported in the general U.S. population: higher serum PFOS and perfluorononanoic acid (PFNA) levels were found among non-Hispanic blacks than whites and other races/ethnicities in multiple National Health and Nutrition Examination Survey (NHANES) cycles when adjusted for other variables (Calafat, Kuklenyik, et al., 2007; Calafat, Wong, et al., 2007; Jain, 2014; Nelson et al., 2012; Park et al., 2019). These differences in serum PFAS could potentially contribute to existing racial health disparities such as the disproportionately high risk of low birth weight, preterm delivery, and mortality in African Americans as compared to whites (Dunlop et al., 2011; Gee & Payne-Sturges, 2004; Giscombé & Lobel, 2005; Kramer & Hogue, 2009). While PFAS exposure has been documented and well-characterized in several studies, few studies have focused on exposure distribution percentile and exposure predictors in African Americans (Boronow et al., 2019; Park et al., 2019), much less pregnant African American women. The limited knowledge among this minority population can restrict our ability to identify critical risk factors for exposure and resulting health outcomes. Furthermore, little information is known about typical chemical exposures in the Southeastern United States. The purpose of this study is to characterize the chemical distribution percentiles and predictors of PFAS exposure in a cohort of pregnant African American women in the Atlanta area, Georgia.

Materials and Methods

Study population

Participants for this study were drawn from the women participating in the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study (Corwin et al., 2017). The study recruited pregnant women from prenatal clinics affiliated with hospitals in Atlanta, Georgia: Emory University Hospital Midtown, a private hospital, which provides service to people with a diverse socioeconomic status (SES), and Grady Memorial Hospital, a county-support public hospital, which provides service mainly to low-income or underserved populations. Inclusion criteria for enrollment into the cohort included: (1) U.S.-born Black woman by self-report; (2) between 8 and 14 weeks gestation with a singleton pregnancy; (3) able to understand and speak English; (4) between 18 and 40 years old; and, (5)

experiencing no chronic medical condition or not taking prescribed chronic medications. The subjects who met the criteria above were asked to provide written informed consent at enrollment. This study was reviewed and approved by Emory's Institutional Review Board (approval reference number 68441).

A total of 453 pregnant African American women were included in this study; these 453 women represented the first consecutively enrolled women in the cohort (enrolled between March 2014 and May 2018) whose pregnancy ended in a birth for whom a blood sample (for PFAS measurement) was available. Women enrolled in the study between January 2016 and May 2018 (n = 232) had the option of enrolling in a sub-study that involved a home visit between 20 and 24 weeks of gestation as part of the Center for Children's Health, the Environment, the Microbiome, and Metabolomics (C-CHEM^2), which seeks to understand the interaction between the prenatal and postnatal environmental toxicant exposures, the microbiome, the metabolome, and their impacts on birth outcomes and infant health and development.

Clinical and questionnaire data collection

The questionnaires were administered to the participants by trained research coordinators, who directly recorded the answers on either paper or tablet computers. Two questionnaires were included in this study: (1) the Sociodemographic Questionnaire, which contains self-report of household income, education, marital or cohabitating status, insurance status during pregnancy, and substance use, which was collected at enrollment at the hospitals; and, (2) the Home Environment and Behavior Questionnaire, which was administrated between 20 and 24 gestation at the participants' home.

Of the 232 participants enrolled from January 2016 to May 2018, 130 participants completed the Home Environment and Behavior Questionnaire during the home visit. The participants were asked about housing characteristics, including age of their home, distance between their home and the nearest industry, dump or waste site, and behavioral factors such as home cleaning behaviors, primary drinking water source (tap/well/bottled water consumption), water consumption in the last 48 hours (tap/well/bottled water), takeout food or deliver pizza consumption last month, microwave popcorn consumption last month, frequency of personal care product use (hair products/shampoo/lotion/sunscreen), and cosmetic product use

(foundation/rouge or blush/lipstick/mascara/nail polish/eye shadow). Additionally, some data were ascertained from the prenatal clinical record, such as maternal age, parity, and BMI (calculated by height and weight measured at prenatal visit).

Quantification of PFAS

A 30-mL venous blood sample was drawn from each participant at enrollment between March 2014 and May 2018. The sample tubes were transported to the laboratory, centrifuged to separate the serum from whole blood, and then stored at −80°C for subsequent analyses. The samples were analyzed at two laboratories in the Children's Health Exposure Analysis Resource (CHEAR): Wadsworth Center/New York University Laboratory Hub (Wadsworth/NYU) and the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) (Emory University). CHEAR is an exposure assessment resource supported by the National Institute of Environmental Health Sciences to expand the ability of including environmental exposure analyses in children's health research. Laboratories in CHEAR have implemented the same rigorous internal quality control procedures to provide harmonized data (Balshaw et al., 2018). All 453 samples were analyzed for perfluorohexane sulfonic acid (PFHxS), PFOS, PFOA, and PFNA from both laboratories, whereas 356 samples were analyzed for perfluorobutane sulfonic acid (PFBS), perfluorooctane sulfonamide (PFOSA), N-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA), n-ethyl perfluorooctane sulfonamido acetic acid (NEtFOSAA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluorododecanoic acid (PFDoA) from Wadsworth/NYU.

Further details of the Wadsworth/NYU method are described elsewhere (Honda et al., 2018). For the LEADER method, each serum sample was spiked with an isotopically labeled standard solution, and deproteinated with formic acid. The supernatant was loaded to the Isolute C18 cartridge (Biotage, Sweden). The cartridge was washed with 1:1(v/v) formic acid and methanol solution, then eluted with methanol. The eluate was evaporated to dryness under a nitrogen stream. The sample was reconstituted with methanol for

the analysis using high-performance liquid chromatography interfaced with tandem mass spectrometry (6460 Triple Quadrupole LC/MS, Agilent Technologies; Santa Clara, CA). In each analytical batch, a matrix-based calibration curve, a blank sample, and quality control samples were prepared and injected alongside 37 unknown serum samples. Quantification was performed using isotope dilution calibration with the limits of detection (LODs) ranging from 0.05 to 0.2 ng/mL. The LODs were defined as the lowest concentrations in the calibration curve, where the signal to noise ratio of the observed signal was ≥3 and the accuracy was within 100 ± 20% (Table S2-1) (FDA, 1994). The method precision, calculated as the relative standard deviation (RSD), was less than 10% for all analytes. Standard reference materials from the National Institute of Standards and Technology (NIST) were analyzed concurrently and the recoveries of target analytes were within 100 ± 20%. Good agreement was observed between the results from the two laboratories using 11 overlapped samples; the Pearson correlation coefficients ranged from 0.88 to 0.93, and the relative percent differences (RPD) ranged from 0.12 to 20.2% with a median of 4.8% (Table S2-2). Additionally, both laboratories successfully participate in and are certified semi-annually for PFAS measurements by the German External Quality Assessment Scheme (g-equas.de).

Statistical analysis

Descriptive analyses were performed for the serum concentrations, including detection frequencies, distribution percentiles, ranges, geometric means (GMs), and geometric standard deviation (GSD). PFAS levels below the LODs were imputed with LOD/$\sqrt{2}$ in the descriptive analyses (Hornumg & Reed, 1990). To conform to normality, we used natural log-transformed values of PFAS concentrations for statistical analyses. The PFAS with low detection frequencies (0-14%), including PFBS, PFOSA, NEtFOSAA, PFHxA, PFHpA, and PFDoA, were excluded from further statistical analyses due to the limited number of quantified measurements.

Pearson correlation coefficients were first calculated to investigate the correlations between the concentrations of individual PFAS. One-way analysis of variance (ANOVA) was performed to examine the concentration differences between different categorical predictors.

We fitted the multivariable linear regression models to assess the associations between serum PFAS levels and potential predictors. In the first stage, the associations between sociodemographic factors and serum PFAS levels were investigated in adjusted models mutually including age (continuous, year), sampling year (2014/2015/2016/2017-2018), parity (0/1-2/3-4), BMI (continuous, kg/m^2), education (less than high school/high school/some college/college and above), poverty income ratio (the ratio of a family's income to the US Census Bureau's poverty threshold; <100/100-150/≥150%), married/cohabiting (yes/no), insurance status during pregnancy (private/Medicaid, a federal-state public health insurance program for low income and disabled people), hospital (Emory/Grady), and tobacco (during pregnancy/not during pregnancy), alcohol (during pregnancy/not during pregnancy), and marijuana use (during pregnancy/not during pregnancy). The covariates were selected based on previous studies (Kingsley et al., 2018; Pitter et al., 2020; Shu et al., 2018; Vuong et al., 2018). In the second stage, the home environment and behavior characteristics were included one at a time with the significant covariates (at a 0.05 α level) in the first stage analysis because the covariates could contribute to the main effects of serum PFAS concentrations. Sampling year was excluded from the models in the second stage because the participants with home environment and behavioral information were mostly enrolled in 2017. PFAS concentrations as outcome variables were natural log-transformed; therefore, we back-transformed coefficient estimates and confidence intervals using $(e^{\beta}-1)\times 100$, and reported effect estimates as a percent change in GM of PFAS concentrations relative to the reference group. Collinearity for the covariates in the models was also assessed by calculating variance inflation factors (James et al., 2013).

Due to missing values on poverty income ratio (missing n = 62; 13.7%), tobacco use (missing n = 6; 1.3%), alcohol use (missing n = 6; 1.3%), marijuana use (missing n = 7; 1.5%), and some home environment and behavior predictors (missing n = 1-92; 0.8-70.8%; Table S2-3), multiple imputations by chained equations with regression imputation approaches were conducted (Buuren & Groothuis-Oudshoorn, 2010). Serum PFAS concentrations below the LODs were multiply imputed using maximum likelihood estimation with a lognormal distribution (Lubin et al., 2004). We imputed 10 datasets up to the full sample size of 453 and

356 for the first stage analyses (based on the availability of PFAS measurements), and 130 for the second stage analyses. The sensitivity analyses included: 1) the comparison between multiple imputations and complete case analysis for multivariate linear regression models to evaluate the impact of the missing values; 2) the comparison between simple imputation with LOD/√2 and multiple imputations using maximum likelihood estimation for PFAS values below LOD. Overall results did not change substantially; thus, multiple imputations are presented because the other methods tended to bias effect estimates away from the null hypothesis in our analyses.

The measured PFAS concentrations in this population were compared with those of the general U.S. population among the same demographic group (non-Hispanic black females between the age of 18 and 40 years in NHANES). Serum PFAS data from four NHANES (2009-2016) cycles (n = 333) were extracted and evaluated accounting for the sampling weights, primary sampling units, and strata due to the complex survey design. The PFAS with more than 50% detection frequencies in this study were compared to the NHANES data. All statistical analyses were conducted in R version 3.6.1, and multiple imputations were done through Multivariate Imputation by Chained Equations (MICE) package.

Results

The demographic characteristics are shown in Table 2-1. Among the participants, the average age was 24.8 years. The majority had a high school or below education (54%), < 150% poverty income ratio (58%), Medicaid as medial insurance (78%), given birth one or more times (52%), and an overweight (21%) or obese (36%) status. PFAS concentrations and summary statistics are presented in Table 2-2. PFHxS, PFOS, PFOA, and PFNA were detected in > 95% samples with PFOS having the highest GM (2.03 ng/mL). NMeFOSAA, PFPeA, PFDA, and PFUnDA were detected in almost 50% of the samples, whereas only a small percentage of samples had detectable levels of PFBS (14%), PFOSA (0%), NEtFOSAA (5%), PFHxA (3%), PFHpA (14%), and PFDoA (2%).

Table 2-3 shows the Pearson correlation coefficients between the natural log-transformed concentrations of PFAS. The four most frequently detected PFAS, i.e., PFHxS, PFOS, PFOA, and PFNA, were moderately to strongly correlated with each other with the coefficients ranging from 0.39 to 0.71 (Schober et al., 2018). Although positive correlations were mostly found between any two PFAS, PFPeA concentrations were negatively associated with most of PFAS with the correlation coefficients ranging from -0.33 to -0.06.

We compared serum PFAS levels in this study with similarly aged, non-Hispanic black women in NHANES. Figure 2-1 shows PFHxS, PFOS, PFOA and PFNA concentrations decrease by sampling year among both NHANES and our study participants. Similar serum PFOS, PFOA, and PFNA concentrations were found among these two populations, whereas PFHxS levels were higher in this study than those in NHANES. The GM of serum PFHxS was 0.63 ng/mL (95% CI 0.07-0.50 ng/mL) and 0.40 ng/mL (95% CI 0.06-0.28 ng/mL) in the 2013-2014 and 2015-2016 NHANES cycles, respectively, which are about 0.4-0.6 times the GM of this study (GM 0.99; 95% CI 0.94-1.06 ng/mL).

The unadjusted GMs and GSDs by different predictors are presented in Table S2-3. PFHxS, PFOS, PFOA, and PFNA concentrations were significantly different by education, poverty income ratio, insurance, sampling year, parity, BMI, tobacco use, marijuana use, age of house, bottle water as primary drinking water source, number of cosmetic product usually worn, usually worn foundation, and usually worn lipsticks in ANOVA analyses. The GM and GSD of NMeFOSAA, PFPEA, PFDA, and PFUnDA were not presented by groups because these PFAS were less frequently detected. The adjusted results using multivariable regression models are presented in Table 2-4 and 2-5. No collinearity was found in the models, and the values of variance inflation factors were between 1.02 and 2.36. Higher education levels were associated with higher PFNA concentrations; the participants with college education and above had a 57.8% (95% CI 14.4-118%) increase in serum PFNA levels compared to those with less than high school education. The concentrations of PFHxS, PFOS, PFOA, PFNA, NMeFOSAA, and PFDA in the samples collected during 2017 and 2018 were 36-90% lower than those collected in 2014. PFPeA and PFUnDA concentrations were higher when the samples were collected in the later years. Lower PFAS concentrations

were found in the women with higher parity, with PFOA having the largest percentage difference in concentrations, yet PFPeA has a different direction of relationship from the other PFAS. Additionally, BMI was negatively associated with PFAS concentrations; a unit changed in BMI was significantly associated with 0.9% (95 CI -1.6, -0.3%), 1.2% (95% CI -2, -0.5%), and 2.4% (95% CI -4.5, -0.3%) decreases in PFHxS, PFOS, and PFUnDA concentrations, respectively. The women using tobacco during pregnancy had lower PFHxS and PFOS concentrations than those not using tobacco during pregnancy, whereas the people using marijuana during pregnancy had higher PFOS, PFOA, and PFNA levels than non-users.

For the home environment and behavioral characteristics (Table 2-6), the participants with ≥ 20 years residential house had 32% and 39% decreases in PFOA and PFNA concentrations than those with <10 years house. The participants who reported bottled water as their primary drinking water source had 65% and 88% increases in PFOS and PFOA concentrations. Additionally, a larger number of cosmetic products usually worn (a 50% increase in PFNA concentrations when comparing 4-6 and 0 products used), and usually worn foundation (52% and 58 % increases in PFOS and PFNA concentrations) were associated with higher serum PFAS. The results of well water consumption and frequency of sunscreen use were not shown since most women reported no exposure to either of these. The results of NMeFOSAA, PFPeA, PFDA, and PFUnDA were not shown due to the small sample size.

Discussion

This is the first study focusing on PFAS exposure and their predictors among pregnant African American women in Atlanta, Georgia. Serum PFHxS, PFOS, PFOA, and PFNA levels in this population were generally lower than the measurements in the other birth cohorts in the United States in which samples were collected in earlier years (Boronow et al., 2019; Kingsley et al., 2018; Lyall et al., 2018; Romano et al., 2016; Sagiv et al., 2015). Serum PFAS levels were more comparable to the concentrations measured in the corresponding NHANES sampling years within a subset of similarly aged, non-Hispanic black females. It is noteworthy that serum PFAS levels among pregnant women, especially women in the late pregnancy, could be lower than non-pregnant women of reproductive age because of physiologic changes during

pregnancy, such as increased blood volume, increased renal plasma flow and glomerular filtration rate, and decreased plasma protein levels (Pan et al., 2017; Soma-Pillay et al., 2016). Although the serum PFAS concentrations among pregnant women could underestimate the concentrations of the women in this population, we expect the effect to be less influential because the serum samples were collected early in pregnancy in this study.

An overall downward trend of serum PFAS concentrations over time was observed in both this population and the matched NHANES population. In addition, sampling year is a significant predictor in the adjusted regression analyses, showing a decrease in serum PFAS levels over the study period (2014-2018). This decline is likely due to the voluntary phase-out by the major manufacturer starting from the year 2000, and followed by EPA PFOA Stewardship Program, which reduced emission and product content of PFOA, and Significant New Use Rule (SNUR), which required a notice and a review before manufacturing, selling, importing, and using long-chain PFAS (EPA, 2020; Land et al., 2018). Previous studies indicated that serum PFAS concentrations among the U.S. population have been declining since the early 2000s, coinciding with the timing of the phase-out (Calafat, Wong, et al., 2007; Kato et al., 2011; Spliethoff et al., 2008).

Higher PFHxS concentrations were found in this population than in NHANES. A study proposed that PFHxS could be a tracer of exposure for PFAS in consumer products rather than the other exposure sources such as seafood consumption (Hu et al., 2018), suggesting consumer products could be important PFAS exposure sources in this population.

The positive correlations observed among most of PFAS suggested similar exposure sources or pathways. However, PFPeA, a short-chain PFAS (≤7 carbons for perfluoroalkyl carboxylic acids (PFCA); ≤5 carbons for perfluoroalkane sulfonic acids (PFSA)) with five carbon atoms (ITRC, 2020), was negatively correlated with the other PFAS such as PFHxS, PFOS, PFOA, PFNA, and PFDA, suggesting the possibility of long-chain PFAS substituted by short-chain alternatives. Moreover, an upward trend of PFPeA concentrations by sampling year was found (Table 2-5), whereas downward trends were shown for most of the long-chain PFAS. Previous studies showed that manufacturers have adopted short-chain PFAS and the other

fluorinated alternatives for the consumer products due to the phase-out of long-chain PFAS (Ateia et al., 2019; Valsecchi et al., 2017). Despite limited environmental and biomonitoring data on PFPeA, several studies have detected PFPeA in precipitation, surface water (Gewurtz et al., 2019), drinking water source (Sun et al., 2016), indoor dust and air (Fraser et al., 2013; Haug et al., 2011; I. Ericson et al., 2012), consumer products (Ministry of Environment and Food of Denmark, 2018), and PFPeA has been the dominant PFAS in influent and effluent water of wastewater treatment plants in some areas during the 2010s (Arvaniti et al., 2012; Zhang et al., 2015). Despite the shorter biological persistence, several concerns about the application of short-chain PFAS were reported by the previous studies. First, short-chain PFAS are lower in technical performance than long-chain PFAS; thus, larger quantities of short-chain PFAS are required in products to reach a similar performance as long-chain PFAS (Lindstrom et al., 2011). Second, the toxicity and the effects on human health remain largely unknown. Third, shorter-chain PFAS could cross the placenta more efficiently than longer-chain PFAS, which has raised more concerns on the exposure among women in pregnancy (Chen et al., 2017; Needham et al., 2011; Zhang et al., 2013). These concerns underscore the need for future research on exposure distribution of PFPeA or its precursor and their potential health effects.

Higher SES has been linked to higher PFAS exposure in previous studies (Buekers et al., 2018; Nelson et al., 2012). A possible explanation is that the people who are wealthier or more highly educated have different lifestyles (e.g. purchasing more PFAS-containing products) and dietary habits (Herzke et al., 2012; Kato et al., 2011). In agreement with these results, we found higher PFAS concentrations in the higher education groups (Table S2-3), and PFNA levels were significantly higher among the participants with college education and above than those with less than a high school education in the adjusted regression models (Table S2-4). Although some studies indicated that family income was a significant or a stronger predictor among the SES-related factors (Buekers et al., 2018; Kato et al., 2014; Nelson et al., 2012; Sagiv et al., 2015; Tyrrell et al., 2013), we found mostly positive but non-significant associations between poverty income ratio and serum PFAS levels. The different results could be due to the different ranges of income

levels among these populations, or the relationship between income and the behaviors linking to PFAS exposure.

Parous women had significantly lower PFHxS and PFOA concentrations in the adjusted models, and decreased GMs of PFAS were mostly shown among the women with more parity, which is consistent with previous findings (Berg et al., 2014; Brantsæter et al., 2013; Kato et al., 2014; Kingsley et al., 2018; Lauritzen et al., 2016; Manzano-Salgado et al., 2016; Sagiv et al., 2015). These results provide evidence of fetal transfer from the mother to her offspring through the placenta or breastfeeding (Kim et al., 2011). BMI was negatively associated with serum PFHxS, PFOS, and PFUnDA, in agreement with some previous findings (Berg et al., 2014; Kato et al., 2014). However, mixed results were found in the literature (Brantsæter et al., 2013; Hölzer et al., 2008; Ji et al., 2012; Jürgen et al., 2008; Rylander et al., 2009; Rylander et al. 2010; Sagiv et al., 2015). In contrast with lipophilic persistent organic pollutants, which are mainly stored in lipid-rich tissues, PFAS are distributed to protein-rich compartments such as the liver, kidneys, and blood; thus, dilution effects due to the amount of adipose tissue might be less significant for PFAS than more lipophilic compounds (The Agency for Toxic Substances and Disease Registry, 2018).

Higher PFAS concentrations were found among the women not using tobacco and the women using marijuana during pregnancy in this study. Previous studies have inconsistently reported the association between smoking status and PFAS (Brantsæter et al., 2013; Fei et al., 2007; Kato et al., 2014; Park et al., 2019; Sagiv et al., 2015), but only limited studies have reported the association between marijuana use and PFAS. In the Health Outcomes and Measures of the Environment (HOME) study, women who reported using marijuana during pregnancy had lower PFOS and PFHxS concentrations than non-users (Vuong et al., 2018). The reasons for these different findings remain unknown, but might be explained by different lifestyles, behaviors, or metabolism between tobacco/marijuana users and non-users (Eriksen et al., 2011; Lauritzen et al., 2016).

Serum PFAS concentrations were not associated with age in this study. Unlike the serum concentrations of lipophilic persistent pollutants which often increase when people aged, serum PFAS concentrations have

shown inconsistent associations with age (Kato et al., 2011; Kato et al., 2014; Manzano-Salgado et al., 2016; Olsen et al., 2003; Rylander et al., 2009). Additionally, the associations between serum PFAS and age were often driven by the greater serum levels in older or younger individuals (Olsen et al., 2017); thus, little changes in PFAS by age were found in this study. The mixed results can also be explained by chemical persistence, and time elapsed after the peak emission (Quinn & Wania, 2012).

We observed that age of house is a significant predictor of serum PFOA and PFNA concentrations; for the other PFAS, although no significant result was found, the GMs of PFHxS and PFOS decreased when the age of house increased. Similar results were presented in previous studies, which showed that PFAS concentrations in indoor air or dust were negatively correlated with age of the residence. This finding can be explained by the difference in building construction and materials, and also the how long the materials have been used (Haug et al., 2011; Kubwabo et al., 2005).

Bottled water as a primary drinking water source was also associated with higher serum PFOS and PFOA concentrations. The result is somewhat unexpected since most studies have shown that the concentrations of PFAS in tap water were higher than in bottled water (Domingo & Nadal, 2019; Kaboré et al., 2018; Ünlü Endirlik et al., 2019), expect for contamination events (Department of Public Health, Bureau of Environmental Health, 2019). An increased PFAS level was shown among reproductive-aged women who mainly consumed tap water than those who mainly consumed bottled water in a highly polluted area in China (Zhou et al., 2019). It is possible that the effect was attributable to residual SES confounding rather than bottled water consumption since no dose-response relationship was found between PFAS levels and the amount of bottled water consumption.

Although positive associations were mostly found between serum PFAS levels and uses of the cosmetics and personal care products, only foundation use was significantly associated with higher PFOA and PFNA. The occurrence of PFAS in cosmetic and personal care products is not well characterized; however, some studies have detected PFAS in various products. For example, PFCAs were found in >86% of the cosmetic products, and >88% of the sunscreen samples in a Japanese study (Fujii et al., 2013). Samples taken from

individuals who use of foundations displayed the highest detection frequencies and concentrations of PFAS among the other personal care products such as hair sprays, body lotions, cream, and powders in Denmark and Sweden (Ministry of Environment and Food of Denmark, 2018; Schultes et al., 2018). The sources of PFCAs in consumer products are postulated to be degradates or the results of biotransformation of polyfluorinated phosphate ester, which could either be an active ingredient or an impurity in cosmetic and personal care products (Fujii et al., 2013; Schultes et al., 2018). Due to higher PFAS concentrations among foundation users in this study and highly detected PFAS in the products, future study is needed to understand whether the use of cosmetic and personal care products, especially foundation, could be an important exposure source to PFAS among pregnant women.

Our study is limited in several ways. First, the questionnaires were not specially designed to evaluate predictors of PFAS exposure, which could limit our ability to identify the associations. For example, no information on the amount or frequency of cosmetic use was collected in the questionnaire; thus, we could not verify if serum PFAS concentrations increase as dose or frequency of foundation use increases. Second, most information was collected at the same time point in the early pregnancy in a cross-sectional study design, with home environment and behavior predictors collected in the mid-pregnancy, which suggests a potential of reverse causality in this study. Third, due to a wide list of predictors being examined, some of the findings might be due to chance. Fourth, diets were shown to be important predictors for serum PFAS (Berg et al., 2014; Domingo & Nadal, 2017; Jain, 2014; Wu & Kannan, 2019); however, we did not include the diet-related predictors for PFAS in this study. Fifth, the relatively small sample size (n = 130) for home environment and behavior predictors could reduce the statistical power, and generalizability in this study. Finally, the relatively low detection frequencies for some PFAS have limited our ability to investigate their association with the predictors.

Conclusions

In summary, our results indicate that PFAS are ubiquitous in the serum of pregnant African American women in Atlanta, Georgia. Characterizing PFAS exposure is important because the health burden and

disparity found in this underserved and underrepresented population may be partly attributable to PFAS exposure, particularly among pregnant women and their fetuses when exposures occur at a relevant time window. We found higher serum PFHxS concentrations among the studied population than NHANES. Serum levels of a short-chain PFAS, PFPeA, increased by year in this population suggesting that PFPeA or a precursor might be a substitute to the long-chain PFAS which have been phased out. Future studies should consider measuring levels of shorter-chain PFAS and their precursors and investigating the associated health effects. Furthermore, we identified several important predictors for serum PFAS concentrations in this population, including parity, BMI, education, tobacco, and marijuana use, age of house, drinking water source, and cosmetic product use. This information can provide data for public health sectors to lower exposure or improve individual and population health by mitigating relevant exposure pathways.

Figures and Tables

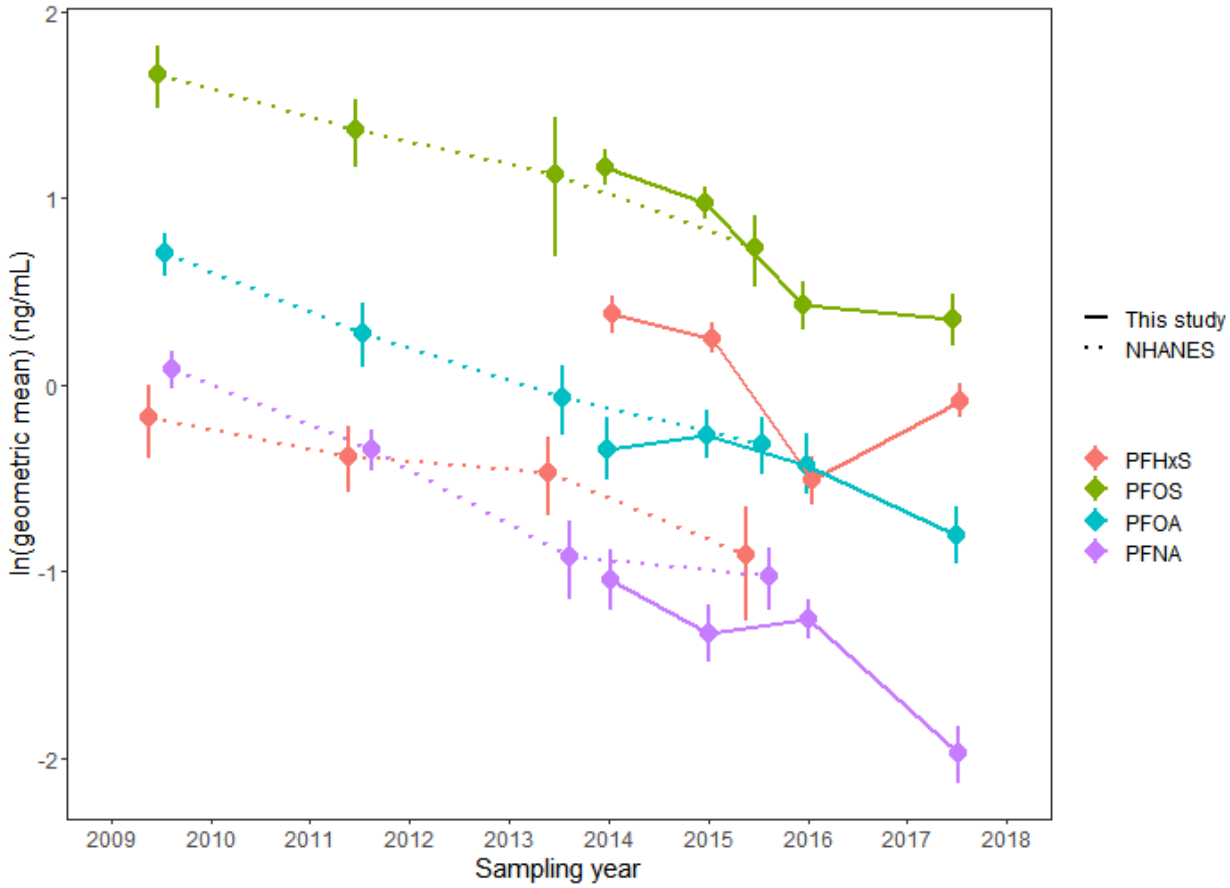

Figure 2-1. PFAS geometric means and 95% confidence interval (ng/mL) among non-Hispanic black females aged 18-40 years in 2009-2016 NHANES and in a cohort of pregnant African American women in the Atlanta area (n=453).

(Note: NHNAES = National Health and Nutrition Examination Survey; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid)

Table 2-1. Demographic characteristics of the participants in pregnant African American women in the Atlanta area, 2014-2018 (n = 453).

Characteristics	n (%)	Characteristics	n (%)
Maternal age at enrollment (years)		**Parity (#)**	
Mean (SD)	24.8 (4.67)	0	216 (47.7%)
Median [Min, Max]	24.0 [18.0, 40.0]	1-2	192 (42.4%)
Education		3-4	45 (9.9%)
Less than high school	72 (15.9%)	**BMI (kg/m^2)**	
High school	172 (38.0%)	< 18.5	16 (3.5%)
Some college	137 (30.2%)	18.5-24.9	176 (38.9%)
College and above	72 (15.9%)	25-29.9	96 (21.2%)
Poverty income ratio (%)		≥ 30	165 (36.4%)
< 100	189 (41.7%)	**Tobacco use**	
100-150	74 (16.3%)	During pregnancy	63 (13.9%)
≥ 150	128 (28.3%)	Not during pregnancy	384 (84.8%)
Missing	62 (13.7%)	Missing	6 (1.3%)
Married/Cohabiting		**Alcohol use**	
Yes	215 (47.5%)	During pregnancy	37 (8.2%)
No	238 (52.5%)	Not during pregnancy	410 (90.5%)
Insurance		Missing	6 (1.3%)
Private	98 (21.6%)	**Marijuana use**	
Medicaid	355 (78.4%)	During pregnancy	99 (21.9%)
Hospital		Not during pregnancy	347 (76.6%)
Emory	179 (39.5%)	Missing	7 (1.5%)
Grady	274 (60.5%)	**Parity (#)**	
Sampling year		0	216 (47.7%)
2014	93 (20.5%)	1-2	192 (42.4%)
2015	124 (27.4%)	3-4	45 (9.9%)
2016	115 (25.4%)		
2017-2018	121 (26.7%)		

Note: SD = standard deviation.

Table 2-2. Summary statistics for PFAS concentrations (ng/mL) measured in pregnant African American women in the Atlanta area, 2014-2018.

	# C	n	% > LOD	GM[a]	GSD[a]	P25	P50	P75	P95	Max
Perfluoroalkane sulfonic acids (PFSAs)										
PFBS	4	356	14	--	--	<LOD	<LOD	<LOD	0.12	0.59
PFHxS	6	453	97	0.99	1.93	0.73	1.10	1.52	2.32	4.80
PFOS	8	453	99	2.03	2.07	1.43	2.17	3.22	5.31	12.4
Perfluoroalkane sulfonamides (FASAs)										
PFOSA	8	356	0	--	--	<LOD	<LOD	<LOD	<LOD	<LOD
NMeFOSAA	11	356	49	--	--	<LOD	<LOD	0.07	0.26	1.46
NEtFOSAA	12	356	5	--	--	<LOD	<LOD	<LOD	0.02	0.11
Perfluoroalkyl carboxylic acids (PFCAs)										
PFPeA	5	356	48	--	--	<LOD	<LOD	0.11	0.22	0.66
PFHxA	6	356	3	--	--	<LOD	<LOD	<LOD	<LOD	0.26
PFHpA	7	356	14	--	--	<LOD	<LOD	<LOD	0.10	0.31
PFOA	8	453	97	0.63	2.35	0.45	0.71	1.07	1.69	4.42
PFNA	9	453	97	0.24	2.35	0.15	0.27	0.42	0.74	2.27
PFDA	10	356	48	--	--	<LOD	<LOD	0.13	0.27	1.06
PFUnDA	11	356	43	--	--	<LOD	<LOD	0.06	0.17	0.53
PFDoA	12	356	2	--	--	<LOD	<LOD	<LOD	<LOD	0.11

Note: #C = number of carbon atoms; n = sample number; LOD = limit of detection; GM= geometric mean; GSD= geometric standard deviation; P25 = the 25th percentile; PFBS = perfluorobutane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOSA = perfluorooctane sulfonamide; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; NEtFOSAA = n-ethyl perfluorooctane sulfonamido acetic acid; PFPeA = perfluoropentanoic acid; PFHxA = Perfluorohexanoic acid; PFHpA = perfluoroheptanoic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFDoA = perfluorododecanoic acid.

[a] Geometric means and standard deviations were not calculated for congeners with detection frequencies < 50%; the values below LODs were imputed with LOD/$\sqrt{2}$.

Table 2-3. Pearson correlation coefficients of natural log-transformed serum PFAS concentrations in pregnant African American women in the Atlanta area, 2014-2018 (n = 453 & 356).

	PFHxS[a]	PFOS[a]	NMeFOSAA[b]	PFPeA[b]	PFOA[a]	PFNA[a]	PFDA[b]	PFUnDA[b]
PFHxS	1.00	0.66**	0.08	-0.25**	0.44**	0.39**	0.44**	0.10
PFOS		1.00	0.15*	-0.31**	0.65**	0.70**	0.55**	0.22*
NMeFOSAA			1.00	-0.06	0.07	0.13*	0.14*	-0.16*
PFPeA				1.00	-0.14*	-0.22**	-0.33**	0.04
PFOA					1.00	0.71**	0.35**	0.17*
PFNA						1.00	0.50**	0.29**
PFDA							1.00	0.11
PFUnDA								1.00

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid.

[a] The values below LODs were imputed with LOD/$\sqrt{2}$; n = 453.

[b] The values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation; n = 356.

* *p*-value < 0.05; ** *p*-value < 0.01.

Table 2-4. Adjusted percent change in natural log-transformed serum PFHxS, PFOS, PFOA, and PFNA by sociodemographic predictors in pregnant African American women in the Atlanta area, 2014-2018 (n = 453).

Predictor[a]	PFHxS	PFOS	PFOA	PFNA
	% difference in PFAS concentration (95% CI)[b,c,d]			
Age (years)	-0.3 (-1.6, 1.1)	-0.2 (-1.8, 1.3)	-0.4 (-2.4, 1.7)	-0.3 (-2.2, 1.6)
Education (Ref = Less than high school)				
High school	4.9 (-10.2, 22.6)	-0.2 (-16.4, 19.2)	6.5 (-15.2, 33.8)	1.2 (-18.4, 25.4)
Some college	5.0 (-11.6, 24.7)	2.1 (-16.1, 24.4)	5.9 (-17.8, 36.4)	5.7 (-16.8, 34.1)
College and above	9.5 (-13.2, 38.2)	25.8 (-3.5, 64.0)	35.7 (-3.5, 90.9)	**57.8 (14.4, 118)****
Poverty income ratio (%) (Ref < 100)				
100-150	8.4 (-6.2, 25.3)	17 (-1.1, 38.3)	19.2 (-3.9, 47.9)	15 (-5.8, 40.5)
≥ 150	14.4 (-2.0, 33.6)	13.8 (-4.2, 35.2)	15.4 (-7.6, 44.2)	13.3 (-8.4, 40.0)
Married or cohabitating (Ref = No)				
Yes	2.5 (-7.8, 14.0)	2.3 (-9.4, 15.6)	6.5 (-8.9, 24.5)	10.7 (-4.4, 28.3)
Insurance (Ref = Private)				
Medicaid	4.6 (-12.8, 25.4)	4.4 (-15.1, 28.4)	-2.6 (-25.4, 27.2)	4.6 (-18.7, 34.5)
Hospital (Ref = Emory)				
Grady	4.2 (-9.8, 20.3)	-0.7 (-15.8, 17.1)	16.4 (-5.7, 43.8)	-0.8 (-18.7, 21.1)
Sampling year (Ref = 2014)				
2015	-9.4 (-22.1, 5.4)	-12.8 (-26.7, 3.6)	12.4 (-10.0, 40.4)	**-20.6 (-35.6, -2.1)***
2016	**-57.6 (-63.7, -50.4)****	**-50.2 (-58.3, -40.5)****	-4.4 (-23.9, 20.1)	-15.1 (-31.5, 5.3)
2017-2018	**-35.5 (-44.7, -24.9)****	**-54.4 (-61.7, -45.7)****	**-37.4 (-50.0, -21.7)****	**-60.3 (-67.8, -51.0)****
Parity (#) (Ref = 0)				
1-2	-10.5 (-20.3, 0.6)	-6.7 (-18.3, 6.5)	**-19.6 (-32.2, -4.6)***	-10.2 (-23.5, 5.5)
3-4	**-19.1 (-33.2, -2.0)***	-6.8 (-25.1, 16.1)	**-25.8 (-44.0, -1.7)***	-11.4 (-32.1, 15.5)
BMI (kg/m^2)	**-0.9 (-1.6, -0.3)****	**-1.2 (-2.0, -0.5)****	-0.3 (-1.3, 0.6)	-0.4 (-1.3, 0.5)
Tobacco use (Ref = Not during pregnancy)				
During pregnancy	**-22.9 (-34.9, -8.7)****	**-25.2 (-38.4, -9.2)****	-18.9 (-36.6, 3.8)	-18.1 (-35.1, 3.3)
Alcohol use (Ref = Not during pregnancy)				
During pregnancy	5.9 (-12.9, 28.6)	20.8 (-3.3, 50.8)	25.8 (-5.4, 67.5)	19.4 (-8.8, 56.3)
Marijuana use (Ref = Not during pregnancy)				
During pregnancy	9.8 (-4.4, 26.1)	**21.8 (3.9, 42.7)***	**38.8 (13.3, 70.0)****	**42.4 (17.6, 72.4)****

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; CI = confidence interval.

[a] The missing values were multiply imputed by chained equations with logistic and polytomous logistic regression imputation approaches.

[b] Percent change in PFAS concentrations associated with each predictor by exponentiating regression coefficients, subtracting 1, and multiplying by 100%.

[c] The models were mutually adjusted for all the variables listed above.

[d] The values below LODs were imputed with LOD/$\sqrt{2}$.

* *p*-value < 0.05; ***p*-value < 0.01.

Table 2-5. Adjusted percent change in natural log-transformed serum NMeFOSAA, PFPeA, PFDA, and PFUnDA by sociodemographic predictors in pregnant African American women in the Atlanta area, 2014-2018 (n = 356).

	NMeFOSAA	PFPeA	PFDA	PFUnDA
Predictor[a]	**% difference in PFAS concentration (95% CI)**[b,c,d]			
Age (years)	-1.0 (-6.1, 4.3)	0.8 (-2.1, 3.8)	-1.0 (-4.5, 2.6)	3.1 (-1.5, 7.8)
Education (Ref = Less than high school)				
High school	-26.0 (-58.0, 30.4)	5.8 (-24.0, 47.3)	13.0 (-23.3, 66.6)	0.5 (-40.2, 69.0)
Some college	-44.4 (-69.4, 0.9)	-2.4 (-31.2, 38.4)	19.5 (-23.7, 87.2)	-2.5 (-46.8, 78.6)
College and above	-17.7 (-63.1, 83.5)	-20.6 (-51.2, 29.1)	47.9 (-19.5, 172)	78.1 (-15.0, 273)
Poverty income ratio (%) (Ref < 100)				
100-150	2.1 (-38.7, 70.0)	4.8 (-24.0, 44.6)	-0.9 (-33.9, 48.6)	42.2 (-13.9, 135)
≥ 150	-21.0 (-54.4, 37.0)	-7.7 (-32.4, 26.0)	5.7 (-26.5, 52.1)	12.3 (-30.4, 81.2)
Married or cohabitating (Ref = No)				
Yes	-14.0 (-40.2, 23.7)	-0.1 (-22.3, 28.5)	5 (-20.3, 38.3)	14.9 (-18.7, 62.2)
Insurance (Ref = Private)				
Medicaid	14.3 (-39.8, 117)	-9.5 (-40.1, 36.8)	-5.9 (-41.6, 51.4)	-19.1 (-54.2, 42.9)
Hospital (Ref = Emory)				
Grady	38.0 (-17.5, 131)	3.3 (-23.9, 40.0)	5.0 (-26.1, 49.3)	-25.2 (-53.8, 21.3)
Sampling year (Ref = 2014)				
2015	**-62.8 (-76.9, -40.1)****	**43 (2.3, 100)***	**-42.9 (-59.5, -19.5)****	**64.6 (5.4, 157)***
2016	**-63.6 (-84.0, -17.3)***	59 (-3.0, 161)	**-87.2 (-93.2, -75.9)****	**110.2 (2.0, 333)***
2017-2018	**-59.4 (-74.4, -35.6)****	**208.8 (119, 337)****	**-89.7 (-92.8, -85.4)****	43.8 (-6.9, 122)
Parity (#) (Ref = 0)				
1-2	-3.8 (-35.9, 44.5)	1.1 (-21.2, 29.8)	-14.1 (-34.9, 13.4)	-12.5 (-42.1, 32.1)
3-4	-9.6 (-54.0, 77.5)	13.9 (-26.3, 76.2)	-22.8 (-53.5, 28.0)	-20 (-47.5, 82.8)
BMI (kg/m^2)	0.1 (-2.1, 2.4)	-0.9 (-2.3, 0.4)	-0.6 (-2.2, 1.1)	**-2.4 (-4.5, -0.3)***
Tobacco use (Ref = Not during pregnancy)				
During pregnancy	20.6 (-34.1, 121)	34.9 (-8.5, 98.9)	-18.5 (-50.6, 34.5)	-26.1 (-58.0, 29.9)
Alcohol use (Ref = Not during pregnancy)				
During pregnancy	28.4 (-34.4, 151)	-32.8 (-57.8, 7.1)	1.6 (-37.9, 66.1)	79.1 (-5.8, 240)
Marijuana use (Ref = Not during pregnancy)				
During pregnancy	41.9 (-11.4, 127)	-8.6 (-33.4, 25.3)	20.9 (-17.8, 77.9)	23.4 (-21.5, 93.9)

Note: NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; CI = confidence interval.

[a] The missing values were multiply imputed by chained equations with logistic and polytomous logistic regression imputation approaches.

[b] Percent change in PFAS concentrations associated with each predictor by exponentiating regression coefficients, subtracting 1, and multiplying by 100%.

[c] The models were mutually adjusted for all the variables listed above.

[d] PFAS values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation.

* *p*-value < 0.05; ***p*-value < 0.01.

Table 2-6. Adjusted percent change in natural log-transformed serum PFHxS, PFOS, PFOA, and PFNA by home environment and behavioral predictors among pregnant African American women in Atlanta area, 2014-2018 (n = 130).

Predictor[a]	PFHxS	PFOS	PFOA	PFNA
	% difference in PFAS concentration (95% CI)[b,c,d]			
Age of house (years) (Ref < 10)				
10-20	-1.4 (-31.4, 41.6)	-13.5 (-40.9, 26.5)	-14.3 (-45.4, 34.5)	0.8 (-34.3, 54.4)
≥ 20	9.1 (-20.2, 49.1)	-25.1 (-45.0, 2.0)	**-31.9 (-53.4, -0.3)***	**-38.5 (-57.1, -11.8)****
Distance to nearest industrial plant, dump, or waste site (meters) (Ref < 400)				
≥ 400	3.4 (-29.7, 52.1)	2.2 (-26.2, 41.6)	1.1 (-33.4, 53.6)	0.6 (-41.6, 73.2)
Frequency of floor cleaning (Ref = Daily)				
A few times a week	5.3 (-19.2, 37.1)	-2.7 (-25.5, 27.0)	1.0 (-27.9, 41.4)	-8.5 (-34.0, 27.0)
Once every couple weeks or less	-0.5 (-31.5, 44.6)	4.7 (-28.2, 52.6)	-6.6 (-42, 50.1)	11.5 (-29.8, 77.1)
Primary drinking water source - tap water (Ref = No)				
Yes	1.9 (-20.3, 30.3)	5.1 (-18.0, 34.8)	11.8 (-18.2, 52.9)	-1.9 (-27.8, 33.2)
Primary drinking water source - bottled water (Ref = No)				
Yes	34.5 (-8.0, 96.8)	**64.6 (12.9, 140)***	**88.3 (17.1, 203)***	57.2 (-1.8, 152)
Tap water consumption in the last 48 hours (8 oz cup) (Ref = None)				
1-5	-9.8 (-31.2, 18.3)	0.7 (-23.4, 32.3)	22.2 (-13.6, 72.6)	28.0 (-8.4, 78.8)
≥ 6	15.4 (-18.7, 63.8)	28.2 (-10.0, 82.5)	19.3 (-23.8, 86.6)	35.7 (-12.1, 109)
Bottled water consumption in the last 48 hours (8 oz cup) (Ref = None)				
1-5	15.4 (-17.6, 61.5)	31.6 (-6.0, 84.4)	12.1 (-27.0, 72.1)	9.2 (-28.2, 66.1)
≥ 6	13.4 (-23.9, 69.1)	30.5 (-12.4, 94.4)	23.7 (-25.5, 105)	2.3 (-37.7, 67.9)
Takeout consumption in the last month (times) (Ref = None)				
1-2	14.0 (-16.5, 55.5)	-1.1 (-27.6, 35.1)	-19.6 (-45.8, 19.3)	-12.6 (-40.4, 28.3)
≥ 3	11.6 (-19.0, 53.6)	-16.1 (-39.2, 15.7)	-13.2 (-42.2, 30.3)	-26.3 (-50.3, 9.4)
Microwave popcorn consumption in the last month (times) (Ref = None)				
1-2	4.2 (-22.5, 40.1)	2.1 (-24.3, 37.8)	-11.7 (-39.5, 28.8)	-11.4 (-38.6, 27.6)
≥ 3	-14.7 (-44.6, 31.4)	3.6 (-33.1, 60.5)	1.6 (-41.4, 76.1)	40.7 (-17.4, 140)
Frequency of cosmetic product use (Ref = Never or Occasionally)				
Daily	-0.3 (-22.8, 28.7)	2.8 (-20.8, 33.5)	-2.0 (-29.2, 35.5)	-6.2 (-31.6, 28.7)
Number of cosmetic products usually worn (#) (Ref = 0)				
1-3	5.3 (-22.4, 43.0)	22.5 (-10.0, 66.7)	14 (-22.4, 67.5)	9.5 (-24.7, 59.3)
4-6	-8.6 (-34.4, 27.4)	18.2 (-15.3, 65.0)	49.1 (-1.8, 126)	**50.4 (0.2, 126)***
Usually worn foundation (Ref = No)				
Yes	-19.8 (-40.3, 7.5)	-3.0 (-28.1, 30.8)	**52.2 (5.2, 120)***	**58.3 (10.5, 127)***
Usually worn rouge or blush (Ref = No)				
Yes	26 (-13.4, 83.2)	14.2 (-21.8, 67.0)	18.7 (-26.4, 91.6)	31.6 (-17.4, 110)
Usually worn lipstick (Ref = No)				
Yes	-1.2 (-24.0, 28.5)	8.0 (-17.2, 40.7)	36.5 (-1.9, 89.8)	36.8 (-0.9, 88.7)
Usually worn mascara (Ref = No)				
Yes	-22.3 (-39.6, 0.0)	7.6 (-17.0, 39.3)	24.3 (-10.1, 71.9)	24.9 (-9.0, 71.3)
Usually worn nail polish (Ref = No)				
Yes	15.2 (-10.7, 48.5)	21.4 (-6.0, 56.8)	27.5 (-7.6, 76.0)	17.9 (-14.1, 61.8)
Usually worn eye shadow (Ref = No)				
Yes	-13.8 (-33.3, 11.5)	-3.3 (-25.5, 25.4)	10.8 (-20.2, 53.7)	19.6 (-13.0, 64.6)
Frequency of lotion use (Ref = Never or Occasionally)				
Daily	-11 (-31.7, 16.0)	-17.1 (-36.5, 8.2)	-15.1 (-39.9, 19.8)	-7.3 (-33.4, 29.1)
Frequency of hair products use (Ref = Never)				
Occasionally or Monthly	18.8 (-20.2, 76.9)	9.9 (-26.5, 64.3)	44.3 (-12.8, 139)	35.3 (-17.4, 122)

Predictor[a]	PFHxS	PFOS	PFOA	PFNA
	% difference in PFAS concentration (95% CI)[b,c,d]			
Weekly or Daily	12.5 (-24.0, 66.7)	13.1 (-24.0, 68.2)	28.6 (-21.8, 112)	25.3 (-23.0, 104)
Frequency of shampoo (Ref = Monthly)				
Biweekly or more often	8.1 (-15.2, 37.9)	21.1 (-5.1, 54.4)	22.3 (-10.1, 66.5)	25.7 (-6.8, 69.7)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; CI = confidence interval.

[a] The missing values were multiply imputed by chained equations with logistic and polytomous logistic regression imputation approaches.

[b] Percent change in PFAS concentrations associated with each predictor by exponentiating regression coefficients, subtracting 1, and multiplying by 100%.

[c] The models were adjusted for education, parity, BMI, tobacco use, and marijuana use.

[d] PFAS values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation.

* *p*-value < 0.05; ***p*-value < 0.01.

Supplementary

Table S2-1. Chemical characteristics of PFAS.

Chemical	Abbr.	# of carbon	Molecular formula	CAS No.	Limit of detection (ng/mL)
Perfluoroalkane sulfonic acids (PFSAs)					
Perfluorobutane sulfonic acid	PFBS	4	$C_4HF_9O_3S$	375-73-5	0.02 (W)
Perfluorohexane sulfonic acid	PFHxS	6	$C_6HF_{13}O_3S$	355-46-4	0.02 (W); 0.2 (E)
Perfluorooctane sulfonic acid	PFOS	8	$C_8HF_{17}O_3S$	1763-23-1	0.02 (W); 0.05 (E)
Perfluoroalkane sulfonamides (FASAs)					
Perfluorooctane sulfonamide	PFOSA	8	$C_8H_2F_{17}NO_2S$	754-91-6	0.02 (W)
N-methyl perfluorooctane sulfonamido acetic acid	NMeFOSAA	11	$C_{11}H_6F_{17}NO_4S$	2355-31-9	0.02 (W)
N-ethyl perfluorooctane sulfonamido acetic acid	NEtFOSAA	12	$C_{12}H_8F_{17}NO_4S$	2991-50-6	0.02 (W)
Perfluoroalkyl carboxylic acids (PFCAs)					
Perfluoropentanoic acid	PFPeA	5	$C_5HF_9O_2$	2706-90-3	0.05 (W)
Perfluorohexanoic acid	PFHxA	6	$C_6HF_{11}O_2$	307-24-4	0.05 (W)
Perfluoroheptanoic acid	PFHpA	7	$C_7HF_{13}O_2$	375-85-9	0.05 (W)
Perfluorooctanoic acid	PFOA	8	$C_8HF_{15}O_2$	335-67-1	0.035 (W); 0.2 (E)
Perfluorononanoic acid	PFNA	9	$C_9HF_{17}O_2$	375-95-1	0.02 (W); 0.1 (E)
Perfluorodecanoic acid	PFDA	10	$C_{10}HF_{19}O_2$	335-76-2	0.035 (W)
Perfluoroundecanoic acid	PFUnDA	11	$C_{11}HF_{21}O_2$	2058-94-8	0.02 (W)
Perfluorododecanoic acid	PFDoA	12	$C_{12}HF_{23}O_2$	307-55-1	0.035 (W)

Note: Abbr. = abbreviation ; # of carbon = number of carbon atoms; W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.

Table S2-2. Overlapped samples concentrations (ng/mL) from the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University and Wadsworth Center/New York University Laboratory Hub.

	PFHxS		PFOS		PFOA		PFNA	
Sample ID	**W**[b]	**E**	**W**[b]	**E**	**W**[b]	**E**	**W**[b]	**E**
1	1.515	1.398	3.506	3.735	1.305	1.299	0.435	0.550
2	0.710	0.587	1.719	1.021	0.492	0.425	0.868	0.811
3	1.055	1.283	1.392	1.215	0.677	0.583	0.372	0.351
4	0.679	0.565	1.733	1.967	0.228	0.376	0.091	0.214
5	0.794	0.601	1.469	1.393	0.848	1.114	0.259	0.419
6	0.999	0.606	5.145	4.509	0.734	0.900	0.197	0.357
7	0.831	0.835	2.478	2.388	0.624	0.566	0.264	0.337
8	1.534	1.645	2.325	1.670	1.013	1.133	0.285	0.401
9	1.332	0.948	2.882	2.559	1.315	1.870	0.402	0.553
10	1.830	1.264	3.965	4.430	1.289	1.845	0.632	0.876
11	1.850	1.835	2.597	2.269	1.350	2.100	0.231	0.410
Pearson correlation coefficients	**0.88**		**0.93**		**0.92**		**0.93**	
Relative percent differences, %RPD; Median (Min-Max)[a]	**4.75 (0.12-12.3)**		**3.17 (0.92-12.7)**		**5.05 (0.12-12.3)**		**8.11 (1.48-20.2)**	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.

[a] %RPD = (Lab 1 result – Lab 2 result)*100/(Lab 1 result + Lab 2 result)/2.

[b]The results from Wadsworth were used in the main analysis for these overlapped samples.

Table S2-3. The geometric means and standard deviations of serum PFASs (ng/mL) by different predictors in pregnant African American women in the Atlanta area, 2014-2018.

			PFHxS			PFOS			PFOA			PFNA		
	n[a]	%[a]	GM[b]	GSD[b]	*p*[c]	GM[b]	GSD[b]	*p*[c]	GM[b]	GSD[b]	*p*[c]	GM[b]	GSD[b]	*p*[c]
Personal characteristics (n = 453)														
Age (years)														
18-25	251	55.4	1.04	1.92	0.11	2.04	1.89	0.57	0.64	2.30	0.87	0.23	2.26	0.62
25-30	117	25.8	1.00	1.88		2.12	2.31		0.61	2.28		0.24	2.34	
30-40	85	18.8	0.87	1.98		1.90	2.29		0.61	2.62		0.26	2.65	
Education														
Less than high school	72	15.9	0.95	2.11	0.52	1.98	2.06	**0.03**	0.60	2.26	0.46	0.23	2.08	**<0.01**
High school	172	38.0	0.96	1.88		1.84	2.06	**	0.61	2.34		0.22	2.55	**
Some college	137	30.2	1.02	1.86		2.08	1.86		0.62	2.25		0.24	2.05	
College and above	72	15.9	1.08	1.97		2.50	2.43		0.73	2.64		0.33	2.55	
Poverty income ratio (%)														
< 100	189	41.7	0.91	1.99	0.26	1.78	2.22	**0.03**	0.54	2.57	**0.03**	0.21	2.45	**0.01**
100-150	74	16.3	0.97	1.89		2.09	2.10	*	0.67	2.17	*	0.25	2.23	*
≥ 150	128	28.3	1.04	1.98		2.21	1.95		0.70	2.26		0.28	2.36	
Married or cohabitating														
Yes	215	47.5	0.98	1.87	0.70	2.03	1.89	0.97	0.60	2.38	0.31	0.23	2.42	0.35
No	238	52.5	1.01	1.98		2.03	2.24		0.65	2.32		0.25	2.28	
Insurance														
Private	98	21.6	1.07	1.96	0.22	2.34	2.00	**0.03**	0.68	2.25	0.25	0.29	2.38	**0.02**
Medicaid	355	78.4	0.97	1.92		1.95	2.09	*	0.61	2.37		0.23	2.33	*
Hospital														
Emory	179	39.5	1.02	1.87	0.57	2.20	1.96	0.06	0.60	2.44	0.42	0.26	2.32	0.09
Grady	274	60.5	0.98	1.96		1.93	2.14		0.64	2.28		0.23	2.36	
Sampling year														
2014	93	20.5	1.46	1.62	**<0.01**	3.21	1.59	**<0.01**	0.71	2.43	**<0.01**	0.35	2.14	**<0.01**
2015	124	27.4	1.28	1.58	**	2.66	1.66	**	0.77	2.03	**	0.26	2.30	**
2016	115	25.4	0.60	2.11		1.53	2.14		0.65	2.38		0.29	1.82	
2017-2018	121	26.7	0.92	1.67		1.42	2.15		0.45	2.36		0.14	2.43	
Parity (#)														
0	216	47.7	1.13	1.90	**<0.01**	2.22	2.00	**0.01**	0.74	2.13	**<0.01**	0.26	2.30	**0.04**
1-2	192	42.4	0.91	1.91	**	1.89	1.99	*	0.55	2.47	**	0.22	2.37	*
3-4	45	9.9	0.77	1.93		1.79	2.72		0.50	2.53		0.22	2.46	
BMI (kg/m^2)														
< 18.5	16	3.5	1.07	1.63	**<0.01**	2.71	1.76	**<0.01**	0.85	1.69	0.19	0.30	1.78	0.20
18.5-25	176	38.9	1.10	1.85	**	2.21	1.95	**	0.64	2.15		0.25	2.22	
25-30	96	21.2	1.00	2.10		2.04	2.22		0.62	2.50		0.23	2.90	
≥ 30	165	36.4	0.88	1.89		1.80	2.11		0.60	2.52		0.23	2.22	
Tobacco use														
Not during pregnancy	384	84.8	1.03	1.91	**<0.01**	2.11	2.05	**<0.01**	0.64	2.34	0.30	0.25	2.31	0.11
During pregnancy	63	13.9	0.78	1.97	**	1.59	2.18	**	0.57	2.41		0.21	2.63	
Alcohol use														
Not during pregnancy	410	90.5	0.99	1.95	0.94	2.00	2.11	0.20	0.62	2.38	0.15	0.24	2.38	0.19
During pregnancy	37	8.2	1.00	1.78		2.35	1.79		0.76	1.99		0.29	2.05	

			PFHxS			PFOS			PFOA			PFNA		
	n[a]	%[a]	GM[b]	GSD[b]	*p*[c]	GM[b]	GSD[b]	*p*[c]	GM[b]	GSD[b]	*p*[c]	GM[b]	GSD[b]	*p*[c]
Marijuana use														
Not during pregnancy	347	76.6	1.00	1.94	0.93	2.02	2.17	0.77	0.59	2.49	**0.02**	0.23	2.45	0.08
During pregnancy	99	21.9	0.99	1.86		2.07	1.78		0.75	1.80	*	0.28	2.00	
Home environment and behavioral characteristics (n=130)[d]														
Age of house (years)														
< 10	26	20.0	0.79	1.87	0.88	1.88	1.96	0.11	0.76	2.93	**0.01**	0.30	1.95	**<0.01**
10-20	20	15.4	0.77	2.33		1.65	1.84		0.73	1.91	*	0.27	1.56	**
≥ 20	41	31.5	0.73	2.04		1.32	1.99		0.45	2.19		0.16	2.44	
Don't know	43	33.1	0.82	1.73		1.33	2.01		0.40	2.38		0.15	2.36	
Distance to nearest industrial plant, dump or, waste site (meters)														
<400	11	8.5	0.75	2.11	0.27	1.48	2.61	0.33	0.45	3.58	0.29	0.19	2.25	0.89
≥ 400	27	20.8	0.81	1.90		1.63	2.11		0.59	2.49		0.22	2.45	
Don't know	32	24.6	0.65	2.17		1.35	1.77		0.68	1.97		0.22	2.52	
Primary cleaner at home														
Yes	114	87.7	0.78	1.92	0.90	1.43	1.91	0.24	0.51	2.43	0.39	0.18	2.37	0.22
No	16	12.3	0.76	2.11		1.78	2.50		0.62	2.55		0.24	1.76	
Frequency of floor cleaning														
Daily	46	35.4	0.74	2.12	0.77	1.42	2.03	0.86	0.51	2.45	0.94	0.19	2.23	0.68
A few times a week	66	50.8	0.81	1.88		1.48	2.05		0.53	2.46		0.18	2.42	
Once every couple weeks or less	18	13.8	0.76	1.75		1.57	1.71		0.50	2.46		0.22	2.13	
Primary drinking water source - tap water														
Yes	47	36.2	0.76	2.06	0.75	1.49	2.08	0.86	0.53	2.23	0.84	0.18	2.27	0.67
No	83	63.8	0.79	1.88		1.46	1.94		0.51	2.57		0.19	2.34	
Primary drinking water source - bottled water														
Yes	115	88.5	0.80	1.86	0.08	1.56	1.79	**0.01**	0.55	2.29	**0.03**	0.20	2.17	**0.04**
No	15	11.5	0.59	2.49		0.94	3.20	*	0.33	3.38	*	0.13	3.17	*
Tap water consumption in the last 48 hours (8 oz cup)														
None	70	53.8	0.81	1.89	0.75	1.45	1.92	0.67	0.49	2.32	0.54	0.17	2.20	0.28
1-5	38	29.2	0.68	2.06		1.40	1.93		0.57	2.66		0.21	2.34	
≥ 6	21	16.2	0.82	1.86		1.60	2.34		0.53	2.49		0.20	2.63	
Bottled water consumption in the last 48 hours (8 oz cup)														
None	21	16.2	0.66	2.37	0.69	1.21	2.52	0.92	0.49	3.30	0.55	0.18	3.07	0.54
1-5 cups	78	60.0	0.81	1.91		1.57	1.82		0.51	2.33		0.20	2.04	
≥ 6 cups	30	23.1	0.79	1.73		1.37	1.97		0.55	2.23		0.16	2.48	
Takeout food or deliver pizza consumption in the last month (times)														
None	31	23.8	0.71	2.05	0.68	1.57	2.18	0.23	0.62	2.52	0.63	0.22	2.02	0.29
1-2	51	39.2	0.81	1.97		1.56	1.72		0.47	2.40		0.20	2.00	
≥ 3	48	36.9	0.79	1.85		1.32	2.13		0.52	2.44		0.17	2.80	
Microwave popcorn consumption in the last month (times)														
None	91	70.0	0.76	1.88	0.59	1.42	2.02	0.86	0.51	2.41	0.94	0.19	2.31	0.39
1-2	28	21.5	0.84	1.93		1.61	1.90		0.52	2.44		0.18	2.25	
≥ 3	11	8.5	0.72	2.55		1.52	2.03		0.58	2.89		0.26	2.40	
Frequency of cosmetic product use														
Never or Occasionally	75	57.7	0.76	2.03	0.94	1.46	1.87	0.26	0.52	2.50	0.47	0.19	2.22	0.66
Daily	26	20.0	0.79	1.64		1.76	1.97		0.63	2.12		0.22	2.43	
Number of cosmetic products usually worn (#)														
0	29	22.3	1.98	1.26	0.76	2.27	0.44	0.41	2.56	0.16	**0.03**	2.42	6.40	0.05
1-3	61	46.9	1.95	1.54		1.86	0.46		2.27	0.17	*	2.14	13.47	

			PFHxS			PFOS			PFOA			PFNA		
	n[a]	**%[a]**	**GM[b]**	**GSD[b]**	***p*[c]**	**GM[b]**	**GSD[b]**	***p*[c]**	**GM[b]**	**GSD[b]**	***p*[c]**	**GM[b]**	**GSD[b]**	***p*[c]**
4-6	40	30.8	1.91	1.52		1.98	0.71		2.47	0.25		2.38	8.83	
Usually worn foundation														
Yes	30	23.1	0.68	1.98	0.20	1.49	2.01	0.91	0.76	2.55	**0.01**	0.28	2.42	**<0.01**
No	100	76.9	0.81	1.92		1.46	1.98		0.46	2.34	*	0.17	2.21	**
Usually worn rouge or blush														
Yes	16	12.3	0.97	1.52	0.16	1.82	1.93	0.19	0.73	2.14	0.11	0.27	2.58	0.07
No	114	87.7	0.75	1.99		1.42	1.99		0.50	2.47		0.18	2.25	
Usually worn lipstick														
Yes	37	28.5	0.79	1.99	0.86	1.60	1.88	0.37	0.71	2.27	**0.01**	0.25	2.32	**0.03**
No	93	71.5	0.77	1.93		1.42	2.03		0.46	2.45	*	0.17	2.26	*
Usually worn mascara														
Yes	42	32.3	0.67	1.98	0.07	1.55	1.99	0.54	0.62	2.55	0.13	0.22	2.31	0.14
No	88	67.7	0.83	1.90		1.43	1.99		0.48	2.37		0.18	2.29	
Usually worn nail polish														
Yes	88	67.7	0.80	1.86	0.41	1.57	1.82	0.10	0.56	2.32	0.21	0.20	2.31	0.27
No	42	32.3	0.72	2.10		1.27	2.29		0.45	2.69		0.17	2.30	
Usually worn eye shadow														
Yes	38	29.2	0.70	1.97	0.27	1.44	2.00	0.87	0.59	2.44	0.27	0.22	2.25	0.18
No	92	70.8	0.81	1.93		1.48	1.99		0.49	2.44		0.18	2.32	
Frequency of lotion use														
Never or Occasionally	42	32.3	0.83	1.48	0.35	1.71	1.59	0.06	0.54	2.43	0.71	0.21	2.20	0.46
Daily	82	63.1	0.74	2.17		1.33	2.17		0.51	2.49		0.18	2.40	
Frequency of hair products use														
Never	14	10.8	0.62	1.94	0.75	1.23	2.17	0.52	0.38	2.70	0.79	0.14	2.72	0.94
Occasionally or Monthly	47	36.2	0.80	1.85		1.45	1.98		0.56	2.38		0.20	2.18	
Weekly or Daily	67	51.5	0.78	2.01		1.53	1.97		0.53	2.46		0.19	2.34	
Frequency of shampoo														
Monthly	52	40.0	0.72	1.89	0.29	1.28	2.17	0.07	0.43	2.47	0.07	0.16	2.36	0.06
Biweekly or more often	77	59.2	0.81	1.98		1.60	1.85		0.58	2.39		0.21	2.24	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; n = sample number; GM = geometric mean; GSD = geometric standard deviation; *p* = *p*-value.

[a] The sample numbers might not be summed up to the total sample size due to missingness.

[b] The values below LODs were replaced by LOD/$\sqrt{2}$.

[c] *P*-values were calculated using ANOVA to evaluate the difference of natural log-transformed serum levels between groups.

[d] Home environment and behavioral characteristics were only available in n =130 participants.

* *p*-value < 0.05; ***p*-value < 0.01.

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Chapter 3. PFAS and Fetal Growth

Associations between Maternal Serum Per- and Polyfluoroalkyl Substance (PFAS) Concentrations at Early Pregnancy and Fetal Growth Outcomes in the African American Birth Cohort.

Abstract

Exposure to per- and polyfluoroalkyl substance (PFAS) has been linked to reduced fetal growth in previous studies. However, studies focusing on the effect of PFAS, with the exception of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), are limited, and even more so in African American populations, who have experienced higher rates of adverse birth outcomes and higher environmental exposures. This study aimed to evaluate the associations of eight PFAS with fetal growth endpoints in a cohort of pregnant African American women. This study included 426 participants with blood samples for serum PFAS measurements collected at 8-14 weeks of gestations (median 11.4 weeks) and birth outcomes. We performed multivariable linear regression analysis to investigate the effects of PFAS on birth weight, sex-specific z-score for gestational age, head circumference, birth length, ponderal index, and preterm birth. We found that serum PFNA and PFOA concentrations were associated with higher odds of small-for-gestational age (SGA) birth – a 2-fold increase in PFNA concentrations was associated with 1.32 times odds of SGA birth (95%CI 0.9-1.49), and 2.22 (95%CI 1.10-4.50), 2.44 (95%CI 1.21-4.92), 2.23 (95%CI 1.10-4.54) times of odds for the 2nd, 3rd, and 4th PFOA exposure quartiles compared to reference group (1st exposure quartile). Significant or marginal significant associations with ponderal index were observed in PFOS (β = -0.05, 95CI% -0.10, 0.01), PFNA (β = -0.06, 95CI% -0.10, -0.01), and PFDA (β = -0.05, 95CI% -0.09, -0.01). However, the same directionality of the results across multiple fetal growth outcomes were only observed for PFNA and PFOA, and the results of PFOS and PFDA were relatively inconclusive. No association between serum PFAS and preterm delivery was found. Our results suggest that reduced fetal growth was associated with serum PFNA and PFOA concentrations in the African American birth cohort.

Keywords: Per- and polyfluoroalkyl substance (PFAS), fetal growth, birth weight, small-for-gestational age (SGA), ponderal index

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a subgroup of persistent organic chemicals manufactured beginning in the 1940s. Because PFAS exhibit both lipophobic and hydrophobic properties, they were used in various consumer products such as food packaging, cookware, fabrics, carpet, upholstery, and personal care products (Paul et al., 2009; The Agency for Toxic Substances and Disease Registry, 2018). Although the primary manufacturers of PFAS voluntarily phased out the production in the U.S in the early 2000s due to their health concerns (US Environmental Protection Agency, 2018), PFAS have been accumulated and widely detected in environment and organisms over the past two decades (Boone et al., 2019; Calafat et al., 2019; Ghisi et al., 2019; Jian et al., 2017).

Reduced fetal growth has been used as a surrogate for the intrauterine conditions of the fetus. Some fetal growth indicators at birth including weight, size (weight-for-gestational-age), length, and ponderal index, were associated with short-term health effects such as infant morbidity and mortality (Madden et al., 2018; Wilcox, 2001), or with long-term health outcomes such as metabolic and cardiovascular diseases (D. J. P. Barker, 2006; Eriksson, 2016; Ramadhani et al., 2006; Risnes et al., 2011). Notably, African American women have shown nearly 50% higher risk of adverse birth outcomes such as preterm birth and low birth weight than white women (13.2% vs 8.9% for preterm birth; 13.2% vs 7% for low birth weight [< 2,500 grams]) (Giscombé & Lobel, 2005; Martin et al., 2017).

Exposure to PFAS has been linked to various biological responses such as interfering with sex steroid and thyroid hormones, perturbing lipid metabolism, inducing oxidative stress and inflammation, and impairing placental functions, which can potentially result in adverse birth outcomes and reduced fetal growth (Abbott et al., 2007; Du et al., 2013; Herrera & Ortega-Senovilla, 2010; Szilagyi et al., 2020). PFAS can also transfer from the mothers to their fetuses − high correlations (Pearson correlations of most PFAS 0.82-0.91; PFHxS 0.05) and partition ratios (0.29-0.74) of umbilical cord to maternal serum were found in previous studies, suggesting a direct impact of chemical exposure to fetuses (Needham et al., 2011). Accordingly, several

systematic reviews and meta-analyses suggested that exposures to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were associated with reduced fetal growth (Bach et al., 2015; Johnson et al., 2014; Koustas et al., 2014; Lam et al., 2014; Negri et al., 2017). However, a more recent meta-analysis for human epidemiological studies indicated that the association between PFOA and reduced birth weight remain inclusive when only including the studies with blood samples collected at early pregnancy (Steenland et al., 2018), and inconsistent findings showing the effects of PFAS on preterm delivery (Ferguson et al., 2013; Stone et al., 2021). Additionally, limited studies have investigated the association of other PFAS exposure (besides PFOA and PFOS) with fetal growth, much less in African American populations, which have been exposed to higher environmental pollutants including PFAS (Calafat, Kuklenyik, et al., 2007; Calafat, Wong, et al., 2007; Nelson et al., 2012; Park et al., 2019). To address these research gaps, this study aimed to investigate the associations between PFAS exposure and birth outcomes including birth weight, sex-specific z-score for gestational age, head circumference, birth length, small-for-gestational age (SGA), ponderal index, and preterm delivery in a cohort of pregnant African American women.

Materials and Methods

Study population

This study utilized samples and data from the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, a prospective birth cohort study in Atlanta, Georgia. The details of this cohort were published previously (Corwin et al., 2017). The participants were enrolled from two hospitals in Atlanta, Georgia – Emory University Hospital and Grady Memorial Hospital between March 2014 and May 2018. Emory University Hospital is a private hospital with women from diverse socioeconomic status, whereas Grady Memorial Hospital is a publicly owned hospital with patients mostly with lower socioeconomic status. The inclusion criteria were U.S.-born African Americans women by self-report, at 8-14 weeks of gestation, aged 18 to 40 years, able to communicate in English, experiencing no chronic

medical condition nor taking prescribed medications. There are 448 women who had available PFAS measurements and birth outcome information of their offspring; however, in the current analyses, we excluded 22 participants who ended with abortion (n=6), stillbirth (n=4), or delivered babies with abnormalities (n=12) in the analyses, resulting in a sample size of 426 participants. Written informed consent was obtained from the participants at enrollment. Our study was reviewed and approved by Emory's Institutional Review Board (approval reference number 68441).

Quantification of PFAS

Blood samples were collected from routine blood drawn at 8-14 weeks gestation for serum PFAS. After sample collection, the samples were transported to the laboratory, processed to obtain serum, and stored at -80 °C until analysis. The samples were then analyzed at two laboratories from the Children's Health Exposure Analysis Resource (CHEAR) − Wadsworth Center/New York University Laboratory Hub (Wadsworth/NYU) and the Laboratory of Exposure Assessment and Development for Environmental Research (LEADER) at Emory University. CHEAR laboratories, supported by the U.S. National Institute of Environmental Health Sciences for the purpose of environmental exposure assessments, have followed the same quality control procedures to provide harmonized and quality data (Balshaw et al., 2017) and passed the German External Quality Assessment Scheme (http://g-equas.de/) twice each year. The analytical methods from the two laboratories were described in the previous literatures (Chang et al., 2020; Honda et al., 2018). The results from these two laboratories have good agreement on 11 overlapped samples with Pearson correlation coefficients ranging from 0.88 to 0.93 and the relative percent differences (RPD) ranging from 0.12% to 20.2% (median 4.8%) (Table S3-1).

All 448 samples were analyzed for PFHxS, PFOS, PFOA, and PFNA by the two laboratories, among which 341 samples were measured for 10 additional PFAS, including perfluorobutane sulfonic acid (PFBS), perfluorooctane sulfonamide (PFOSA), n-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA), n-ethyl perfluorooctane sulfonamido acetic acid (NEtFOSAA), perfluoropentanoic acid (PFPeA),

perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoA) by Wadsworth/NYU.

Birth outcomes and confounder ascertainment

The clinical data collection was completed by the research team using a standardized chart abstraction tool to ascertain the following characteristics, conditions, and birth outcomes during two prenatal visits at 8-14 and 24-30 weeks of gestation, and post-delivery. Gestational weeks 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. All participants received early pregnancy dating by last menstrual period (LMP) and/or early ultrasound. Preterm birth is the birth of an infant at > 20 and < 37 completed weeks of gestation. Birth weight, birth length, and head circumference were determined from the first physical measurement in the delivery room. Percentiles and z-scores of birth weights were calculated based on gestational age and at delivery and infant's sex using the population reference from the U.S. natality files for singleton in 2017 (Aris et al., 2019), which has provided an updated and nationally representative birth weight to gestational age reference based on obstetrical estimates of gestational age instead of traditional LMP-based method. Infants with birth weight < 10^{th} percentiles were defined as SGA. Ponderal index is calculated by birth weight divided by the third power of birth length (g/cm^3), and the value below 2 was considered low ponderal index (Sharma et al., 2016). Information of parity, body mass index (BMI: calculated by height and weight measured at prenatal visit at 8-14 weeks), and pregnancy complications was gathered from medical chart abstraction. Additionally, a survey based on maternal self-report and prenatal administrative record review was used to ascertain maternal age at enrollment, education, marital and cohabiting status health insurance, income-to-poverty ratio, and substance use status (tobacco and marijuana use).

Statistical analysis

Six less frequently detected PFAS (< 15%), including PFBS, PFOSA, PFHxSA, PFHpA, NEtFOSAA, and PFDoA, were excluded from further analyses, resulting in a total of eight PFAS with higher detection

frequency (> 43%) in the analyses. Serum PFAS concentrations including detection frequencies, geometric means (GMs), geometric standard deviations (GSDs), and distribution percentiles were calcuated, and the concentrations below the limit of detections (LODs) were imputed with LOD/$\sqrt{2}$ for descriptive statistics (Hornung & Reed, 1990). All PFAS concentrations were $\log_2$-transformed to reduce the potential effects from outliers in the analyses. No data transformation was conducted for birth weight, birth length, head circumference, and ponderal index because the distributions of these variables were approximately normal. Pearson correlations were calculated among $\log_2$-transformed PFAS concentrations.

We conducted multivariable linear regressions to evaluate the associations of serum PFAS concentrations with birth weight, sex-specific z-score for gestational age, birth length, head circumference, and ponderal index, and multivariable logistic regressions for SGA and preterm birth. A directed acyclic graph (DAG) was used to identify confounders in the causal relationship between serum PFAS concentrations and birth outcomes (Figure S3-1). The models with the outcomes including birth weight, head circumference, birth length, and ponderal index were adjusted for maternal age (continuous; years), education (categorical; less than high school, high school, some college, college and above), BMI (categorical; < 18.5, 18.5-25, 25-30, ≥ 30 kg/m^2), parity (categorical; 0, 1, ≥2), infant's sex, tobacco use (categorical; during pregnancy, not during pregnancy), and marijuana use (categorical; during pregnancy, not during pregnancy); these models were restricted to the women who delivered term birth infants to remove the effect of length of gestation. Head circumference models were additionally adjusted for delivery mode (C-section, vaginal delivery). The models of sex-specific z-scores for gestational age and SGA were adjusted for the same variables above expect for infant's sex. The women who delivered preterm and term births were included in the models with the outcomes including sex-specific z-scores, SGA, and preterm birth. We considered dose-response relationships by using categorical PFAS concentration groups divided by quantiles as exposure variables – the PFAS with detection frequencies > 90% were grouped by quartiles, and the PFAS with 40-50% detection frequencies were categorized into three groups including < LODs, and low and high exposure groups divided by median values of detectable levels. We used *p*-values < 0.05 as the cut-off for statistical

significance of the associations between serum PFAS and birth outcomes. Test for trend was performed by using median serum PFAS concentrations of each exposure group as a continuous variable, and *p*-values for trend < 0.05 were considered as monotonic effects.

We performed two stratified analyses by sex and tobacco use because previous studies have shown that these variables can modify the association between PFAS and birth outcomes. Although the mechanisms remain unclear, female infants and infants whose mothers smoked during pregnancy have been more vulnerable to PFAS exposure in relation to fetal growth (Andersen et al., 2010; Bach et al., 2016; Govarts et al., 2018; Kashino et al., 2020; Manzano-Salgado et al., 2017; Starling et al., 2019; Wikström et al., 2020). Moreover, interaction terms (created by multiplying PFAS and the stratified variables; PFAS × sex and PFAS × tobacco use) were included in the models to assess statistical interaction.

We performed single imputation with LOD/$\sqrt{2}$ for the PFAS with detection frequencies between 97-99%, and multiple imputation procedure for the PFAS with lower detection frequencies (40-50%). For multiple imputation procedure, we imputed the values below LOD by randomly sampling from a lognormal distribution with the estimated parameters from maximum likelihood estimates. Ten datasets were created based on different estimated parameters from bootstrapping to incorporate the uncertainty. We fitted models to each of the datasets and combined the results to reflect variabilities of the imputation process (Lubin et al., 2004). All the analyses were performed in R (version 3.6.1).

Sensitivity analysis

We performed sensitivity analyses to investigate different approaches to control for length of gestation, including the models adjusted for gestational weeks at delivery as a covariate and restricted to only term births. The models of sex-specific z-score for gestational age and SGA were not included in this analysis since length of gestation was already accounted for in the outcome variables. Additionally, we conducted regressions before and after including hypertension (including both pregnancy-induced hypertension and preeclampsia; categorical; yes, no) and gestational diabetes (categorical; yes, no) as covariates, and

excluding the subjects with either hypertension or gestational diabetes. Maternal height was adjusted in the birth length models but not included in the final model because it did not materially change the estimates (data not shown).

Results

Table 3-1 shows the characteristics of this study population. Among 426 women included in this study, the average age is 24.7 years (standard deviation [SD] = 4.66), and the majority had high school education or less (n = 227; 56%), had income-to-poverty ratio < 100% (n = 180; 42%), had no married or cohabiting partner (n = 227; 53%), and was supported by Medicaid (n = 332; 79%) instead of private medical insurance during prenatal care. There are 95 (22%) and 56 (13%) women reporting using marijuana and tobacco during pregnancy. The average of gestational week at delivery was 38.6 weeks (SD = 2.32) with 56 (13%) women delivered their infants preterm, the average of birth weight was 3,040 grams with 50 (12%) low birth weight infants (< 2,500 grams), and the average of sex-specific z-score and percentile for gestational age was -0.64 (SD = 1.03) and 32.7 (SD = 26.2) with 113 (27%) women delivered SGA infants.

PFHxS, PFOS, PFOA, and PFNA were detected in > 97% of serum samples, and PFPeA, PFDA, PFUnDA, and NMeFOSAA were detected in 43-50% of serum samples. The GMs of PFHxS, PFOS, PFOA and PFNA were 1.02 (GSD = 1.90), 2.05 (GSD = 2.05), 0.63 (GSD = 2.34), and 0.24 (GSD = 2.30) ng/mL, respectively. Concentrations of most serum PFAS were positively correlated with each other (Pearson correlation coefficients (*r)*, 0.02-0.67), except for the correlations between NMeFOSAA and PFUnDA (r = -0.16), and between PFPeA and most PFAS (r = -0.31 to -0.04).

Figure 3-1 shows the associations between serum PFAS concentrations and different fetal growth endpoints. Under the adjusted models, a 32% increase in odds of SGA birth (odds ratio [OR] = 1.32; 95% CI = 1.07, 1.63) was associated with a 2-fold increase in serum PFNA concentrations, and a 0.06 and a 0.05 g/cm^3 decrease in ponderal index were associated with a 2-fold increase in serum PFNA and PFDA concentrations.

No significant result was found for serum PFAS concentrations (as log-transformed variables) in relation to birth weight, sex-specific z-score for gestational age, head circumference, birth length, and preterm birth.

Dose-response relationships were observed in the associations between PFDA and ponderal index (*p* for trend = 0.01), and between PFNA and odds of SGA (*p* for trend = 0.04) (Table 3-2 and 3-3). Lower ponderal index was found in some PFOS exposed groups, the 2nd (β = -0.18, 95CI% -0.34, -0.02) and the 4th quartile group (β = -0.20, 95% CI -0.36, -0.03), than in the reference group (the 1st quartile). Higher odds of SGA birth were also observed in the 2nd (OR = 2.22, 95% CI 1.10, 4.50), the 3rd (OR = 2.44, 95% CI 1.21, 4.92), and the 4th (OR = 2.23, 95CI% 1.10, 4.54) exposure quartile of PFOA compared with the reference group, with borderline dose-response relationships (PFOS and ponderal index *p* for trend = 0.05; PFOA and SGA *p* for trend = 0.06). The other fetal growth endpoints also differed between exposed and reference groups, but most results were not consistent across exposed groups nor showed dose-response relationships. For example, birth weights were significantly lower in the 2nd exposure quartile of PFOA than the reference group (β = -126, 95%CI -241, -10), but no association was found for the 3rd (β = -44, 95%CI -162, 73) and the 4th exposure quartile (β = -107 , 95%CI -227, 13) (*p* for trend = 0.23). Inconsistent findings were also found for the associations between PFOA concentrations and head circumference and between PFOS, PFNA, and PFDA concentrations and birth length.

No significant effect modification was observed when including interaction terms in the analyses (i.e., *p*-values for interaction terms > 0.1) for either infant's sex or tobacco use during pregnancy. When stratifying the analyses by infant's sex and tobacco use during pregnancy, the estimated effects of serum PFAS concentrations on fetal growth endpoints did not materially change, and the statistical significance remains similar (data not shown). For sensitivity analyses, two approaches to control for length of gestation were examined. First, we restricted the analyses to the women with term births. Second, the analyses were adjusted for gestational week at delivery as a covariate (Figure S3-2 and Table S3-4). The effect estimates were similar in these models, but the confidence intervals were generally wider in the models restricted to women with term births, which can be explained by the smaller sample size. Figure S3-3, Table S3-5, and

Table S3-6 show the results of different approaches to control for pregnancy-induced hypertension, preeclampsia, and gestational diabetes. Despite some differences in statistical significance, the effect estimates were comparable across the models.

Discussion

Serum PFAS concentrations in this cohort were generally lower than the measurements in the other birth cohorts in the U.S (Boronow et al., 2019; Kingsley et al., 2018; Lyall et al., 2018; Romano et al., 2016; Sagiv et al., 2015). Since higher PFAS exposures have been linked to higher socioeconomic status and earlier sampling years due to the major phase-out starting in 2002, lower PFAS concentrations observed in the current study might be explained by the recent sample collection and lower socioeconomic status among the study participants (Buekers et al., 2018; Nelson et al., 2012). We investigated the associations between eight PFAS and fetal growth endpoints in this study. We found that serum PFNA and PFOA concentrations were associated with higher odds of SGA birth and with other fetal growth endpoints. The effect estimates of most fetal growth outcomes had the same directionality (i.e., reduced fetal growth) despite some non-significant results. For example, we found negative associations of serum PFOA and PFNA concentrations with birth weight, sex-specific z-score for gestational age, head circumference, and ponderal index, but the significant results were only detected in the associations between PFNA and ponderal index. Although some evidence indicated that PFOS and PFDA concentrations may link to fetal growth, in particular ponderal index, the results across different fetal growth outcomes were inconsistent – the physical measurements did not decrease by different exposure groups and no dose-response relationship was observed. No associations of serum PFHxS, PFPeA, PFUnDA, and NMeFOSAA concentrations with fetal growth endpoints were discovered, given the inconsistent results across fetal growth endpoints.

Of the eight PFAS included in this study, we observed the strongest and most consistent associations in PFNA concentrations. Since the majority of previous studies have focused on PFOS and PFOA, the information on the associations between exposure to the other PFAS (including PFNA) and fetal growth are relatively limited and remains inconclusive. Additionally, epidemiological studies which measured

multiple PFAS often had relatively smaller samples sizes, resulting in lower statistical power. PFNA concentrations have been associated with reduced fetal growth (including reduced birth weight, z-score for gestational age, head circumference, and/or SGA) in the birth cohorts in Colorado (USA) (Starling et al., 2017), Japan (Kashino et al., 2020), Sweden (Gyllenhammar et al., 2018; Wikström et al., 2020), Taiwan (Y. Wang et al., 2016), and Denmark (Meng et al., 2018), whereas no association was discovered in the other studies (Bach et al., 2016; Chen et al., 2012; Manzano-Salgado et al., 2017; Shi et al., 2017; Shoaff et al., 2018). Great differences in PFNA concentrations were shown in different regions and timing of sample collections (early pregnancy, late pregnancy, or cord blood), thus the inconsistency may be explained by the heterogeneity of study designs, study populations as well as exposure ranges across studies.

Previous systemic reviews and meta-analyses have concluded that developmental exposure to PFOA could reduce fetal growth in both human and animal studies (Bach et al., 2015; Johnson et al., 2014; Koustas et al., 2014; Lam et al., 2014; Negri et al., 2017). However, the statistically significant associations were mostly demonstrated when median serum or plasma PFOA levels were above 3 ng/mL (Bach et al., 2015). In the present analyses, we observed higher odds of SGA birth among the exposed groups than the reference group, and negative but insignificant associations of serum PFOA concentration with birth weight, sex-specific z-score for gestational age, head circumference, and birth length at much lower serum PFOA levels (median = 0.71 ng/mL; range < 0.02 to 4.42 ng/mL). Similar low-dose effects of PFOA on birth weight and SGA birth were also reported by recent studies in Sweden (median 1.61 ng/mL; range <0.02 to 3.18) (Wikström et al., 2020) and in seven European cohorts (median 0.55ng/mL; range 25th to 75th percentiles 0.29 to 1.2 ng/mL) (Govarts et al., 2018), suggesting a need to determine a safe lower PFOA concentrations among pregnant women.

Higher PFOS exposure was also linked to impaired fetal growth in toxicology literature (Lau et al., 2004, 2006, 2007) and in previous systematic reviews for human epidemiological studies, despite higher level of uncertainties as compared with the evidence in PFOA (Bach et al., 2015; Negri et al., 2017). No consistent evidence linking serum PFOS concentrations to reduced fetal growth outcomes was found in the current

analysis, which may be explained by lower exposure doses observed in this population. PFOS concentrations in the previous studies which reported significant associations between serum PFOS and fetal growth endpoints were at higher levels than in this cohort (GM = 2.05 ng/mL; median = 2.19 ng/mL; range <0.02 to 12.4 ng/mL) (Bach et al., 2015; Maisonet et al., 2012; Manzano-Salgado et al., 2017; Souza et al., 2020). For example, Maisonet et al. (2012) reported a GM of 13.2 ng/mL and a range between 3.8 and 112 ng/mL, Manzano-Salgado et al. (2017) showed a median plasma concentrations of 6.05 ng/mL, and Souza et al. (2020) had a GM of 3.41 ng/mL and a range between 1.06 and 106 ng/mL.

Ponderal index is a measurement for body thinness and proportionality. Lower ponderal index has been linked to reduced fetal growth (Vik, Markestad, et al., 1997; Vik, Vatten, et al., 1997), poor neonatal outcomes, higher perinatal mortality and morbidity (Dombrowski et al., 1994; Fay et al., 1991), and the development of diabetes and cardiovascular disease later in life (D. J. Barker et al., 1993; Forsén et al., 1997). Moreover, ponderal index allows the differentiation between symmetric and asymmetric growth restriction – low ponderal index suggests asymmetric growth in infants (Sparks et al., 1998). Symmetric growth restriction is defined as a proportional reduction in both weight and length, whereas asymmetric growth restriction shows more reduction in weight than length. Increased severity of growth restriction often associated with increased growth asymmetry (Kramer et al., 1989) – most asymmetrical growth restrictions result from placental insufficiency and other extrinsic factors, whereas more symmetrical growth restrictions are considered normal and healthy with some suffering from genetic and infectious insults at early pregnancy (Soothill et al., 1987). Our results show that serum PFOS, PFNA, and PFDA concentrations were associated with lower ponderal index, suggesting a tendency of asymmetric growth in relation to the exposure, although no corresponding effects on birth weight or birth length were found. Previous studies have reported both inverse (Apelberg et al., 2007; Chen et al., 2012; Minatoya et al., 2017; H. Wang et al., 2019) and no associations between PFAS exposure and ponderal index (Hamm et al., 2010; Lee et al., 2013; Shi et al., 2017).

Although this and previous studies have observed some evidence linking PFAS exposure to reduced fetal growth, it is worth noting that the effective extrapolated serum concentration derived from animal studies are much higher than the current doses reported in human epidemiological studies, which reduces the biological plausibility of this relationship. A good understanding of mode of action is essential when performing this interspecies extrapolation. However, the mode of action for the relationship between PFAS exposure and fetal growth has not yet been clearly identified and agreed (Negri et al., 2017). Thus, future studies are warranted to investigate the key biological events and processes involved in order to characterize the causal relationship.

Our study has several strengths. First, blood samples were collected in the early pregnancy (median = 11.4 weeks; 74% at first trimester, 26% at early second trimester), which may reduce the possibilities of reverse causality and confounding effect from pregnancy hemodynamics such as plasma volume expansion and glomerular filtration rate (GFR) (Sagiv et al., 2015; Steenland et al., 2018; Verner et al., 2015). Second, multiple serum PFAS besides PFOA and PFOS were measured in this study. This is particularly important because there are over 4,700 PFAS identified and PFOA and PFOS have been phased out and replaced by the other PFAS (Ateia et al., 2019; Sun et al., 2016; Valsecchi et al., 2017). Additionally, we were able to ascertain quality clinical outcomes by using early pregnancy gestational age dating and medical chart abstraction. There are some potential limitations to this study. First, although we have controlled for the most important confounders, it is likely that other unmeasured or residual confounders, such as diet, may bias the associations reported in this study. For example, certain diet patterns, such as seafood and packaged food consumption, might have an impact on fetal growth and link to higher exposure levels. However, we did not find the associations of takeout food and microwave popcorn consumption with serum PFAS concentrations (Chang et al., 2020), nor the associations of seafood and fish consumption with serum PFAS in this population (data not shown). Second, although we included multiple fetal growth outcomes, we recognized that these outcomes at birth are only proxies to measure fetal growth. When feasible, repeated ultrasound measurement of fetal anthropometrics should be used since it is recognized as the ‘gold’ standard

(Smarr et al., 2013). However, these physical measurements including birth weight, z-score for gestational age, head circumference, and birth length are commonly utilized since they are very accessible variables, allowing for comparison of results in this study with the previous studies. Another potential limitation is that we only included eight PFAS in this study. It is possible that other PFAS or co-exposure chemicals may also yield similar results. Finally, our study population represented a group of people with higher risk factors for adverse birth outcome than the U.S. population, suggesting a limited generalizability with our results.

Conclusion

Taken together, our results suggested that reduced fetal growth was associated with serum PFNA and PFOA concentrations, inconclusive findings for PFOS and PFDA, and null associations for PFHxS, PFPeA, PFUnDA, and NMeFOSAA. Although only some results were statistically significant, the trend of directionality was consistent across multiple fetal growth endpoints in the results of PFNA and PFOA, even in this low background exposure population. We did not find effect modification by infant's sex or maternal smoking behavior during pregnancy nor association between PFAS concentrations and preterm delivery. This study indicated that continued utilization of PFAS in consumer products and widespread PFAS pollution in the environment may lead to adverse effects, especially fetal growth, to this pregnant African American population.

Figures and Tables

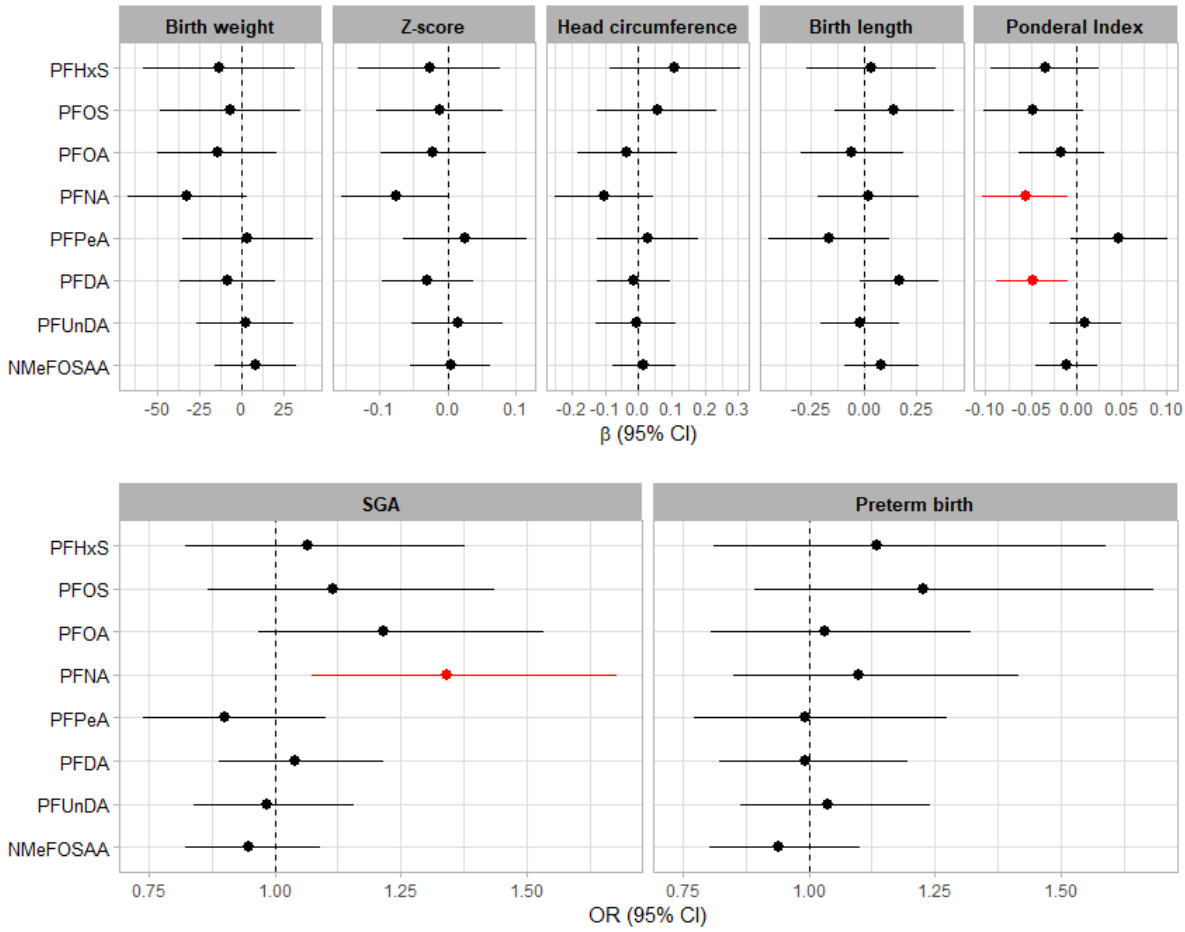


Figure 3-1. Adjusted associations between maternal serum PFAS and fetal growth in pregnant African American women in the Atlanta area, 2014-2018.
Each model was adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex. Head circumference models were additionally adjusted for delivery modes. Z-score and SGA models were not adjusted for infant's sex. The models of birth weight, head circumference, birth length and ponderal index were restricted to term births. (Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; SGA = small-for-gestational age; OR = odds ratio; CI = confidence interval)

Table 3-1. Demographic characteristics of the participants in pregnant African American women in the Atlanta area, 2014-2018 (n = 426).

Characteristics	n (%)[a]	Characteristics	n (%)[a]
Age (years)		**Tobacco use**	
Mean ± SD	24.7 ± 4.66	Not during pregnancy	370 (87%)
18-25	239 (56%)	During pregnancy	56 (13%)
25-30	108 (25%)	**Infant's sex**	
30-35	62 (15%)	Male	206 (48%)
≥35	17 (4%)	Female	220 (52%)
Education		**Gestational week at delivery (weeks)**	
Less than high school	66 (16%)	Mean ± SD	38.6 ± 2.32
High school	161 (38%)	**Preterm birth (< 37 gestational weeks)**	
Some college	131 (31%)	No	370 (87%)
College and above	68 (16%)	Yes	56 (13%)
Income-to-poverty ratio (%)		**Birth weight (grams)**	
< 100	180 (42%)	Mean ± SD	3040 (558)
100-150	67 (16%)	**Low birth weight (LBW) (< 2,500 grams)**	
150-300	68 (16%)	No	376 (88%)
≥ 300	52 (12%)	Yes	50 (12%)
Married or cohabiting		**Sex-specific z-score for gestation age**	
Yes	199 (47%)	Mean ± SD	-0.64 (1.03)
No	227 (53%)	**Sex-specific percentile for gestation age**	
Insurance		Mean ± SD	32.7 (26.2)
Private	94 (22%)	**Small-for-gestational age (SGA) (<10th percentiles)**	
Medicaid	332 (78%)	No	313 (74%)
Hospital		Yes	113 (27%)
Emory	172 (40%)	**Head circumference (cm)**	
Grady	254 (60%)	Mean ± SD **(missing = 68)**	33.1 (2.14)
Parity (#)		**Birth length (cm)**	
0	201 (47%)	Mean ± SD **(missing = 8)**	48.1 (3.46)
1	120 (28%)	**Ponderal index (g/cm^3) (missing = 8)**	
≥ 2	105 (25%)	Mean ± SD	2.72 (0.54)
BMI (kg/m^2)		Normal	406 (97%)
< 18.5	14 (3%)	Low ponderal index (<2)	12 (3.0%)
18.5-24.9	170 (40%)	**Gestational diabetes**	
25-29.9	90 (21%)	No	415 (97%)
≥ 30	152 (36%)	Yes	11 (2.6%)
Marijuana use		**Pregnancy-induced hypertension/preeclampsia**	
Not during pregnancy	331 (78%)	No	357 (83.8%)
During pregnancy	95 (22%)	Pregnancy-induced hypertension	45 (10.6%)
		Preeclampsia	24 (5.6%)

Note: SD = standard deviation

[a] The sample numbers do not be summed up to the total sample size due to missingness in some cases.

Table 3-2. Adjusted difference in fetal growth endpoints by PFAS concentration quantiles in pregnant African American women in the Atlanta area, 2014-2018.

PFAS (ng/mL)	Birth weight (g)[a] (n = 370)	Z-score[b] (n=426)	Head circumference (cm)[a,c] (n = 317)	Birth length (cm)[a] (n = 363)	Ponderal index (g/cm^3)[a] (n = 363)
			β (95%CI)		
PFHxS					
Q1: < LOD-0.75	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Q2: 0.75-1.10	-36 (-154, 83)	-0.03 (-0.29, 0.23)	0.19 (-0.31, 0.68)	-0.36 (-1.18, 0.45)	-0.01 (-0.17, 0.15)
Q3: 1.10-1.53	5 (-112, 123)	-0.09 (-0.36, 0.18)	0.41 (-0.10, 0.91)	0.12 (-0.69, 0.93)	-0.09 (-0.25, 0.07)
Q4: 1.53-4.80	-54 (-173, 66)	-0.08 (-0.35, 0.19)	-0.02 (-0.52, 0.49)	0.10 (-0.72, 0.92)	-0.14 (-0.30, 0.02)
p for trend[d]	0.50	0.52	0.99	0.58	0.06
Per log_2-unit	-14 (-58, 31)	-0.03 (-0.13, 0.08)	0.11 (-0.09, 0.31)	0.04 (-0.27, 0.34)	-0.03 (-0.09, 0.03)
PFOS					
Q1: < LOD-1.44	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Q2: 1.44-2.19	78 (-40, 196)	0.17 (-0.10, 0.43)	-0.11 (-0.60, 0.39)	**0.99 (0.18, 1.80)***	**-0.18 (-0.34, -0.02)***
Q3: 2.19-3.24	20 (-98, 138)	0.05 (-0.22, 0.32)	0.14 (-0.35, 0.63)	0.50 (-0.31, 1.31)	-0.15 (-0.31, 0.01)
Q4: 3.24-12.40	-16 (-136, 105)	0.03 (-0.24, 0.31)	0.01 (-0.48, 0.51)	0.61 (-0.22, 1.43)	**-0.20 (-0.36, -0.03)***
p for trend[d]	0.48	0.88	0.78	0.42	0.05
Per log_2-unit	-7 (-48, 34)	-0.01 (-0.11, 0.08)	0.06 (-0.12, 0.24)	0.14 (-0.14, 0.43)	-0.05 (-0.10, 0.01)
PFOA					
Q1: < LOD-0.45	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Q2: 0.45-0.71	**-126 (-241, -10)***	-0.25 (-0.51, 0.01)	**-0.80 (-1.28, -0.32)****	-0.74 (-1.53, 0.06)	-0.03 (-0.19, 0.12)
Q3: 0.71-1.07	-44 (-162, 73)	-0.07 (-0.33, 0.19)	-0.24 (-0.72, 0.24)	-0.18 (-0.98, 0.63)	-0.07 (-0.23, 0.09)
Q4: 1.07-4.42	-107 (-227, 13)	-0.18 (-0.45, 0.09)	-0.44 (-0.93, 0.06)	-0.53 (-1.35, 0.30)	-0.06 (-0.23, 0.10)
p for trend[d]	0.23	0.44	0.46	0.47	0.41
Per log_2-unit	-14 (-49, 21)	-0.02 (-0.10, 0.06)	-0.03 (-0.18, 0.12)	-0.06 (-0.30, 0.19)	-0.02 (-0.06, 0.03)
PFNA					
Q1: < LOD-0.16	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Q2: 0.16-0.27	-41 (-159, 77)	-0.09 (-0.36, 0.17)	-0.14 (-0.63, 0.35)	**0.98 (0.18, 1.79)***	**-0.28 (-0.44, -0.12)****
Q3: 0.27-0.42	-48 (-165, 69)	-0.06 (-0.33, 0.20)	-0.28 (-0.77, 0.20)	0.24 (-0.56, 1.04)	-0.14 (-0.30, 0.02)
Q4: 0.42-2.27	-106 (-227, 14)	-0.26 (-0.53, 0.01)	-0.22 (-0.71, 0.28)	0.23 (-0.59, 1.05)	**-0.20 (-0.35, -0.04)***
p for trend[d]	0.09	0.07	0.40	0.89	0.11
Per log_2-unit	-32 (-67, 3)	-0.08 (-0.16, 0.00)	-0.10 (-0.25, 0.04)	0.02 (-0.22, 0.26)	**-0.06 (-0.10, -0.01)***
	(n=293)	**(n=340)**	**(n=258)**	**(n=288)**	**(n=288)**
PFPeA					
< LOD	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Low: 0.05-0.11	-86 (-204, 32)	0.03 (-0.23, 0.30)	-0.10 (-0.60, 0.40)	-0.24 (-1.08, 0.60)	0.02 (-0.15, 0.19)
High: 0.11-0.66	62 (-58, 181)	0.12 (-0.15, 0.38)	0.20 (-0.30, 0.70)	-0.31 (-1.16, 0.55)	0.14 (-0.03, 0.32)
p for trend[d]	0.40	0.39	0.40	0.46	0.11
Per log_2-unit	4 (-35, 42)	0.02 (-0.07, 0.10)	0.03 (-0.12, 0.18)	-0.16 (-0.45, 0.12)	0.05 (-0.01, 0.10)
PFDA					
< LOD	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Low: 0.04-0.13	34 (-86, 154)	-0.01 (-0.28, 0.26)	-0.10 (-0.60, 0.30)	**1.10 (0.27, 1.94)****	**-0.23 (-0.40, -0.06)****
High:0.13-1.06	-45 (-165, 75)	-0.19 (-0.45, 0.08)	0.00 (-0.50, 0.50)	0.68 (-0.15, 1.52)	**-0.20 (-0.37, -0.03)***
p for trend[d]	0.52	0.17	0.82	0.07	**0.01***
Per log_2-unit	-8 (-37, 20)	-0.04 (-0.10, 0.02)	-0.01 (-0.12, 0.09)	0.17 (-0.02, 0.36)	**-0.05 (-0.09, -0.01)***
PFUnDA					
< LOD	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Low: 0.02-0.06	-3 (-128, 123)	0.15 (-0.12, 0.43)	-0.20 (-0.70, 0.40)	-0.08 (-0.97, 0.80)	0.02 (-0.16, 0.20)
High:0.06-0.53	38 (-88, 163)	0.10 (-0.18, 0.38)	0.10 (-0.40, 0.70)	-0.05 (-0.94, 0.84)	0.08 (-0.10, 0.26)
p for trend[d]	0.55	0.48	0.59	0.91	0.39
Per log_2-unit	2 (-26, 31)	0.02 (-0.04, 0.08)	-0.01 (-0.13, 0.11)	-0.02 (-0.21, 0.17)	0.01 (-0.03, 0.05)
NMeFOSAA					
< LOD	0 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)	0.00 (Ref)
Low: 0.02-0.07	-34 (-154, 86)	-0.20 (-0.47, 0.06)	-0.20 (-0.70, 0.30)	-0.03 (-0.88, 0.82)	0.02 (-0.15, 0.19)
High: 0.07-1.46	27 (-92, 145)	-0.08 (-0.34, 0.19)	0.10 (-0.40, 0.60)	0.38 (-0.46, 1.21)	-0.08 (-0.25, 0.09)
p for trend[d]	0.58	0.73	0.59	0.35	0.30

PFAS (ng/mL)	Birth weight (g)[a] (n = 370)	Z-score[b] (n=426)	Head circumference (cm)[a,c] (n = 317)	Birth length (cm)[a] (n = 363)	Ponderal index (g/cm^3)[a] (n = 363)
			β (95%CI)		
Per log_2-unit	8 (-16, 32)	0.00 (Ref)	0.02 (-0.08, 0.11)	0.08 (-0.09, 0.26)	-0.01 (-0.04, 0.02)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval.

[a] Adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex; restricted to term births.

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

[c] Additionally adjusted for delivery mode.

[d] Median serum PFAS concentrations of each exposure group were used as a continuous exposure variable.

* *p*-value < 0.05; ** *p*-value < 0.01.

Table 3-3. Adjusted odds ratios (ORs) of small-for-gestational age (SGA) and preterm birth by PFAS concentration quantiles in pregnant African American women in the Atlanta area, 2014-2018.

PFAS (ng/mL)	SGA[a] (n = 426)	Preterm birth[b] (n = 426)	PFAS (ng/mL)	SGA[a] (n = 340)	Preterm birth[b] (n = 340)
	OR (95%CI)			OR (95%CI)	
PFHxS			**PFPeA**		
Q1: < LOD-0.75	1.00 (Ref)	1.00 (Ref)	< LOD	1.00 (Ref)	1.00 (Ref)
Q2: 0.75-1.10	1.36 (0.71, 2.61)	2.07 (0.91, 4.73)	Low: 0.05-0.11	0.69 (0.36, 1.32)	1.09 (0.50, 2.39)
Q3: 1.10-1.53	1.35 (0.70, 2.61)	0.91 (0.36, 2.34)	High: 0.11-0.66	0.66 (0.34, 1.27)	0.91 (0.40, 2.05)
Q4: 1.53-4.80	1.11 (0.57, 2.17)	1.75 (0.72, 4.27)			
p for trend[c]	0.84	0.51	*p* for trend[c]	0.19	0.83
Per log_2-unit	1.10 (0.85, 1.42)	1.13 (0.81, 1.59)	Per log_2-unit	0.93 (0.77, 1.12)	0.99 (0.77, 1.27)
PFOS			**PFDA**		
Q1: < LOD-1.44	1.00 (Ref)	1.00 (Ref)	< LOD	1.00 (Ref)	1.00 (Ref)
Q2: 1.44-2.19	0.92 (0.47, 1.78)	1.59 (0.67, 3.74)	Low: 0.04-0.13	0.90 (0.46, 1.75)	0.61 (0.25, 1.52)
Q3: 2.19-3.24	1.32 (0.69, 2.53)	1.64 (0.67, 4.01)	High:0.13-1.06	1.18 (0.63, 2.21)	1.07 (0.50, 2.33)
Q4: 3.24-12.40	1.09 (0.56, 2.13)	1.78 (0.74, 4.29)			
p for trend[c]	0.65	0.26	*p* for trend[c]	0.62	0.95
Per log_2-unit	1.12 (0.88, 1.42)	1.23 (0.89, 1.68)	Per log_2-unit	1.04 (0.90, 1.20)	0.99 (0.82, 1.20)
PFOA			**PFUnDA**		
Q1: < LOD-0.45	1.00 (Ref)	1.00 (Ref)	< LOD	1.00 (Ref)	1.00 (Ref)
Q2: 0.45-0.71	**2.22 (1.10, 4.50)***	0.88 (0.37, 2.06)	Low: 0.02-0.06	0.78 (0.40, 1.51)	1.76 (0.81, 3.81)
Q3: 0.71-1.07	**2.44 (1.21, 4.92)***	1.36 (0.60, 3.06)	High:0.06-0.53	0.86 (0.44, 1.70)	1.13 (0.47, 2.71)
Q4: 1.07-4.42	**2.23 (1.10, 4.54)***	1.52 (0.64, 3.60)			
p for trend[c]	0.06	0.22	*p* for trend[c]	0.68	0.78
Per log_2-unit	1.20 (0.97, 1.49)	1.03 (0.81, 1.32)	Per log_2-unit	0.99 (0.85, 1.15)	1.04 (0.87, 1.24)
PFNA			**NMeFOSAA**		
Q1: < LOD-0.16	1.00 (Ref)	1.00 (Ref)	< LOD	1.00 (Ref)	1.00 (Ref)
Q2: 0.16-0.27	1.73 (0.87, 3.43)	1.75 (0.76, 4.04)	Low: 0.02-0.07	0.92 (0.48, 1.76)	1.06 (0.49, 2.30)
Q3: 0.27-0.42	1.72 (0.87, 3.40)	1.22 (0.50, 2.99)	High: 0.07-1.46	0.92 (0.49, 1.74)	0.54 (0.22, 1.32)
Q4: 0.42-2.27	**2.22 (1.12, 4.38)***	1.84 (0.76, 4.42)			
p for trend[c]	**0.04***	0.29	*p* for trend[c]	0.82	0.16
Per log_2-unit	**1.32 (1.07, 1.63)***	1.10 (0.85, 1.42)	Per log_2-unit	0.97 (0.84, 1.11)	0.94 (0.80, 1.10)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; OR = odds ratio; CI = confidence interval.

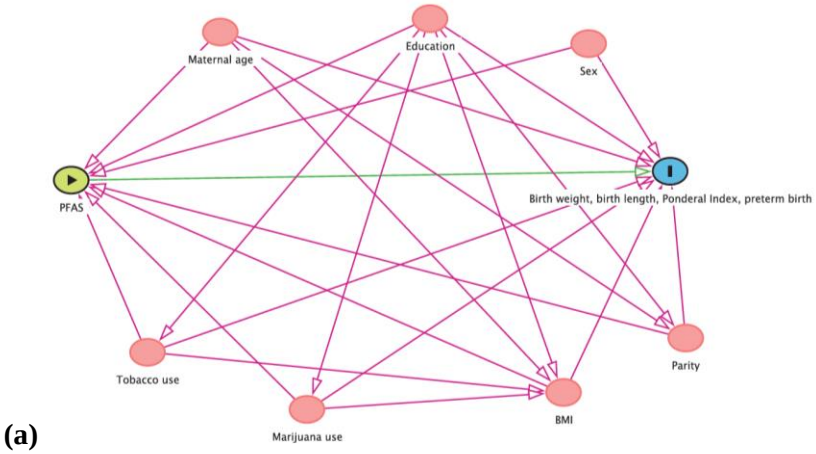
[a] Adjusted for maternal age, education, BMI, parity, tobacco use, and marijuana use.

[b] Additionally adjusted for infant's sex.

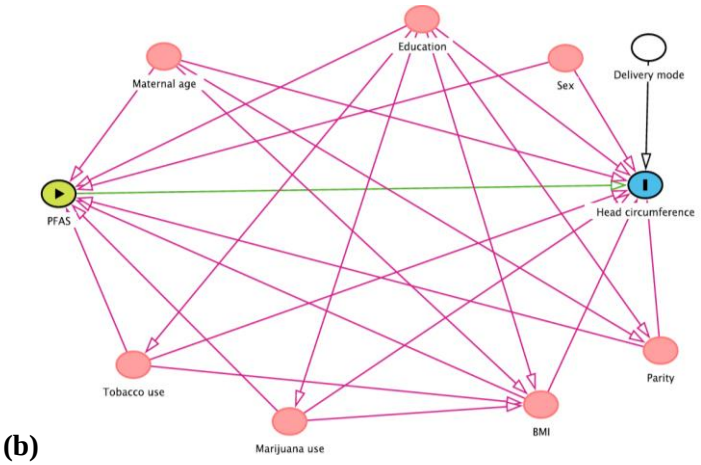
[c] Median serum PFAS concentrations of each exposure group were used as a continuous exposure variable.

* *p*-value < 0.05.

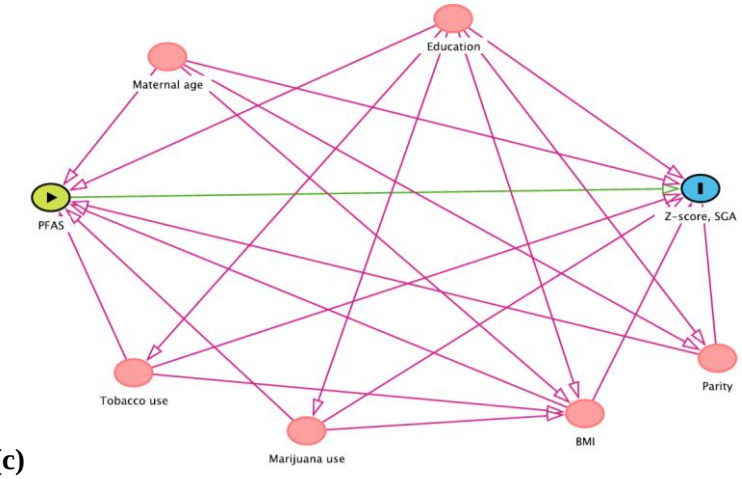
Supplementary



(a)



(b)



(c)

Figure S3-1. Directed acyclic graph (DAG) showing the associations of PFAS exposure with (a) birth weight, birth length, ponderal index (restricting to term births), and preterm birth, (b) head circumference, and (c) sex-specific z-score for gestational age and small-for-gestational age (SGA).

Green lines represent causal paths and red lines were biasing paths. Delivery mode in the head circumference models was served as a precision variable for the outcome.

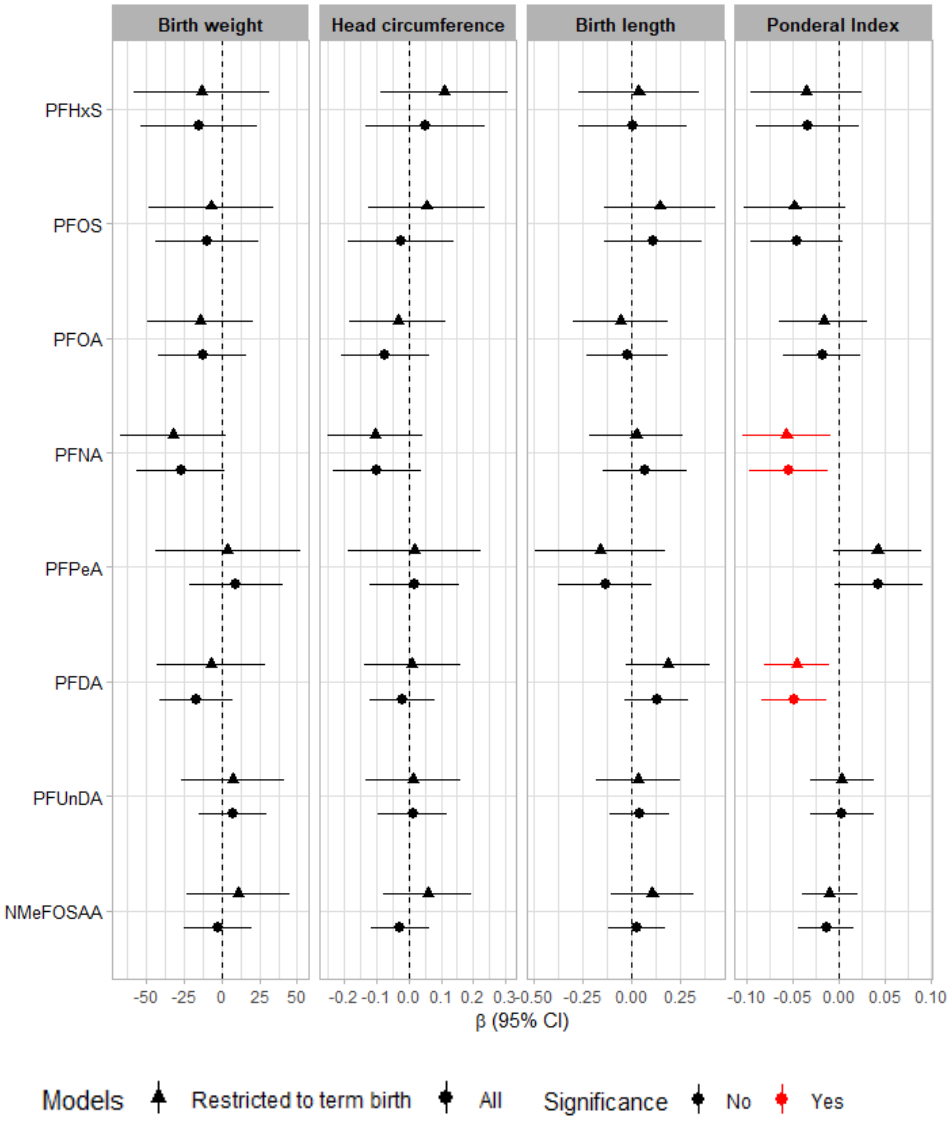


Figure S3-2. Associations between maternal serum PFAS and fetal growth controlled for length of gestation in pregnant African American women in the Atlanta area, 2014-2018.

The models including all participants were adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, gestational week at delivery, and infant's sex. Head circumference models were additionally adjusted for delivery modes. The models restricted to term births were adjusted for the same set of variables expect for gestational week at delivery. (Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; SGA = small-for-gestational age; OR = odds ratio; CI = confidence interval)

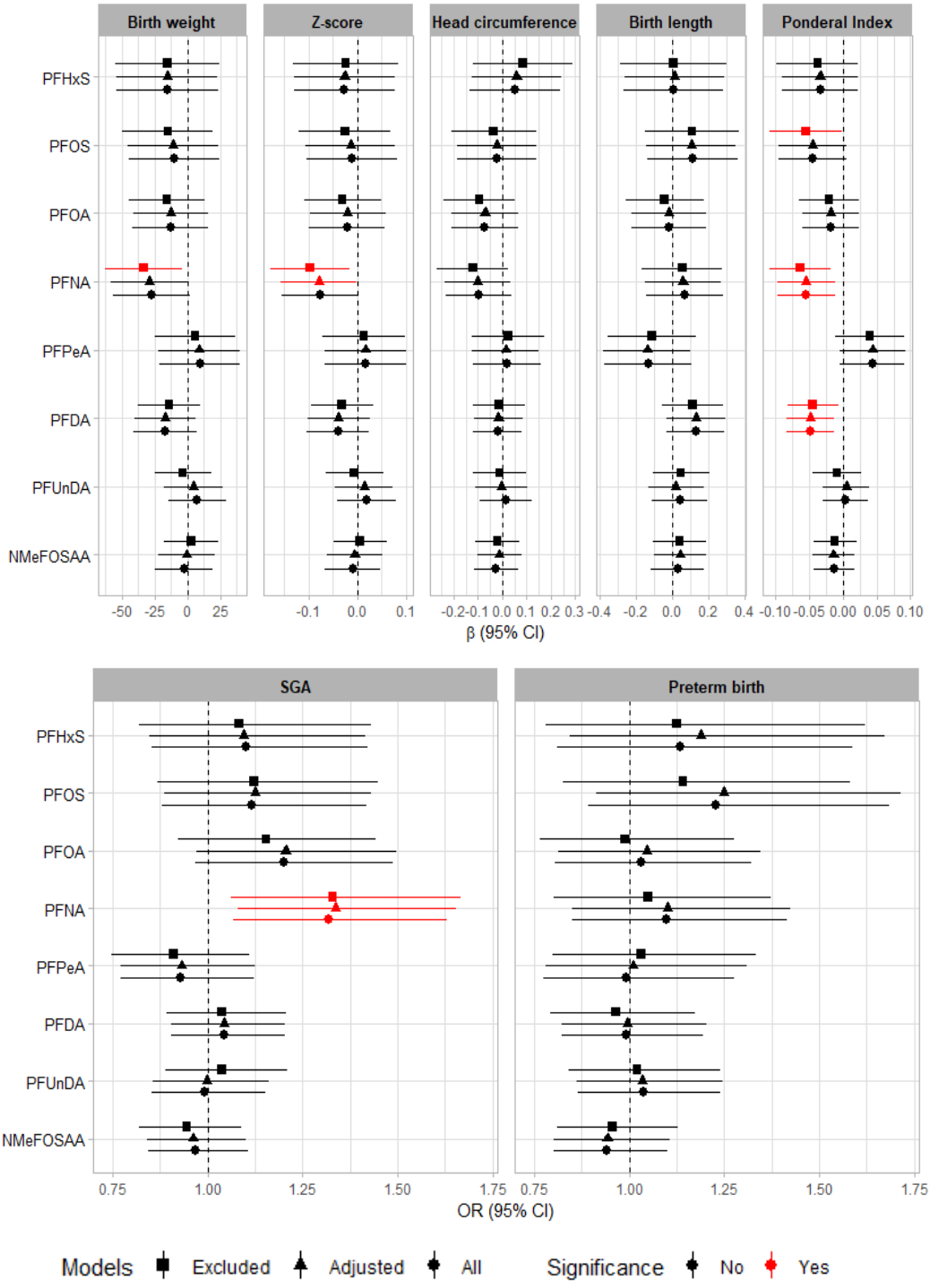


Figure S3-3. Associations between maternal serum PFAS and fetal growth controlled for pregnancy-induced hypertension/preeclampsia and gestational diabetes in pregnant African American women in the Atlanta area, 2014-2018.

The models including all participants (All) were adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, gestational week at delivery, and infant's sex. Head circumference models were additionally adjusted for delivery modes. SGA models were not adjusted for infant's sex or gestational

week at delivery, and preterm birth models were not adjusted for length of gestation at delivery. We controlled for pregnancy-induced hypertension/preeclampsia and gestational diabetes by 1) excluding the participants with the pregnancy complications in the analyses (Excluded) and 2) additionally adjusting for variables of pregnancy-induced hypertension/preeclampsia and gestational diabetes as covariates (Adjusted). (Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; SGA = small-for-gestational age; OR = odds ratio; CI = confidence interval)

Table S3-1. Overlapped samples concentrations (ng/mL) from the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University and Wadsworth Center/New York University Laboratory Hub.

	PFHxS		PFOS		PFOA		PFNA	
Sample ID	**W**[b]	**E**	**W**[b]	**E**	**W**[b]	**E**	**W**[b]	**E**
1	1.515	1.398	3.506	3.735	1.305	1.299	0.435	0.550
2	0.710	0.587	1.719	1.021	0.492	0.425	0.868	0.811
3	1.055	1.283	1.392	1.215	0.677	0.583	0.372	0.351
4	0.679	0.565	1.733	1.967	0.228	0.376	0.091	0.214
5	0.794	0.601	1.469	1.393	0.848	1.114	0.259	0.419
6	0.999	0.606	5.145	4.509	0.734	0.900	0.197	0.357
7	0.831	0.835	2.478	2.388	0.624	0.566	0.264	0.337
8	1.534	1.645	2.325	1.670	1.013	1.133	0.285	0.401
9	1.332	0.948	2.882	2.559	1.315	1.870	0.402	0.553
10	1.830	1.264	3.965	4.430	1.289	1.845	0.632	0.876
11	1.850	1.835	2.597	2.269	1.350	2.100	0.231	0.410
Pearson correlation coefficients	**0.88**		**0.93**		**0.92**		**0.93**	
Relative percent differences, %RPD; Median (Min-Max)[a]	**4.75 (0.12-12.3)**		**3.17 (0.92-12.7)**		**5.05 (0.12-12.3)**		**8.11 (1.48-20.2)**	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.

[a] %RPD = (Lab 1 result – Lab 2 result)*100/(Lab 1 result + Lab 2 result)/2.

[b]The results from Wadsworth were used in the main analysis for these overlapped samples .

Table S3-2. Serum PFAS concentrations (ng/mL) in pregnant African American women in the Atlanta area, 2014-2018.

PFAS	n	LOD	% > LOD	GM[a]	GSD[a]	P25	P50	P75	Max
PFHxS	426	0.02 (W); 0.2 (E)	97	1.02	1.90	0.76	1.10	1.53	4.80
PFOS	426	0.02 (W); 0.05 (E)	99	2.05	2.05	1.45	2.19	3.24	12.4
PFOA	426	0.035 (W); 0.2 (E)	97	0.63	2.34	0.45	0.71	1.07	4.42
PFNA	426	0.02 (W); 0.1 (E)	97	0.24	2.30	0.16	0.27	0.42	2.27
PFPeA	340	0.05 (W)	48	--	--	<LOD	<LOD	0.11	0.66
PFDA	340	0.035 (W)	48	--	--	<LOD	<LOD	0.13	1.06
PFUnDA	340	0.02 (W)	43	--	--	<LOD	<LOD	0.06	0.53
NMeFOSAA	340	0.02 (W)	50	--	--	<LOD	<LOD	0.07	1.46

Note: LOD = limits of detection; GM = geometric mean; GSD = geometric standard deviation; P25 = the 25th percentile; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; W = Wadsworth Center/New York University Laboratory Hub; E = Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.
[a] Geometric means and standard deviations were not calculated for congeners with detection frequencies <50%; the values below LODs were replaced by LOD/√2.

Table S3-3. Pearson correlation coefficients of log_2-transformed serum PFAS concentrations in pregnant African American women in the Atlanta area, 2014-2018.

	PFHxS[a]	PFOS[a]	PFOA[a]	PFNA[a]	PFPeA[b]	PFDA[b]	PFUnDA[b]	NMeFOSAA[b]
PFHxS	1.00	0.67**	0.46**	0.35**	-0.23**	0.41**	0.12*	0.06
PFOS		1.00	0.67**	0.65**	-0.29**	0.49**	0.24**	0.11
PFOA			1.00	0.72**	-0.13*	0.34**	0.19*	0.06
PFNA				1.00	-0.21**	0.46**	0.31**	0.10
PFPeA					1.00	-0.31**	0.02	-0.04
PFDA						1.00	0.09	0.13*
PFUnDA							1.00	-0.16*
NMeFOSAA								1.00

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid.

[a] The values below LODs were imputed with LOD/$\sqrt{2}$.

[b] The values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation.

* p-value < 0.05; ** p-value < 0.01.

Table S3-4. Associations between maternal serum PFAS and fetal growth controlled for length of gestation in pregnant African American women in the Atlanta area, 2014-2018.

	Birth weight (g) β (95%CI)		Head circumference (cm) β (95%CI)	
PFAS (ng/mL)	**Model 1[a] (n = 370)**	**Model 2[b] (n = 426)**	**Model 1[a,c] (n = 317)**	**Model 2[b.,c] (n = 358)**
PFHxS[d]	-14 (-58, 31)	-15 (-54, 23)	0.11 (-0.09, 0.31)	0.05 (-0.13, 0.24)
PFOS[d]	-7 (-48, 34)	-10 (-44, 25)	0.06 (-0.12, 0.24)	-0.02 (-0.19, 0.14)
PFOA[d]	-14 (-49, 21)	-13 (-42, 16)	-0.03 (-0.18, 0.12)	-0.07 (-0.21, 0.06)
PFNA[d]	-32 (-67, 3)	-27 (-56, 3)	-0.10 (-0.25, 0.04)	-0.10 (-0.23, 0.04)
	(n=293)	**(n=340)**	**(n=258)**	**(n=291)**
PFPeA[e]	4 (-35, 42)	10 (-21, 40)	0.03 (-0.12, 0.18)	0.02 (-0.12, 0.16)
PFDA[e]	-8 (-37, 20)	-17 (-41, 7)	-0.01 (-0.12, 0.09)	-0.02 (-0.12, 0.08)
PFUnDA[e]	2 (-26, 31)	8 (-15, 30)	-0.01 (-0.13, 0.11)	0.01 (-0.09, 0.12)
NMeFOSAA[e]	8 (-16, 32)	-2 (-25, 20)	0.02 (-0.08, 0.11)	-0.03 (-0.12, 0.06)
	Birth length (cm) β (95%CI)		**Ponderal index (g/cm^3) β (95%CI)**	
PFAS (ng/mL)	**Model 1[a] (n = 363)**	**Model 2[b] (n = 418)**	**Model 1[a] (n = 363)**	**Model 2[b] (n = 418)**
PFHxS[d]	0.04 (-0.27, 0.34)	0.01 (-0.26, 0.29)	-0.03 (-0.09, 0.03)	-0.03 (-0.09, 0.02)
PFOS[d]	0.14 (-0.14, 0.43)	0.10 (-0.14, 0.35)	-0.05 (-0.10, 0.01)	-0.05 (-0.10, 0.01)
PFOA[d]	-0.06 (-0.30, 0.19)	-0.02 (-0.23, 0.18)	-0.02 (-0.06, 0.03)	-0.02 (-0.06, 0.02)
PFNA[d]	0.02 (-0.22, 0.26)	0.07 (-0.14, 0.28)	**-0.06 (-0.10, -0.01)***	**-0.05 (-0.10, -0.01)***
	(n=288)	**(n=334)**	**(n=288)**	**(n=334)**
PFPeA[e]	-0.16 (-0.45, 0.12)	-0.14 (-0.38, 0.10)	0.05 (-0.01, 0.10)	0.04 (0.00, 0.09)
PFDA[e]	0.17 (-0.02, 0.36)	0.13 (-0.03, 0.29)	**-0.05 (-0.09, -0.01)***	**-0.05 (-0.08, -0.01)****
PFUnDA[e]	-0.02 (-0.21, 0.17)	0.04 (-0.11, 0.20)	0.01 (-0.03, 0.05)	0.00 (-0.03, 0.04)
NMeFOSAA[e]	0.08 (-0.09, 0.26)	0.03 (-0.12, 0.17)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval.

[a] Adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, and infant's sex; restricted to the participants with term delivery.

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

[c] Additionally adjusted for delivery mode.

[d] The values below LODs were imputed with LOD/$\sqrt{2}$.

[e] The values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation.

* p-value < 0.05; ** p-value < 0.01.

Table S3-5. Associations between maternal serum PFAS and fetal growth controlled for pregnancy-induced hypertension/preeclampsia and gestational diabetes in pregnant African American women in the Atlanta area, 2014-2018.

	Birth weight (g) β (95%CI)			Z-score β (95%CI)		
PFAS (ng/mL)	**Model 1[a,b] (n = 348)**	**Model 2[a,c] (n = 426)**	**Model 3[a] (n = 426)**	**Model 1[a,b] (n = 348)**	**Model 2[a,c] (n = 426)**	**Model 3[a] (n = 426)**
PFHxS[e]	-15 (-55, 24)	-15 (-54, 23)	-15 (-54, 23)	-0.02 (-0.13, 0.08)	-0.03 (-0.13, 0.08)	-0.03 (-0.13, 0.08)
PFOS[e]	-15 (-49, 20)	-11 (-45, 23)	-10 (-44, 25)	-0.03 (-0.12, 0.07)	-0.01 (-0.11, 0.08)	-0.01 (-0.10, 0.08)
PFOA[e]	-16 (-45, 13)	-12 (-41, 16)	-13 (-42, 16)	-0.03 (-0.11, 0.05)	-0.02 (-0.10, 0.06)	-0.02 (-0.10, 0.06)
PFNA[e]	**-33 (-63, -4)***	-29 (-58, 0)	-27 (-56, 2)	**-0.10 (-0.18, -0.02)***	**-0.08 (-0.16, 0.00)***	-0.08 (-0.16, 0.00)
	(n = 285)	**(n = 340)**	**(n = 340)**	**(n = 285)**	**(n = 340)**	**(n = 340)**
PFPeA[f]	6 (-25, 37)	9 (-22, 40)	10 (-21, 40)	0.01 (-0.07, 0.10)	0.02 (-0.07, 0.10)	0.02 (-0.07, 0.10)
PFDA[f]	-14 (-37, 10)	-17 (-40, 7)	-17 (-41, 7)	-0.03 (-0.10, 0.03)	-0.04 (-0.10, 0.03)	-0.04 (-0.10, 0.02)
PFUnDA[f]	-3 (-25, 18)	5 (-17, 27)	8 (-15, 30)	-0.01 (-0.07, 0.05)	0.01 (-0.05, 0.07)	0.02 (-0.04, 0.08)
NMeFOSAA[f]	3 (-17, 23)	-1 (-22, 21)	-2 (-25, 20)	0.01 (-0.05, 0.06)	-0.01 (-0.06, 0.05)	-0.01 (-0.07, 0.05)

	Head circumference (cm) β (95%CI)			Birth length (cm) β (95%CI)		
PFAS (ng/mL)	**Model 1[a,b,d] (n = 288)**	**Model 2[a,c,d] (n = 358)**	**Model 3[a,d] (n = 358)**	**Model 1[a,b] (n = 340)**	**Model 2[a,c] (n = 418)**	**Model 3[a] (n = 418)**
PFHxS[e]	0.08 (-0.12, 0.29)	0.06 (-0.13, 0.24)	0.05 (-0.13, 0.24)	0.01 (-0.29, 0.30)	0.01 (-0.27, 0.29)	0.01 (-0.27, 0.28)
PFOS[e]	-0.04 (-0.21, 0.14)	-0.02 (-0.19, 0.14)	-0.02 (-0.19, 0.14)	0.11 (-0.15, 0.37)	0.10 (-0.14, 0.35)	0.11 (-0.14, 0.36)
PFOA[e]	-0.10 (-0.24, 0.05)	-0.07 (-0.21, 0.06)	-0.07 (-0.21, 0.06)	-0.04 (-0.26, 0.17)	-0.02 (-0.23, 0.19)	-0.02 (-0.23, 0.19)
PFNA[e]	-0.12 (-0.27, 0.02)	-0.10 (-0.24, 0.03)	-0.10 (-0.23, 0.04)	0.05 (-0.17, 0.28)	0.06 (-0.15, 0.27)	0.07 (-0.15, 0.28)
	(n = 242)	**(n = 291)**	**(n = 291)**	**(n = 280)**	**(n = 334)**	**(n = 334)**
PFPeA[f]	0.02 (-0.12, 0.17)	0.01 (-0.12, 0.15)	0.02 (-0.12, 0.16)	-0.12 (-0.36, 0.13)	-0.14 (-0.38, 0.10)	-0.14 (-0.38, 0.10)
PFDA[f]	-0.01 (-0.12, 0.09)	-0.02 (-0.12, 0.08)	-0.02 (-0.12, 0.08)	0.11 (-0.06, 0.28)	0.13 (-0.03, 0.29)	0.13 (-0.03, 0.29)
PFUnDA[f]	-0.01 (-0.12, 0.10)	0.00 (-0.11, 0.10)	0.01 (-0.09, 0.12)	0.05 (-0.11, 0.20)	0.02 (-0.13, 0.17)	0.04 (-0.11, 0.20)
NMeFOSAA[f]	-0.02 (-0.11, 0.07)	-0.01 (-0.10, 0.08)	-0.03 (-0.12, 0.06)	0.04 (-0.11, 0.19)	0.04 (-0.10, 0.19)	0.03 (-0.12, 0.17)

	Ponderal index (g/cm^3) β (95%CI)		
PFAS (ng/mL)	**Model 1**[a,b] **(n = 340)**	**Model 2**[a,c] **(n = 418)**	**Model 3**[a] **(n = 418)**
PFHxS[e]	-0.04 (-0.10, 0.02)	-0.03 (-0.09, 0.02)	-0.03 (-0.09, 0.02)
PFOS[e]	**-0.05 (-0.11, 0.00)***	-0.05 (-0.10, 0.00)	-0.05 (-0.10, 0.00)
PFOA[e]	-0.02 (-0.07, 0.02)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)
PFNA[e]	**-0.06 (-0.11, -0.02)****	**-0.05 (-0.10, -0.01)***	**-0.05 (-0.10, -0.01)***
	(n = 280)	**(n = 334)**	**(n = 334)**
PFPeA[f]	0.04 (-0.01, 0.09)	0.04 (0.00, 0.09)	0.04 (0.00, 0.09)
PFDA[f]	**-0.04 (-0.08, -0.01)***	**-0.05 (-0.08, -0.01)****	**-0.05 (-0.08, -0.01)***
PFUnDA[f]	-0.01 (-0.04, 0.03)	0.00 (-0.03, 0.04)	0.00 (-0.03, 0.04)
NMeFOSAA[f]	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval.

[a] Adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, gestational week for delivery, and infant's sex

[b] Restricted to the participants without pregnancy-induced hypertension/preeclampsia, or gestational diabetes.

[c] Additionally adjusted for pregnancy-induced hypertension/preeclampsia and gestational diabetes as covariates.

[d] Additionally adjusted for delivery mode.

[e] The values below LODs were imputed with LOD/$\sqrt{2}$.

[f] The values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation.

* p-value < 0.05; ** p-value < 0.01.

Table S3-6. Associations of maternal serum PFAS with small-for-gestational age (SGA) risk and preterm birth controlled for pregnancy-induced hypertension/preeclampsia and gestational diabetes in pregnant African American women in the Atlanta area, 2014-2018.

	SGA OR (95%CI)			Preterm birth OR (95%CI)		
PFAS (ng/mL)	**Model 1[a.b] (n = 348)**	**Model 2[a.c] (n = 426)**	**Model 3[a] (n = 426)**	**Model 1[a.b.d] (n = 348)**	**Model 2[a.c,d] (n = 426)**	**Model 3[a,d] (n = 426)**
PFHxS[e]	1.08 (0.82, 1.43)	1.09 (0.85, 1.41)	1.10 (0.85, 1.42)	1.13 (0.78, 1.62)	1.19 (0.84, 1.67)	1.13 (0.81, 1.59)
PFOS[e]	1.12 (0.87, 1.45)	1.12 (0.88, 1.43)	1.12 (0.88, 1.42)	1.14 (0.82, 1.58)	1.25 (0.91, 1.71)	1.23 (0.89, 1.68)
PFOA[e]	1.15 (0.92, 1.44)	1.21 (0.97, 1.50)	1.20 (0.97, 1.49)	0.99 (0.77, 1.28)	1.05 (0.81, 1.35)	1.03 (0.81, 1.32)
PFNA[e]	**1.33 (1.06, 1.67)***	**1.34 (1.08, 1.66)****	**1.32 (1.07, 1.63)***	1.05 (0.80, 1.37)	1.10 (0.85, 1.43)	1.10 (0.85, 1.42)
	(n = 285)	**(n = 340)**	**(n = 340)**	**(n = 285)**	**(n = 340)**	**(n = 340)**
PFPeA[f]	0.91 (0.75, 1.11)	0.93 (0.77, 1.12)	0.93 (0.77, 1.12)	1.03 (0.80, 1.34)	1.01 (0.78, 1.31)	0.99 (0.77, 1.27)
PFDA[f]	1.04 (0.89, 1.21)	1.04 (0.90, 1.20)	1.04 (0.90, 1.20)	0.96 (0.79, 1.17)	0.99 (0.82, 1.20)	0.99 (0.82, 1.19)
PFUnDA[f]	1.04 (0.89, 1.21)	1.00 (0.86, 1.16)	0.99 (0.85, 1.15)	1.02 (0.84, 1.24)	1.04 (0.86, 1.25)	1.04 (0.86, 1.24)
NMeFOSAA[f]	0.95 (0.82, 1.09)	0.96 (0.84, 1.10)	0.97 (0.84, 1.11)	0.96 (0.81, 1.13)	0.94 (0.80, 1.11)	0.94 (0.80, 1.10)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; SGA = small-for-gestational age; OR = odds ratio; CI = confidence interval.

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

[b] Restricted to the participants without pregnancy-induced hypertension/preeclampsia or gestational diabetes.

[c] Additionally adjusted for pregnancy-induced hypertension/preeclampsia and gestational diabetes.

[d] Additionally adjusted for infant's sex.

[e] The values below LODs were imputed with LOD/$\sqrt{2}$.

[f] The values below LODs were imputed by a lognormal probability distribution and maximum likelihood estimation.

* p-value < 0.05; ** p-value < 0.01.

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Chapter 4. PFAS and Vitamin D

Associations of Single and Multiple Per- and Polyfluoroalkyl Substance (PFAS) Exposure with Vitamin D Biomarkers in African American Women during Pregnancy.

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Abstract

Vitamin D has been linked to various physiological functions in pregnant women and their fetuses. Previous studies have suggested that some per- and polyfluoroalkyl substances (PFAS) may alter serum vitamin D concentrations. However, no study has investigated the relationship between PFAS and vitamin D in pregnant women. This study aims to evaluate the associations of serum PFAS with serum total and free 25-hydroxyvitamin D (25(OH)D) during pregnancy in a cohort of African American women in Atlanta, GA. Blood samples from 442 participants were collected in early pregnancy (8-14 weeks of gestation) for PFAS and 25(OH)D measurements, and additional samples were collected in late pregnancy (24-30 weeks) for the second 25(OH)D measurements. We fit multivariable linear regressions and weighted quantile sum (WQS) regressions to estimate the associations of individual PFAS and their mixtures with 25(OH)D concentrations. We found mostly positive associations of total 25(OH)D with PFHxS (perfluorohexane sulfonic acid), PFOS (perfluorooctane sulfonic acid), PFDA (perfluorodecanoic acid), and NMeFOSAA (n-methyl perfluorooctane sulfonamido acetic acid), and negative associations with PFPeA (perfluoropentanoic acid). For free 25(OH)D, positive associations were observed with PFHxS, PFOS, PFOA (perfluorooctanoic acid), and PFDA, and a negative association with PFPeA among the women with male fetuses in the models using 25(OH)D measured in late pregnancy. In mixture models, a quartile increase in WQS index was associated with 2.88 ng/mL (95%CI 1.14-4.59) and 5.68 ng/mL (95%CI 3.31-8.04) increases in total 25(OH)D measured in the early and late pregnancy, respectively. NMeFOSAA, PFDA, and PFOS contributed the most to the overall effects among the eight PFAS. No association was found between free 25(OH)D and the PFAS mixture. These results suggest that PFAS may affect vitamin D biomarker concentrations in pregnant African American women, and some of the associations were modified by fetal sex.

Keywords: Vitamin D, per- and polyfluoroalkyl substance (PFAS), chemical mixtures, weighted quantile sum (WQS) regression, endocrine disruptors

Introduction

Per- and polyfluoroalkyl substances (PFAS) have been manufactured and used from the 1940s. Due to their unique hydrophobic and lipophobic properties, PFAS have been applied in numerous consumer products such as stain- and water-resistant fabrics and textiles, nonstick coatings on food wrappers and cookware, personal care products, and firefighting foams (Herzke et al., 2012; Sunderland et al., 2018). Because of their ubiquity as well as the persistence, previous studies have commonly detected PFAS in the environment and biological samples, leading to critical concerns to the public (Harris et al., 2017; Kim et al., 2020; Lau et al., 2007; Paul et al., 2009). Thus, major manufacturers have voluntarily phased out the production of PFAS since 2002. However, over 98% the participant in the 2015-2016 National Health and Nutrition Examination Survey (NHANES) have detectable serum perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) concentrations (US Department of Health and Human Services, 2019). Additionally, exposure to PFAS has been associated with endocrine disruption (Abbott et al., 2007; Li et al., 2020; White et al., 2011), metabolic syndrome (Frisbee et al., 2010), reduced immune function (Grandjean et al., 2012; Granum et al., 2013; Stein et al., 2016), developmental issues (Abbott et al., 2007; Lam et al., 2014), and adverse skeletal health (Koskela et al., 2016, 2017) in experimental and observational studies (Ballesteros et al., 2017; Cluett et al., 2019; Frisbee et al., 2010; Hu et al., 2019; Johnson et al., 2014; Khalil et al., 2016; Kim et al., 2018; Lau et al., 2006; Di Nisio, et al., 2020a; Rappazzo et al., 2017; Steenland et al., 2010).

Vitamin D significantly contributes to development and progression of chronic diseases, such cancers, autoimmune diseases, metabolic diseases, and cardiovascular diseases in addition to maintaining skeletal health (Bikle, 2014; Mousavi et al., 2019; Norman & Powell, 2014). During pregnancy, vitamin D homeostasis is essential for placentation and maintaining maternal and fetal health (Luk et al., 2012; Ponsonby et al., 2010; Wagner & Hollis, 2018; Zehnder et al., 2002). For example, vitamin D controls the secretion of some placental hormones, reduces infection, limits the production of pro-inflammatory cytokines, and supports intrauterine growth by providing calcium and phosphorous, and enhancing skeletal

ossification (Barrera et al., 2007, 2008; Shin et al., 2010). In two meta-analyses of epidemiological studies, levels of serum 25-hydroxyvitamin D (25(OH)D), a metabolite of vitamin D, during pregnancy were inversely associated with risks of adverse pregnancy and birth outcomes including pre-eclampsia, gestational diabetes, preterm birth, and small-for-gestational age (Aghajafari et al., 2013; Wei et al., 2013). The effect of PFAS exposure on vitamin D hemostasis during pregnancy is of interest because perturbation of maternal hormone levels in this susceptible window can result in profound health risks in both pregnant women and their fetuses (Wagner & Hollis, 2018).

Environmental endocrine-disrupting chemicals (EDCs) have been proven to affect steroid and thyroid hormone metabolisms via different actions, such as interaction with hormone receptors and serum protein transporters, and influences on steroidogenesis and clearance (Ghassabian & Trasande, 2018; Sanderson, 2006; Yang et al., 2015). Vitamin D metabolism may also be altered by EDCs through similar pathways because its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), is akin to the molecular structure of classic steroid hormones, and vitamin D receptor belongs to the same superfamily of steroid and thyroid receptors (Norman, 2008; Pike & Meyer, 2010; Schug et al., 2011). Previous epidemiological studies have shown that vitamin D metabolism was disturbed by exposures to EDCs including polychlorinated biphenyls (Morales et al., 2013), organochlorine pesticides (Yang et al., 2012), bisphenol A, and phthalates (Erden et al., 2014; Johns et al., 2016, 2017). Additionally, studies suggest that PFAS can affect bone mineral density in both adults and children (Khalil et al., 2016, 2018; Cluett et al., 2019; Hu et al., 2019), and the disturbance of vitamin D by PFAS exposure could be a potential explanation (Di Nisio et al., 2020b). Therefore, we hypothesized that PFAS, which act as EDCs to disrupt sex steroids and thyroid hormones (Benninghoff et al., 2011; Li et al., 2020; Weiss et al., 2009), may also affect vitamin D metabolism.

Only few epidemiological studies have investigated the association between PFAS and the vitamin D system. These studies have shown inconsistent results and some of them have suffered from limited statistical power due to small sample sizes (Di Nisio et al. 2020b; Etzel et al., 2019; Khalil et al., 2018). Additionally, we are not aware of any study investigating the association in pregnant women. In the present

study, we aimed to investigate the association of individual and combined serum PFAS levels with circulating serum total and free 25(OH)D concentrations in a population-based cohort of pregnant women.

Materials and Methods

Study population

This study utilized samples and data from the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Study, a prospective birth cohort study in Atlanta, Georgia. The details of this cohort were described in a previous report (Corwin et al., 2017). The participants were enrolled from two hospitals, Emory University Hospital (privately owned) and Grady Memorial hospital (publicly run), to enhance a wider coverage of socioeconomic status. The inclusion criteria were U.S.-born African Americans women by self-report, between 8-14 weeks of gestation, between age 18-40 years, able to communicate in English, experiencing no chronic medical condition nor taking prescribed medications. In the present study, we analyzed data from 442 women enrolled between March 2014 and May 2018. These subjects represent the first group of the participants in the cohort, whose pregnancy ended with a live birth with blood samples collected for PFAS and 25(OH)D measurements. Written informed consent was obtained from the participants at enrollment. Our study was reviewed and approved by Emory's Institutional Review Board (approval reference number 68441).

Data collection

Data were collected at two routine clinical visits (Enrollment/Visit 1, at 8-14 weeks of gestation; Visit 2, at 24-30 weeks of gestation) through questionnaire administration and medical record abstraction. Sociodemographic information such as education, marital and cohabiting status, insurance status, income-to-poverty ratio, and tobacco, marijuana, and alcohol use was gathered by self-report and prenatal clinical records. Clinical data including maternal age, parity, fetal sex, and gestational age at time of sampling were ascertained from prenatal clinical records, and body mass index (BMI) was derived from height and weight measured at Visit 1.

A Food Frequency Questionnaire (FFQ) was administrated on a subset of women (n=292) at Visit 1 and Visit 2 to collect the information of fish and vitamin D supplement intake over the previous three months. Vitamin D supplement intake information was extracted by the question "*how often did you take vitamin D supplements*", and fish intake information was obtained from the question "*how often did you eat fish*". The FFQ used in this study was modified to collect intake information from pregnant women and validated in various low-income pregnant women population (Baer et al., 2005).

Biological specimens and assays

For blood sample collections, the laboratory technicians extracted additional blood from the routine blood draws at two prenatal clinical visits for research purposes. The blood samples were transported to the laboratory and centrifuged for serum separation. The serum samples were then stored at -80°C for future analyses. PFAS were measured in the serum samples from Visit 1, and total and free 25(OH)D were measured in the serum samples from both prenatal visits.

Quantification of PFAS

Aliquots of maternal serum were measured at two laboratories from the Children's Health Exposure Analysis Resource (CHEAR) − Wadsworth Center/New York University Laboratory Hub (Wadsworth/NYU) and the Laboratory of Exposure Assessment and Development for Environmental Research (LEADER) at Emory University. CHEAR laboratories, supported by the U.S. National Institute of Environmental Health Sciences for the purpose of environmental exposure assessments, have followed the same quality control procedures to provide harmonized and quality data (Balshaw et al., 2017). All 442 samples were analyzed for PFHxS, PFOS, PFOA, and PFNA by these two laboratories, among which 351 samples were measured for 10 additional PFAS, including perfluorobutane sulfonic acid (PFBS), perfluorooctane sulfonamide (PFOSA), n-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA), n-ethyl perfluorooctane sulfonamido acetic acid (NEtFOSAA), perfluoropentanoic acid (PFPeA),

perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoA) by Wadsworth/NYU.

Further details of the analytical methods were described previously (Chang et al., 2020; Honda et al., 2018). Briefly, each sample was spiked with internal standards, extracted by solid phase extraction, and analyzed by liquid chromatography interfaced with tandem mass spectrometry (LC-MS/MS). Quantification of PFAS was performed using isotope dilution calibration. Wadsworth/NYU and LEADER have been certified by the German External Quality Assessment Scheme (http://g-equas.de/) twice each year for PFAS measurements. The results from these two laboratories have good agreements on 11 overlapped samples with Pearson correlation coefficients ranging from 0.88 to 0.93 and the relative percent differences (RPD) ranging from 0.12% to 20.2% (median 4.8%) (Table S4-1).

Quantification of vitamin D biomarkers

Serum total and free 25(OH)D were analyzed in the Vitamin D Research Laboratory at Emory University School of Medicine. The automated competitive binding chemiluminescence 25(OH)D assay (Immunodiagnostic Systems Ltd, Fountain Hills, AZ) was utilized to measure total 25(OH)D, with a detection range of 7-120 ng/mL. A competitive enzyme-linked immunosorbent assay (DIAsource ImmunoAssays, Louvain-la-Neuve, Belgium), calibrated against a symmetrical dialysis method, was used to measured free 25(OH)D. This method allows a direct measurement of the free fraction of 25(OH)D with a detection range of 2.4 –17.1 pg/mL and the limit of blank of 1.5 pg/mL. The laboratory participates in Vitamin D Metabolites Quality Assurance Program at the National Institute of Standards and Technology (Lippa et al., 2020) and the Vitamin D External Quality Assessment Scheme (http://www.deqas.org/), providing interlaboratory comparisons to warrant the reliability of 25(OH)D measurements. The definition of vitamin D deficiency was based on the reference range for the general population prescribed by the Endocrine Society: total 25(OH)D ≤ 20 ng/mL (Holick et al., 2011).

Statistical analysis

All the analyses were performed in R (version 3.6.1). Arithmetic means and standard deviations of total and free 25(OH)D at two prenatal visits were tabulated by selected population characteristics, and the measurements of serum 25(OH)D and PFAS below the limits of detection (LODs) were imputed as LOD/$\sqrt{2}$ in the descriptive analysis (Hornung & Reed, 1990). No data transformation was performed for total and free 25(OH)D concentrations because the empirical histograms approximated normal distributions in this population. Due to right-skewed distribution, PFAS concentrations were natural-log transformed to reduce the impact of outliers before further analyses. PFBS, PFOSA, PFHxSA, PFHpA, NEtFOSAA, and PFDoA, which were less frequently detected (<15%), were excluded from this analysis, resulting in a total of eight PFAS were analyzing in this study. We used paired t-test to compare the means of total and free 25(OH)D concentrations from Visit 1 and Visit 2. Pearson correlation coefficients were calculated to investigate the correlations between the serum concentrations of total and free 25(OH)D at two visits and serum PFAS.

Next, we fit multivariable linear regressions to estimate the associations between PFAS exposure and 25(OH)D concentrations measured at two prenatal visits individually. For the Visit 1 models, both the exposure and the outcome were collected at the same prenatal visit (Visit 1), which was considered as a cross-sectional study design; whereas for the Visit 2 models, the exposure and outcome were collected at Visit 1 and Visit 2, respectively, which was considered as a prospective cohort study design. To explore the dose-response relationships, we modeled difference in total and free 25(OH)D concentrations with successive exposure categories (the PFAS with >90% detection frequencies grouped into quartiles; the PFAS with 40-50% detection frequencies were categorized into three groups: <LODs, and low and high exposure groups divided by median values of detectable levels) using multivariable linear regressions. Moreover, test for trend was performed by modeling the exposure categories as ordinal variables and used *p*-values for trend <0.05 as the criteria for monotonic effects. The odds of vitamin D deficiency were also modeled using multivariable logistic regressions.

We chose covariates guided by a directed acyclic graph (DAG) to identify potential confounding variables in the causal association between PFAS exposure and 25(OH)D concentrations (Figure S4-1) (Greenland et al., 1999). The covariates include maternal age (continuous, years), education (less than high school, high school, some college, college and above), BMI (<18.5, 18.5-25, 25-30, ≥30 kg/m^2), parity (0, 1, ≥2), fetal sex (male, female), marijuana use (during pregnancy, not during pregnancy), tobacco use (during pregnancy, not during pregnancy), and season of sample collection for 25(OH)D (spring: March to May, summer: June to August, fall: September to November, winter: December to February). We assessed potential effect modification of PFAS by fetal sex because vitamin D metabolism through the placenta could be modulated by sex steroid hormones, leading to differential sensitivities to PFAS exposure by fetal sex (Liu et al., 2018; Olmos-Ortiz et al., 2016). We included interaction terms in the models and used *p*-value <0.10 as the cut-off for significance. Moreover, the effect estimates by fetal sex were derived from the model with the interaction term.

Additionally, a weighted quantile sum (WQS) regression was performed to evaluate the joint associations of the highly correlated eight PFAS levels with serum 25(OH)D concentrations using the R *gwqs* and *miWQS* packages. WQS can address the collinearity issues of highly correlated exposures and identify the major contributors of pollutant mixture. Details of the WQS methods can be found elsewhere (Carrico et al., 2015). Specifically, we divided the dataset into a training set (40%) and a validation set (60%). Each exposure is empirically assigned a weight based on their associations with the outcomes by using bootstrap samples in a training set. We categorized the eight PFAS into quartiles and the bootstrapped weights are multiplied by these PFAS quantiles. The products of quartiles and weights were then summed to create an index for the PFAS mixtures. Next, the index is used to estimate the overall effect of the PFAS mixture on 25(OH)D using a validation set. The basic WQS regression model is:

$$g(\mu) = \beta_0 + \beta_1 \times (\sum_{j=1}^{8} \omega_j \times PFASq_{ji}) + z_i'\varphi$$

where β_0 is the intercept, β_1 is the regression coefficient for the WQS index and can be interpreted as the effect of the PFAS mixture on the outcomes, z_i' represents the values of other covariates from the i^{th} subject, and φ denotes the corresponding regression coefficients. The term $\sum_{j=1}^{8} \omega_j \times PFASq_{ji}$ represents the WQS index for each participant, ω_j is the weight for the j^{th} PFAS ($0 \leq \omega_j \leq 1, \sum_{j=1}^{8} \omega_j = 1$) and can be used to identify the important chemicals in the PFAS mixture (*a priori* cut-point = 1/number of chemicals = 1/8 =0.125); $PFASq_{ji}$ is the quantile for the j^{th} PFAS from the i^{th} subject. $g(\mu)$ is a monotonic link function linking the predictor to the mean of the continuous outcome variables. The effects of the PFAS mixture on 25(OH)D were estimated for both directions separately, using the analysis constrained in either positive or negative directions. Effect modification by fetal sex was evaluated by including an interaction term with the WQS index in the model, and the interaction term was removed if the *p*-value was higher than 0.10 (Brunst et al., 2017; Preston et al., 2020).

Single imputation with LOD/$\sqrt{2}$ was conducted for the biomarkers with higher detection frequencies (87-99%), i.e., PFHxS, PFOS, PFOA, PFNA, and total and free 25(OH)D. A multiple imputation framework was adopted for the biomarkers with lower detection frequencies (40-50%), i.e., PFPeA, PFDA, PFUnDA, and NMeFOSAA, because a high percentage of the measurements below the LODs could introduce biases into the analyses. We imputed the values below the LOD by randomly sampling from a lognormal distribution with the estimated parameters from maximum likelihood estimates (Gilbert, 1987). To incorporate the uncertainty, ten datasets were created based on the different estimated parameters from bootstrapping. These datasets were independently analyzed in the statistical models and combined to reflect variabilities of the imputation process (Hargarten & Wheeler, 2020; Lubin et al., 2004). Analyses investigating the association between PFAS and 25(OH)D which produced *p*-values less than 0.05 were considered statistically significant.

Several sensitivity analyses were performed to ensure the robustness of our results. First, to assess the impact of the potential diet confounders, which were only available for some participants in the study, we performed sensitivity analyses on a subset of participants (n=292) to adjust for fish (yes/no) and vitamin D

supplement intake (yes/no) in addition to the other covariates in the main models. Additionally, to remove the effect of vitamin D supplement intake completely, we further excluded the participants who took vitamin D supplement during pregnancy in the analyses (n=160). Second, because both PFAS and 25(OH)D can bind to albumin and circulate in the blood (Bikle & Schwartz, 2019; Forsthuber et al., 2020), we used data from NHANES 2011-2014 to examine the influence of albumin on the association between PFAS and 25(OH)D. The detailed statistical analyses for NHANES data were described in the supplement. Third, the results of single imputation with LOD/$\sqrt{2}$ and multiple imputation for the biomarkers with lower detection frequencies, i.e., PFPeA, PFDA, PFUnDA, and NMeFOSAA, were compared to determine the impact of different imputation methods.

Results

Distribution of study variables

The demographic characteristics of the cohort are described in Table 4-1. The majority of the participating women were between 18 and 25 years of age, and predominantly at a lower socioeconomic status – about 55% with a high school or below education, 57% below 150% income-to-poverty ratio, 78% with Medicaid as medical insurance, and 60% enrolled at a public hospital. A total of 52% of the participants had given birth one or more times; 21% were overweight, and 36% were obese.

In the paired t-test analyses, the mean concentrations of total and free 25(OH)D significantly increased from Visit 1 to Visit 2 ($p < 0.01$ for both total and free). On average, the total 25(OH)D levels were 19.5 ng/mL (standard deviation (SD) = 8.7) and 23.3 ng/mL (SD = 11.1), and the free 25(OH)D concentrations were 3.7 pg/mL (SD = 1.4) and 4.0 pg/mL (SD = 1.7) at Visit 1 and Visit 2, respectively. Similar concentrations were found in the subset of the participants with FFQ data. Total and free 25(OH)D concentrations were generally higher among people in high socioeconomic groups, including the women with college and above education, ≥300% income-to-poverty ratio, private insurance, and being enrolled in the private hospital. In addition, higher 25(OH)D concentrations were mostly observed among the women with no partner, less parity, and with male fetuses. The other variables shown different 25(OH)D

concentrations include the consumption of marijuana, tobacco, and vitamin D supplement, and the season of sample collection. Among two prenatal visits, detection frequencies for total and free 25(OH)D were in a range of 87-94% in this study population (Table S4-2).

The exposure distributions of PFAS of this cohort have been described in detail previously (Chang et al., 2020). Briefly, PFHxS, PFOS, PFOA, and PFNA were detected in >95% samples with PFOS having the highest geometric mean (2.03 ng/mL, geometric SD = 2.08). NMeFOSAA, PFPeA, PFDA, and PFUnDA were detected in approximately 40-50% of the participants (Table S4-3). Total 25(OH)D was positively correlated with most PFAS (r = 0.10-0.34), and negatively correlated with PFPeA (r = -0.23 and -0.21). Free 25(OH)D was weakly correlated with PFDA (r = 0.13 and 0.15) and PFOS (r = 0.10) but showed no correlation with the other PFAS. Moderate and strong correlations were found between total and free 25(OH)D concentrations with the coefficients ranging from 0.43 to 0.77 (Schober et al., 2018) (Table S4-4).

Association between individual PFAS and vitamin D biomarkers

The associations between serum PFAS and 25(OH)D are presented in Table 4-2 by fetal sex. Each natural-log unit increase in PFHxS, PFOS, PFDA, and NMeFOSAA was associated with a significant increase in total 25(OH)D concentrations among the women with either male or female fetuses, except for NMeFOSAA in the Visit 1 model. PFHxS in the Visit 2 models showed the largest effects (β_{male} = 4.71, 95%CI 2.28-7.14; β_{female} = 3.53, 95%CI 1.28-5.77). Negative associations between total 25(OH)D and serum PFPeA were observed among the women with male fetuses in both the Visit 1 and 2 models (Visit 1: β_{male} = -2.23, 95%CI -3.50, -0.95, p_{int} = 0.14; Visit 2: β_{male} = -3.53, 95%CI -5.68, -1.38, p_{int} = 0.08), and null associations among those with female fetuses (Visit 1: β_{female} = -0.88, 95%CI –2.21, 0.45; Visit 2: β_{female} = -0.77, 95%CI -2.70, 1.16). Additionally, some significant but inconsistent associations of total 25(OH)D across the Visit 1 and 2 models were found in PFOA, PFNA, and PFUnDA. For free 25(OH)D, positive associations were found in PFHxS, PFOS, PFOA, and PFDA, and negative association was found in PFPeA only among the women with male fetuses in the Visit 2 models. PFHxS also showed the largest effects for

free 25(OH)D (β_{male} = 0.41, 95%CI 0.03-0.79, p_{int} = 0.05). The associations between PFAS and 25(OH)D among all participants were presented in Table S4-5.

Table S4-6 and Figure S4-2 show the dose-response relationships between PFAS and 25(OH)D by fetal sex. Monotonic responses of total 25(OH)D were generally found in PFHxS, PFOS, PFDA, and NMeFOSAA among the women with both fetal sexes, and PFPeA and PFUnDA among the women with male fetuses. For free 25(OH)D, significant *p*-values for trend were observed in PFOS, PFPeA, and PFDA among the women with male fetuses.

There were 238 (54%) and 140 (32%) participants at Visit 1 and Visit 2 who were vitamin D deficient. As shown in Table S4-7, increases in serum PFHxS, PFOS, PFDA, and NMeFOSAA concentrations were generally associated with decreased odds of vitamin D deficiency among the women with both fetal sexes, with PFHxS among the women with female fetuses in the Visit 1 model showing the largest effects (OR_{female} = 0.32, 95%CI 0.19-0.54). However, an increase in PFPeA concentrations was associated with increased odds of vitamin D deficiency among the women with male fetuses (OR_{male} = 1.58, 95%CI 1.00-2.48 for Visit 1; OR_{male} = 2.06, 95%CI 1.24-3.44 for Visit 2). Some significant but inconsistent findings were shown in PFOA, PFNA, and PFUnDA across the Visit 1 and 2 models.

Association between the PFAS mixture and vitamin D biomarkers

Figure 4-1 summarizes the results of WQS regression analyses (see also Table S4-8). Because there was no significant effect modification by fetal sex in the WQS analyses, we presented the effects of the PFAS mixtures on 25(OH)D for all the participants collectively. The WQS index was positively associated with total 25(OH)D. More specifically, a quartile increase in the WQS index was associated with increases of 2.88 ng/mL (95%CI 1.14-4.59) and 5.68 ng/mL (95%CI 3.31-8.04) total 25(OH)D in the Visit 1 and Visit 2 models, respectively. Within the PFAS mixture, NMeFOSAA (weight = 0.36 for Visit 1; 0.38 for Visit 2), PFDA (weight = 0.41 for Visit 1; 0.17 for Visit 2), and PFOS (weight = 0.24 for Visit 2) had weights exceeding the cut-point of 0.125, suggesting major contributions of these PFAS to the overall effect of the mixture. No negative regression coefficients in the bootstrapped models for total 25(OH)D were found;

thus, we were unable to present the results of negative direction models. Additionally, no association was found between free 25(OH)D concentrations and the PFAS mixture in both directionalities.

Sensitivity analysis

In the sensitivity analyses, additionally adjusting for fish and vitamin D supplement intake or excluding the participants taking vitamin D supplement did not substantially change the results among a subset of the participants (Table S4-9). Similarly, additional adjustment for albumin had little impact on the estimates in the NHANES 2011-2014 participants, even when stratifying by age, race/ethnicity, and sex (Table S4-10). Table S4-8 and S4-11 shows the difference between single imputation with LOD/$\sqrt{2}$ and multiple imputation for the values below the LODs. Changes in estimates were calculated between the two imputation methods, and the range of percentage change [$(\beta_{multiple}-\beta_{single})/\beta_{single}$] was between -133% and 33% with a median of -35%. We observed overall larger effect sizes using single rather than multiple imputation.

Discussion

In this cohort of 442 healthy pregnant African American women, we report general findings of positive associations of circulating total 25(OH)D with PFHxS, PFOS, PFDA, and NMeFOSAA concentrations. We noted positive associations of total 25(OH)D with PFOA and PFNA, and negative associations of total 25(OH)D with PFPeA and PFUnDA among certain fetal sex. Although the statistical significance levels of these findings were inconsistent between the Visit 1 and the Visit 2 models for PFOA, PFNA, PFUnDA, and NMeFOSAA, the direction of associations remains consistent for the same PFAS. For free 25(OH)D, we observed positive associations with PFHxS, PFOS, PFOA, and PFDA, and an inverse association with PFPeA among the women with male fetuses in the Visit 2 models. A joint effect of the eight PFAS was also positively associated with total 25(OH)D concentrations, with NMeFOSAA, PFDA, and PFOS as the most important contributors, explaining 79-85% of the total weight. No significant association between free 25(OH)D and the PFAS mixture was found.

To date, limited human research have evaluated the associations between PFAS exposure and vitamin D biomarkers. Altered vitamin D levels associated with serum PFAS concentrations were observed in the general U.S. population using the data from NHANES 2003-2010 participants (n=7040), where a positive association of total 25(OH)D with PFHxS and an inverse association with PFOS concentrations were found predominantly in non-Hispanic whites than the other races/ethnicities (Etzel et al., 2019). No association between total 25(OH)D and PFAS was reported by Khalil et al. (2018) or Di Nisio et al. (2020b) with their smaller cohorts of obese children aged 8-12 years (n=47) and healthy males aged 18-21 years (n=100), respectively. The inconsistent findings across these studies suggest that more epidemiological studies with larger samples size are needed.

It is somewhat unexpected to observe that most PFAS exposures are associated with elevated 25(OH)D concentrations given the majority of environmental pollutants showing inverse associations with total 25(OH)D (Johns et al., 2016, 2017; Morales et al., 2013; Yang et al., 2012). Although the positive associations could be due to residual confounding, including behaviors and socioeconomic status, it is also possible that our results only partially captured non-monotonic dose-response relationships, which have been observed in numerous EDCs, especially given the relatively narrow range of serum PFAS concentrations in an environmental exposure cohort or lower 25(OH)D concentrations in African Americans (Ginde et al., 2010; Li et al., 2007; Vandenberg et al., 2012). Additionally, the positive associations also suggest that PFAS may have different actions in the vitamin D system from the other environmental pollutants (Etzel et al., 2019).

PFOA was shown to disrupt vitamin D activity in silico and in vitro by competing for vitamin D receptors with 1,25(OH)2D, the active metabolite of vitamin D (Di Nisio et al., 2020b) (Figure S4-3). The competition may reduce the activation of vitamin D receptor on the responsive gene expression and cause a functional hypovitaminosis D. For example, CYP24A1, a major 25(OH)D-inactivating cytochrome P450 enzyme in the liver, can be transcriptionally upregulated by activated vitamin D receptors (Jones et al., 2012; Ohyama et al., 1993). The antagonistic activity of PFOA via receptor competition may result in

downregulation of CYP24A1, thus elevation of circulating 25(OH)D. Similarly, PFOA may dysregulate CYP27B1 in the kidney, and lead to altered levels of 1,25(OH)2D and of 25(OH)D (Bikle, 2014; Johnson et al., 2014). It is worth noting that besides acting as a passive antagonist through competing for receptor binding, an EDC ligand bound to the hormone receptor may also function as agonist or active antagonist to induce or repress gene expression through recruiting coactivators or corepressors in a tissue context-dependent manner (Li et al., 2007; Smith et al., 1997; Smith & O'Malley, 2004). It is therefore possible that the biological effects of PFAS through vitamin D receptors can be bidirectional. Moreover, the homeostasis of vitamin D is also tightly regulated through feedbacks involving parathyroid hormone, calcium, phosphorus, and fibroblast growth factor; thus, it is possible for PFAS to influence vitamin D levels through interacting with the concentrations of these metabolites (Christakos et al., 2010; Johns et al., 2017). However, future studies are necessary to establish the actions of PFAS on the vitamin D system and elucidate the clinical and public health relevance of these findings.

The effects of PFAS on free 25(OH)D were not as predominant and consistent as those on total 25(OH)D across the Visit 1 and the Visit 2 models. Free 25(OH)D is present at very low concentrations (i.e., parts-per-trillion, 10-12) with low variance, which makes their measurements very challenging; the current immunoassay method has not been rigorously validated in a broad human population with various physiological conditions (Feldman et al., 2017; Jukic et al., 2018). Thus, the potential measurement errors coupled with relatively small variance could bias the associations between PFAS and free 25(OH)D to the null. Additionally, the associations between PFAS and total 25(OH)D may be driven by vitamin D binding proteins (DBP) or the affinity of DBP for 25(OH)D, which, in turn, may largely impact the levels of total 25(OH)D but not free 25(OH)D since approximately 85% of total 25(OH)D is bound to DBP and <1% is in its free form. Previous studies have shown that DBP production increases with elevated estrogen, glucocorticoids, and certain cytokine such as IL-6, and the affinity of DBP for 25(OH)D was also affected by estrogen concentrations (Best et al., 2019; Bikle & Schwartz, 2019; Pop et al., 2015). Since these physiological factors were also associated with PFAS exposure (Benninghoff et al., 2011; Li et al., 2020;

Liu et al., 2020; Pereiro, 2014; Son et al., 2009), it is possible that PFAS only indirectly influence DBP and total 25(OH)D through affecting endocrine systems or immune responses.

Some evidence of effect modification by fetal sex was observed in this study. Generally, we found larger effects on both total and free 25(OH)D among the women with male fetuses in the Visit 2 models. The heterogeneous effect by fetal sex may be due to the differences in vitamin D systems in the placenta. Previous studies have shown the levels of vitamin D receptors and CYP24A1 gene expression were higher in the placentas of women with male than female fetuses (Liu et al., 2018). Moreover, testosterone, which is higher on average in male fetuses, stimulates CYP24A1 and inhibits CYP27B1 gene expressions in the placenta (Olmos-Ortiz et al., 2016). It is thus likely that the clearance of 25(OH)D through CYP24A1 may be higher in the women with male fetuses than female fetuses, rendering it more sensitive to perturbations by PFAS as discussed above. Additionally, we observed larger effects of PFAS on both total and free 25(OH)D concentrations in the Visit 2 than the Visit 1 models. These findings could be explained by the higher means of total and free 25(OH)D concentrations measured at Visit 2 than at Visit 1. Although a higher mean of total 25(OH)D at Visit 2 is expected due to the increase in DBP during pregnancy, it is unclear why free 25(OH)D is also higher since its concentrations often remain the same or decrease during gestation (Bikle & Schwartz, 2019; Tsuprykov et al., 2019).

In addition to the single-PFAS models, we also investigated the associations between the PFAS mixtures and 25(OH)D concentrations. We identified that PFHxS has the strongest positive association with total 25(OH)D in the single-PFAS models but found PFHxS contributed little weight to the overall effects in the WQS regression models. Accordingly, PFPeA was inversely associated with total 25(OH)D in the single-chemical models, but no overall negative association between the PFAS mixture and total 25(OH)D was found. Although free 25(OH)D was significantly associated with some individual PFAS in women with male fetuses in the Visit 2 models, no significant effects nor significant effect modifications were observed in the mixture models. The inconsistent results between the mixture and single chemical models indicate the possibility of confounding effects among PFAS in the single-chemical models and also highlight the

importance of incorporating mixture analysis when there are high correlations and similar biological functions among the exposures of interest (Carrico et al., 2015).

Although we found that serum PFAS were associated with decreased odds of vitamin D deficiency, it is unlikely that PFAS would be "protective" to the vitamin D system. Because of its longer half-life, 25(OH)D is considered the best indicator to monitor vitamin D status compared with the other metabolites in the vitamin D system such as 1,25(OH)2D. Accordingly, vitamin D deficiency, which is associated with many adverse health outcomes, was often diagnosed by low serum total 25(OH)D concentrations (e.g., $\leq$ 20 ng/mL) (Holick et al., 2011). However, the reference level of vitamin D deficiency remains controversial, especially among African Americans due to genetic polymorphisms (Powe et al., 2018). Thus, the clinical implication of this finding remains unknown and needs further investigation. However, our findings in the models with the continuous 25(OH)D indicate that elevated PFAS concentrations were associated with changes in 25(OH)D concentrations and may cause perturbation on the vitamin D system.

Although the use of serum biomarkers to assess PFAS exposure is advantageous because of their ability to provide an integrated internal dose, many physiological conditions that influence serum biomarker concentrations may also affect or be affected by the health outcomes of interest, suggesting a possibility of introducing confounding effects. For example, it is possible that the observed associations were partly confounded by a third unknown factor which transports, metabolizes, or excretes both serum PFAS and 25(OH)D in the same fashion. This confounding issue is especially concerning in a cross-sectional study design (Fitz-Simon et al., 2013; Savitz & Wellenius, 2018; Steenland et al., 2009). A strength of our study was the repeated 25(OH)D measurements, which provide an opportunity to examine the associations in not only a cross-sectional (the Visit 1 models) but a prospective cohort (the Visit 2 models) study design. Confounding is less problematic in a cohort study design because the third unknown confounding factor may not simultaneously affect the exposure and outcome measured at two different time points. Additionally, the mixture models, which mutually adjusted for the other PFAS, can remove the confounding

effects if the physiological parameters (e.g., transportation, metabolism, and excretion) regulating the eight PFAS are correlated (Fletcher & Webster, 2020).

Our study was limited in several ways. First, several potential confounders that were either not measured or only measured in a subset of the participants, such as vitamin D supplement intake, fish intake, and albumin concentrations, were not included in the main analyses. However, we performed sensitivity analyses on either a subset of our cohort or different sub-populations in the NHANES to evaluate the impact of these covariates. The results show little impact of these variables on the associations between PFAS and 25(OH)D. Second, the low detection frequencies of PFPeA, PFDA, PFUnDA, and NMeFOSAA could bias the results. We found single imputation with LOD/$\sqrt{2}$ biased the effect estimates away from the null hypothesis in this study; thus, we presented the results using multiple instead of single imputation to mitigate the impact of the measurements below their respective LODs. Third, information on more vitamin D related metabolites such as calcium, phosphorous, parathyroid hormone, and DBP, which we did not measure, will improve our understandings of how PFAS may disturb vitamin D metabolism. Finally, our results from pregnant African American women limit the generalizability to other populations.

Conclusions

Our study provides suggestive evidence that exposure to PFAS might disturb vitamin D metabolism among pregnant African American women and that some of these effects might be modified by fetal sex. These results show potential explanations of the relationships between PFAS exposure and some adverse health effects reported by the previous studies, such as adverse skeletal health, and pregnancy and birth outcomes. Future experimental and observational studies are warranted to understand the underlying biological mechanisms, to confirm the findings in different populations, and to determine the implications of these findings to clinical practice and public health.

Figures and Tables

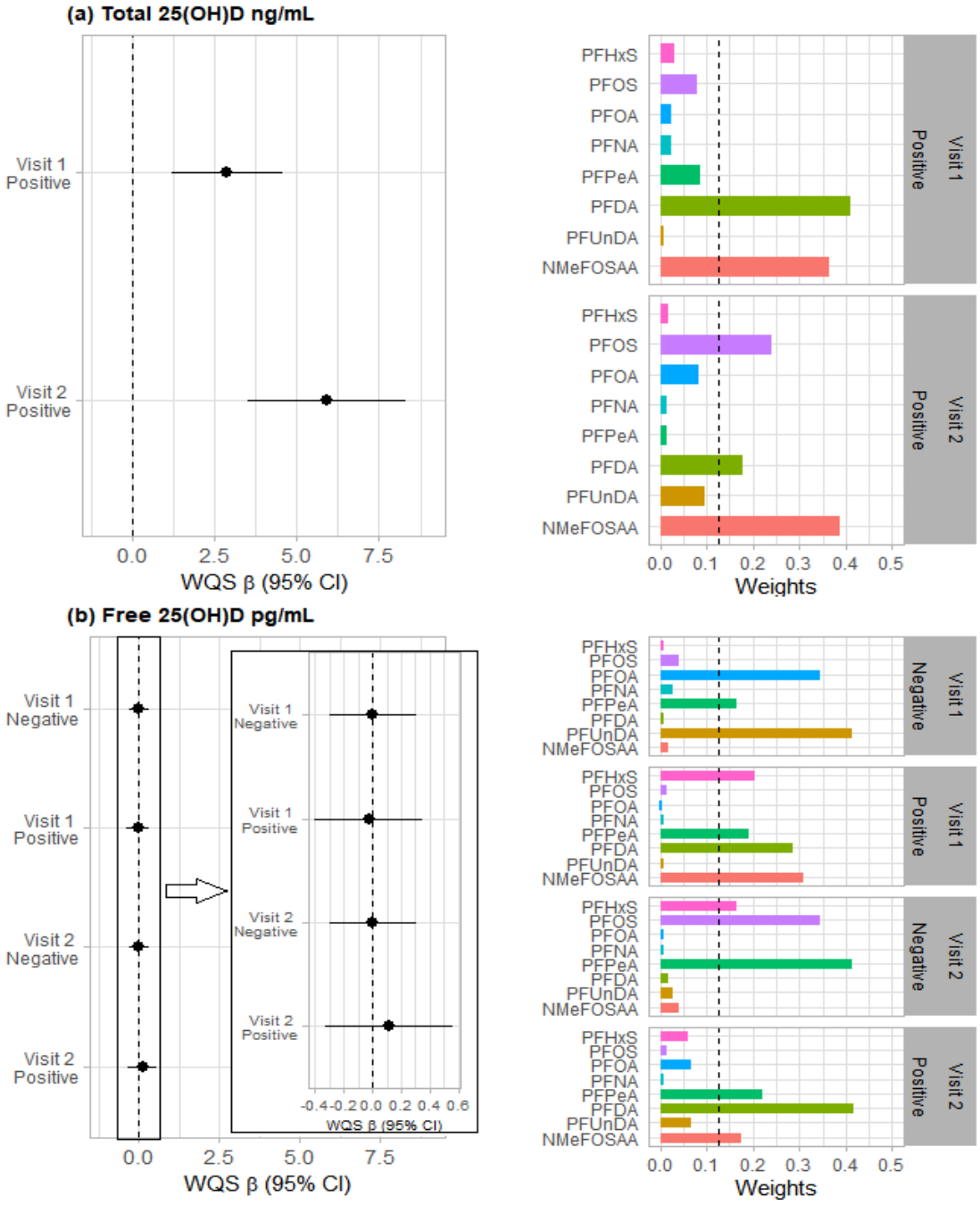

Figure 4-1. Associations of the PFAS mixture with (a) total 25(OH)D concentrations, and with (b) free 25(OH)D concentrations in pregnant African American women in the Atlanta area (2014-2018) based on weighted quantile sum regression (WQS) analyses.

The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D. We ran each model twice, one in positive and one in

negative direction of effects. Sample numbers are 346, 261, 348, and 264 for the models of total 25(OH)D at Visit 1, total 25(OH)D at Visit 2, free 25(OH)D at Visit 1, and free 25(OH)D at Visit 2, respectively. (Note: Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid. The dashed line on the right bar chart represent the *a priori* cut-point for identification of important agents: 1/numer of chemicals = 1/8 = 0.125)

Table 4-1. Serum 25(OH)D concentrations by selected population characteristics in pregnant African American women in the Atlanta area, 2014-2018 (n=442).

	Visit 1				Visit 2			
Characteristics	**n (%)[a]**	**Total 25(OH)D[b] (ng/mL) Mean ± SD**	**n (%)[a]**	**Free 25(OH)D[b] (pg/mL) Mean ± SD**	**n (%)[a]**	**Total 25(OH)D[b] (ng/mL) Mean ± SD**	**n (%)[a]**	**Free 25(OH)D[b] (pg/mL) Mean ± SD**
All participants (N=442)	436 (98.6)	19.5 ± 8.7	439 (99.3)	3.7 ± 1.4	337 (76.2)	23.3 ± 11.1	341 (77.1)	4.0 ± 1.7
Age (year)								
18-25	241 (54.5)	18.9 ± 8.5	241 (54.5)	3.6 ± 1.4	184 (41.6)	22.7 ± 10.6	186 (42.1)	3.9 ± 1.7
25-30	111 (25.1)	20.1 ± 8.8	114 (25.8)	3.7 ± 1.3	81 (18.3)	23.3 ± 10.9	84 (19.0)	3.9 ± 1.5
30-35	65 (14.7)	20.7 ± 8.8	65 (14.7)	3.9 ± 1.5	57 (12.9)	24.1 ± 10.9	57 (12.9)	4.2 ± 1.7
≥35	19 (4.3)	20.8 ± 11.8	19 (4.3)	4.4 ± 2.3	15 (3.4)	27.5 ± 17.1	14 (3.2)	4.6 ± 2.1
Education								
Less than high school	71 (16.1)	19.6 ± 8.1	71 (16.1)	3.6 ± 1.2	50 (11.3)	23.5 ± 9.8	50 (11.3)	4.0 ± 1.5
High school	166 (37.6)	17.6 ± 8.3	167 (37.8)	3.4 ± 1.4	126 (28.5)	21.9 ± 11.2	129 (29.2)	3.7 ± 1.5
Some college	130 (29.4)	20.2 ± 8.6	130 (29.4)	3.7 ± 1.3	102 (23.1)	22.4 ± 10.2	103 (23.3)	4.1 ± 1.8
College and above	69 (15.6)	22.9 ± 9.6	71 (16.1)	4.2 ± 1.7	59 (13.3)	27.5 ± 12.4	59 (13.3)	4.5 ± 1.7
Income-to-poverty ratio (%)								
<100	184 (41.6)	19.2 ± 9.2	186 (42.1)	3.6 ± 1.3	144 (32.6)	22.0 ± 10.8	147 (33.3)	3.8 ± 1.5
100-150	67 (15.2)	16.9 ± 7.1	68 (15.4)	3.3 ± 1.0	50 (11.3)	21.6 ± 9.6	51 (11.5)	3.6 ± 1.2
150-300	93 (21.0)	18.6 ± 7.8	94 (21.3)	3.7 ± 1.4	75 (17.0)	21.1 ± 9.3	75 (17.0)	4.0 ± 1.4
≥300	31 (7.0)	24.6 ± 9.9	31 (7.0)	4.7 ± 2.1	28 (6.3)	30.5 ± 14.7	28 (6.3)	4.7 ± 2.2
Married or cohabitating								
No	207 (46.8)	20.5 ± 8.8	209 (47.3)	3.8 ± 1.5	163 (36.9)	24.2 ± 11.7	165 (37.3)	4.1 ± 1.7
Yes	229 (51.8)	18.7 ± 8.7	230 (52.0)	3.6 ± 1.3	174 (39.4)	22.5 ± 10.4	176 (39.8)	3.9 ± 1.6
Insurance								
Private	95 (21.5)	22.5 ± 9.1	96 (21.7)	4.1 ± 1.7	81 (18.3)	25.5 ± 12.1	80 (18.1)	4.3 ± 1.8
Public (Medicaid)	341 (77.1)	18.7 ± 8.5	343 (77.6)	3.6 ± 1.3	256 (57.9)	22.6 ± 10.7	261 (59.0)	3.9 ± 1.6
Hospital								
Private (Emory)	175 (39.6)	21.3 ± 9.1	175 (39.6)	3.9 ± 1.5	140 (31.7)	24.3 ± 11.1	140 (31.7)	4.1 ± 1.6
Public (Grady)	261 (59.0)	18.4 ± 8.3	264 (59.7)	3.5 ± 1.4	197 (44.6)	22.6 ± 11.0	201 (45.5)	3.9 ± 1.7
Parity								
0	210 (47.5)	19.7 ± 8.3	210 (47.5)	3.7 ± 1.5	148 (33.5)	25.2 ± 11.8	148 (33.5)	4.3 ± 1.8
1	119 (26.9)	20.4 ± 9.5	121 (27.4)	3.8 ± 1.5	101 (22.9)	23.3 ± 10.6	103 (23.3)	3.9 ± 1.6
≥2	107 (24.2)	18.3 ± 8.7	108 (24.4)	3.5 ± 1.2	88 (19.9)	20.1 ± 9.7	90 (20.4)	3.5 ± 1.
BMI (kg/m^2)								
< 18.5	15 (3.4)	18.0 ± 10.1	15 (3.4)	3.8 ± 1.8	13 (2.9)	23.2 ± 12.5	13 (2.9)	4.3 ± 1.9
18.5-25	169 (38.2)	20.3 ± 8.1	171 (38.7)	3.7 ± 1.4	129 (29.2)	25.4 ± 10.2	132 (29.9)	4.1 ± 1.9
25-30	94 (21.3)	20.4 ± 9.8	94 (21.3)	3.8 ± 1.6	68 (15.4)	24.7 ± 12.8	70 (15.8)	3.9 ± 1.7
≥ 30	158 (35.7)	18.4 ± 8.5	159 (36.0)	3.6 ± 1.3	127 (28.7)	20.4 ± 10.3	126 (28.5)	3.9 ± 1.3
Fetal Sex								
Male	208 (47.1)	20.5 ± 9.4	208 (47.1)	3.9 ± 1.6	163 (36.9)	24.4 ± 11.5	165 (37.3)	4.1 ± 1.8
Female	225 (50.9)	18.6 ± 8.1	228 (51.6)	3.5 ± 1.2	174 (39.4)	22.3 ± 10.6	176 (39.8)	3.9 ± 1.5
Marijuana Use								

Characteristics	Visit 1				Visit 2			
	n (%)[a]	Total 25(OH)D[b] (ng/mL) Mean ± SD	n (%)[a]	Free 25(OH)D[b] (pg/mL) Mean ± SD	n (%)[a]	Total 25(OH)D[b] (ng/mL) Mean ± SD	n (%)[a]	Free 25(OH)D[b] (pg/mL) Mean ± SD
Not during pregnancy	343 (77.6)	20.1 ± 8.6	344 (77.8)	3.8 ± 1.5	261 (59.0)	24.4 ± 11.1	264 (59.7)	4.1 ± 1.7
During pregnancy	93 (21.0)	17.5 ± 9.1	95 (21.5)	3.3 ± 1.1	76 (17.2)	19.6 ± 10.3	77 (17.4)	3.5 ± 1.4
Tobacco Use								
Not during pregnancy	377 (85.3)	19.9 ± 8.6	378 (85.5)	3.7 ± 1.4	289 (65.4)	23.6 ± 11.2	291 (65.8)	4.1 ± 1.7
During pregnancy	59 (13.3)	17.5 ± 9.3	61 (13.8)	3.5 ± 1.3	48 (10.9)	21.4 ± 9.8	50 (11.3)	3.7 ± 1.4
Alcohol Use								
Not during pregnancy	400 (90.5)	19.5 ± 8.7	403 (91.2)	3.7 ± 1.4	307 (69.5)	23.3 ± 11.1	311 (70.4)	4.0 ± 1.6
During pregnancy	36 (8.1)	19.5 ± 8.8	36 (8.1)	3.6 ± 1.6	30 (6.8)	23.4 ± 11.0	30 (6.8)	4.0 ± 1.9
Sampling season[c]								
Winter (Dec-Feb)	79 (17.9)	15.9 ± 7.5	81 (18.3)	3.4 ± 1.3	115 (26.0)	21.4 ± 11.1	119 (26.9)	3.7 ± 1.5
Spring (Mar-May)	96 (21.7)	17.7 ± 7.0	96 (21.7)	3.5 ± 1.2	70 (15.8)	23.0 ± 12.6	71 (16.1)	3.8 ± 1.6
Summer (Jun-Aug)	123 (27.8)	24.6 ± 8.0	121 (27.4)	4.2 ± 1.4	70 (15.8)	24.0 ± 10.1	70 (15.8)	4.3 ± 1.8
Fall (Sep-Nov)	138 (31.2)	18.4 ± 9.3	141 (31.9)	3.5 ± 1.5	81 (18.3)	25.7 ± 10.1	81 (18.3)	4.3 ± 1.7
Vitamin D deficiency (Total 25(OH)D ≤ 20 ng/mL)								
No	198 (44.8)	27.1 ± 6.2	196 (44.3)	4.3 ± 1.5	197 (44.6)	30.5 ± 8.2	196 (44.3)	4.7 ± 1.8
Yes	238 (53.8)	13.3 ± 4.6	238 (53.8)	3.2 ± 1.1	140 (31.7)	13.1 ± 4.7	140 (31.7)	3.1 ± 0.9
The participants with Food frequency questionnaires (n=292)	287 (98.3)	19.6 ± 8.9	291 (99.7)	3.8 ± 1.5	238 (81.5)	23.3 ± 11.5	243 (83.2)	4.0 ± 1.7
Vitamin D supplement in the past three months[c]								
No	158 (55.1)	18.4 ± 8.2	161 (55.3)	3.6 ± 1.3	129 (54.2)	21.7 ± 11.7	131 (53.9)	3.8 ± 1.5
Yes	129 (44.9)	21.1 ± 9.5	130 (44.7)	4.0 ± 1.6	109 (45.8)	25.2 ± 11.0	112 (46.1)	4.3 ± 1.9
Fish consumption in the past three months[c]								
No	56 (19.5)	21.2 ± 8.3	55 (18.9)	4.0 ± 1.5	89 (37.4)	22.6 ± 11.2	91 (37.4)	3.9 ± 1.5
Yes	231 (80.5)	19.3 ± 9.0	236 (81.1)	3.7 ± 1.5	149 (62.6)	23.7 ± 11.6	152 (62.6)	4.1 ± 1.8

Note: Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; n = sample number; SD = standard deviation.

[a] The sample numbers might not be summed up to the total sample number due to missingness.

[b] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[c] Longitudinal variables: data were collected at two visits.

Table 4-2. Adjusted differences in serum 25(OH)D concentrations with per natural-log unit increase of serum PFAS concentrations (ng/mL) by fetal sex in pregnant African American women in the Atlanta area, 2014-2018 (n=442) .

25(OH)D		PFHxS[a]		PFOS[a]		PFOA[a]		PFNA[a]		PFPeA[b]		PFDA[b]		PFUnDA[b]		NMeFOSAA[b]	
		β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Total[a] Visit 1 (ng/mL)	**M**[c]	**2.58 (0.86, 4.30)**	**<0.01**	**2.79 (1.28, 4.31)**	**<0.01**	0.39 (-0.86, 1.64)	0.54	0.26 (-0.92, 1.45)	0.66	**-2.23 (-3.50, -0.95)**	**<0.01**	**1.59 (0.62, 2.57)**	**<0.01**	**-1.00 (-1.92, -0.08)**	**0.03**	**1.21 (0.34, 2.08)**	**0.01**
	F[c]	**4.15 (2.58, 5.72)**	**<0.01**	**3.56 (2.01, 5.12)**	**<0.01**	1.20 (-0.17, 2.56)	0.09	**1.49 (0.12, 2.87)**	**0.03**	-0.88 (-2.21, 0.45)	0.20	**1.88 (0.95, 2.81)**	**<0.01**	0.25 (-0.65, 1.16)	0.58	0.77 (-0.09, 1.62)	0.08
	p_{int}	0.18		0.48		0.39		0.18		0.14		0.67		**0.05**		0.49	
	n	433		433		433		433		346		346		346		346	
Total[a] Visit 2 (ng/mL)	**M**[c]	**4.71 (2.28, 7.14)**	**<0.01**	**4.64 (2.43, 6.84)**	**<0.01**	**2.91 (1.15, 4.67)**	**<0.01**	1.42 (-0.36, 3.19)	0.12	**-3.53 (-5.68, -1.38)**	**<0.01**	**2.59 (1.12, 4.06)**	**<0.01**	-1.17 (-2.56, 0.23)	0.10	**1.90 (0.60, 3.20)**	**0.01**
	F[c]	**3.53 (1.28, 5.77)**	**<0.01**	**2.65 (0.69, 4.61)**	**0.01**	0.76 (-1.13, 2.66)	0.43	1.11 (-0.84, 3.06)	0.26	-0.77 (-2.70, 1.16)	0.44	**2.52 (1.06, 3.99)**	**<0.01**	0.02 (-1.36, 1.40)	0.98	**1.31 (0.03, 2.60)**	**0.05**
	p_{int}	0.48		0.18		**0.10**		0.82		**0.08**		0.95		0.22		0.54	
	n	336		336		336		336		261		261		261		261	
Free[a] Visit 1 (pg/mL)	**M**[c]	0.13 (-0.18, 0.43)	0.42	0.18 (-0.09, 0.45)	0.19	0.07 (-0.14, 0.28)	0.52	0.00 (-0.21, 0.20)	0.97	-0.21 (-0.47, 0.06)	0.13	0.14 (-0.05, 0.34)	0.14	-0.14 (-0.31, 0.04)	0.12	0.08 (-0.07, 0.23)	0.29
	F[c]	-0.17 (-0.44, 0.10)	0.21	-0.19 (-0.44, 0.05)	0.12	-0.09 (-0.32, 0.13)	0.43	-0.17 (-0.40, 0.06)	0.14	0.06 (-0.16, 0.28)	0.60	0.05 (-0.12, 0.22)	0.57	-0.03 (-0.18, 0.12)	0.70	0.04 (-0.11, 0.19)	0.59
	p_{int}	0.14		**0.04**		0.30		0.28		0.14		0.48		0.36		0.70	
	n	436		436		436		436		348		348		348		348	
Free[a] Visit 2 (pg/mL)	**M**[c]	**0.41 (0.03, 0.79)**	**0.03**	**0.40 (0.06, 0.74)**	**0.02**	**0.29 (0.03, 0.56)**	**0.03**	0.20 (-0.07, 0.47)	0.14	**-0.37 (-0.72, -0.02)**	**0.04**	**0.28 (0.05, 0.52)**	**0.02**	-0.12 (-0.36, 0.13)	0.36	0.05 (-0.15, 0.25)	0.62
	F[c]	-0.10 (-0.45, 0.25)	0.57	-0.07 (-0.38, 0.23)	0.63	-0.01 (-0.30, 0.28)	0.94	-0.23 (-0.52, 0.07)	0.13	0.23 (-0.05, 0.52)	0.11	0.04 (-0.18, 0.26)	0.72	0.01 (-0.20, 0.22)	0.93	0.04 (-0.15, 0.22)	0.71
	p_{int}	**0.05**		**0.04**		0.13		**0.04**		**0.01**		0.15		0.45		0.91	
	n	341		341		341		341		264		264		264		264	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; M = male fetus; F = female fetus; p_{int} = *p*-value for interaction term; n = sample number.

[a] The values below limits of detection (LODs) were replaced by LOD/√2.

[b] The values below LODs were multiply imputed by a lognormal distribution and maximum likelihood estimation due to their lower detection frequencies (43-49%).

[c] The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D, and PFAS × fetal sex interaction term. The effect estimates (β) by fetal sex were derived from the model with the interaction term.

Supplementary

Method of the National Health and Nutrition Examination Survey (NHANES) data analyses

We included the National Health and Nutrition Examination Survey (NHANES) 2011-2014 participants with PFAS and vitamin D biomarkers in this sensitivity analyses to investigate the impact of albumin on the PFAS-vitamin D relationships. NHANES is a national representative cross-sectional survey collecting health information and biological samples from noninstitutionalized population in the U.S.

For PFAS analysis, solid phase extraction (SPE) coupled to high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was performed for quantifying serum perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and n-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA) concentrations. In the 2013-2014 cycle, both linear and branched isomer of PFOS and PFOA were analyzed. The detail analytical method was presented in Kuklenyik, et al. (2005). For total 25-hydrozyvitamin D (25(OH)D) analysis, serum samples were extracted from aqueous layer to hexane layer using liquid-liquid extraction, and then analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Quantitative detection of $25(OH)D_3$ and $25(OH)D_2$ was performed (CDC, 2010). A bichromatic digital endpoint method (DcX800), monitoring the changes in absorbance at 600nm, was used to quantified albumin levels (CDC, 2013).

Seven PFAS with the detection frequencies between 37% to 100% were included in the analyses − PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, and NMeFOSAA. In the 2013-2014 cycle, linear and branched isomers were summed for the total concentrations of PFOA and PFOS. PFAS values below limit of detections (LODs) were imputed with $LOD/\sqrt{2}$ (Hornung & Reed, 1990). Since PFAS concentrations were right-skewed, natural log-transformation was conducted to reduce the impact of potential outliers. Total 25(OH)D concentrations were calculated by summing vitamin D_2 and D_3 concentrations. To get demographically approximate populations to compare with our study population, in addition to all

participants, we performed the analyses on non-Hispanic black females, females between age 18 to 40 years old, and non-Hispanic black females between age 18 to 40 years old. Multivariable linear regressions were conducted to examine the association between PFAS and total 25(OH)D after adjusting for education levels (less than high school/high school/college/college and above), age (continuous, year), 6-month examination time period (during November 1st to April 30th/May 1st to October 31st), urine cotinine (continuous, ng/mL), BMI (<18.5/18.5-25/25-30/≥30, kg/m^2), and vitamin D supplement intake in the past 30 days (yes/no), race/ethnicity (Mexican American/other Hispanic/non-Hispanic white/non-Hispanic black/other), sex (male/female), and albumin (continuous, g/dL) The models were not adjusted for fish intake in the past 30 days due to the small number of participants with the information in the current analyses. Survey design was not considered in the analyses due to the small sample sizes among some subgroups.

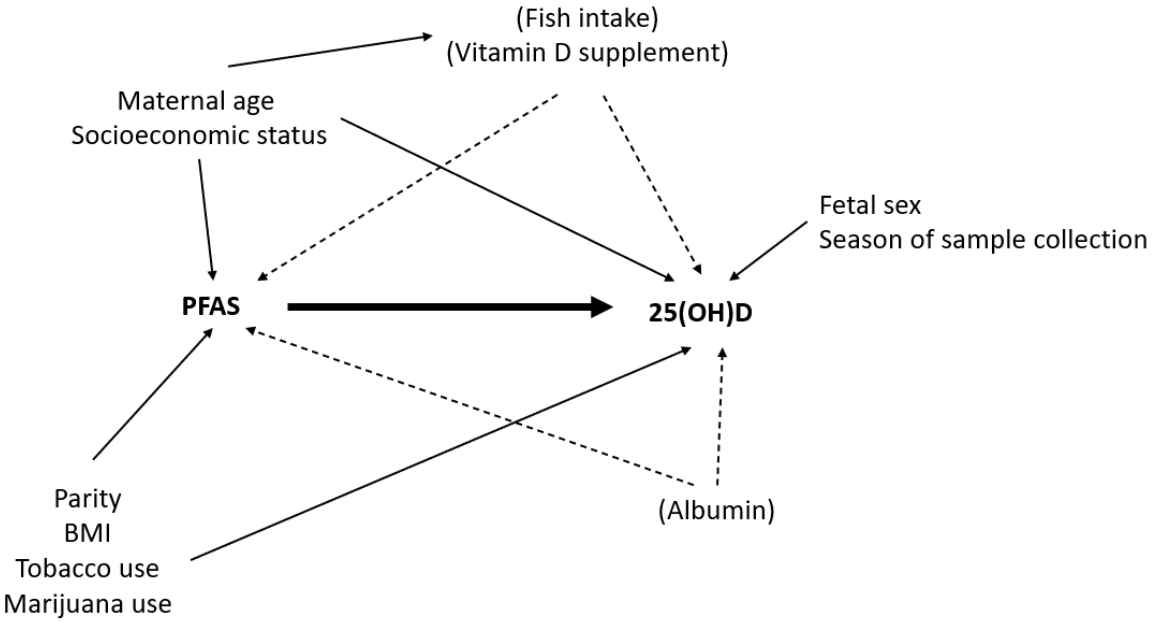

Figure S4-1. Directed acyclic graph showing the association between PFAS exposure and 25(OH)D.

Variables with parenthesis are potential confounders not controlled in the main analyses, but their impact was examined using sensitivity analyses. Fetal sex and season of sample collection were precision variables which predict the outcomes.

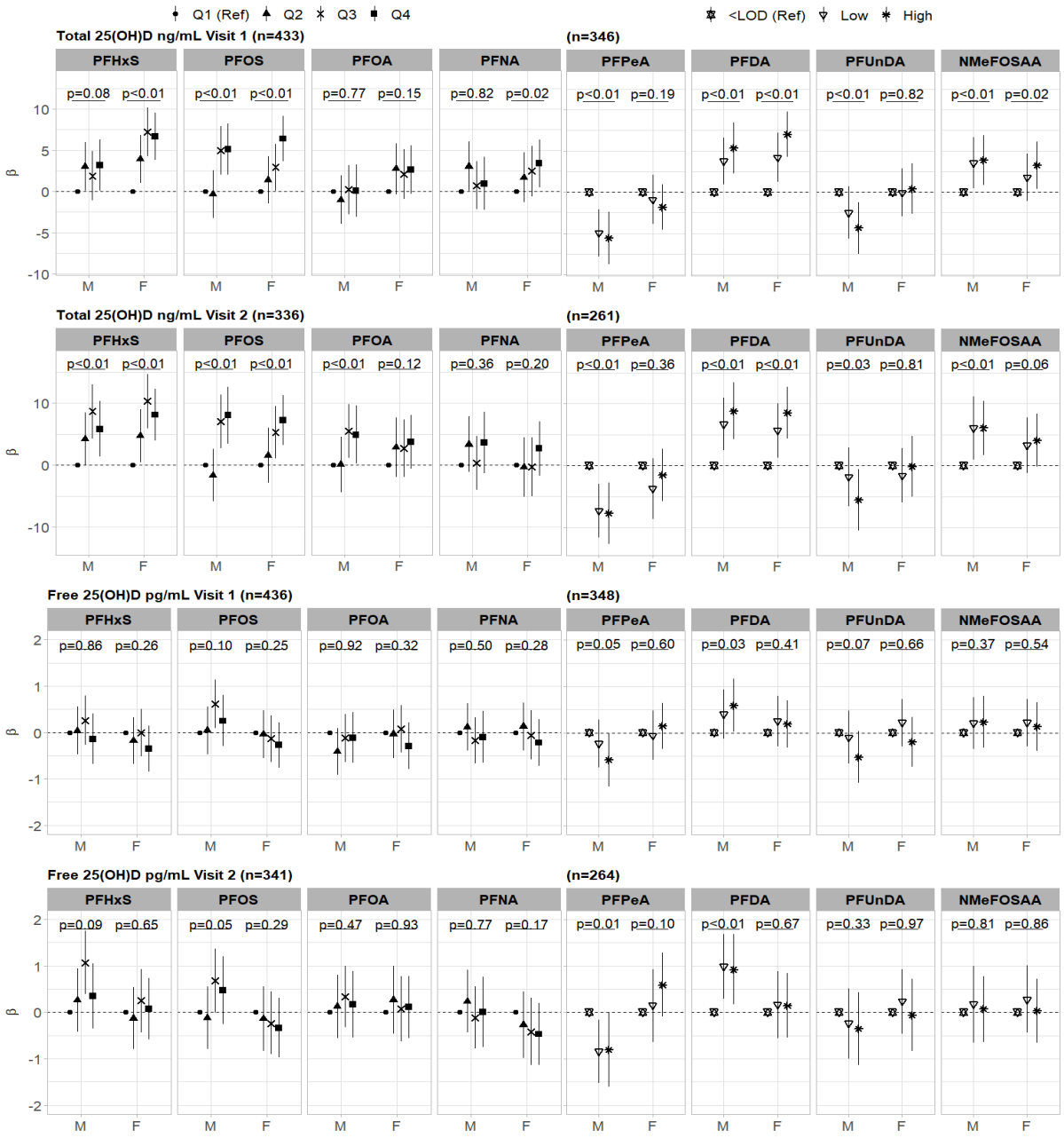

Figure S4-2. Differences and 95% confidence intervals in serum 25(OH)D concentrations among PFAS exposure groups by fetal sex in pregnant African American women in the Atlanta area, 2014-2018.

The models were adjusted for maternal age, education, BMI, parity, tobacco use, marijuana use, and season of sample collection for 25(OH)D. Q1, Q2, Q3, Q4 were exposure groups devided by quartiles, and low and high were exposure groups devided by medians. (Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; <LOD = the concentrations below the limits of detection; *p* = *p*-value for trend test; n = sample number; M = male fetus, F = female fetus; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation)

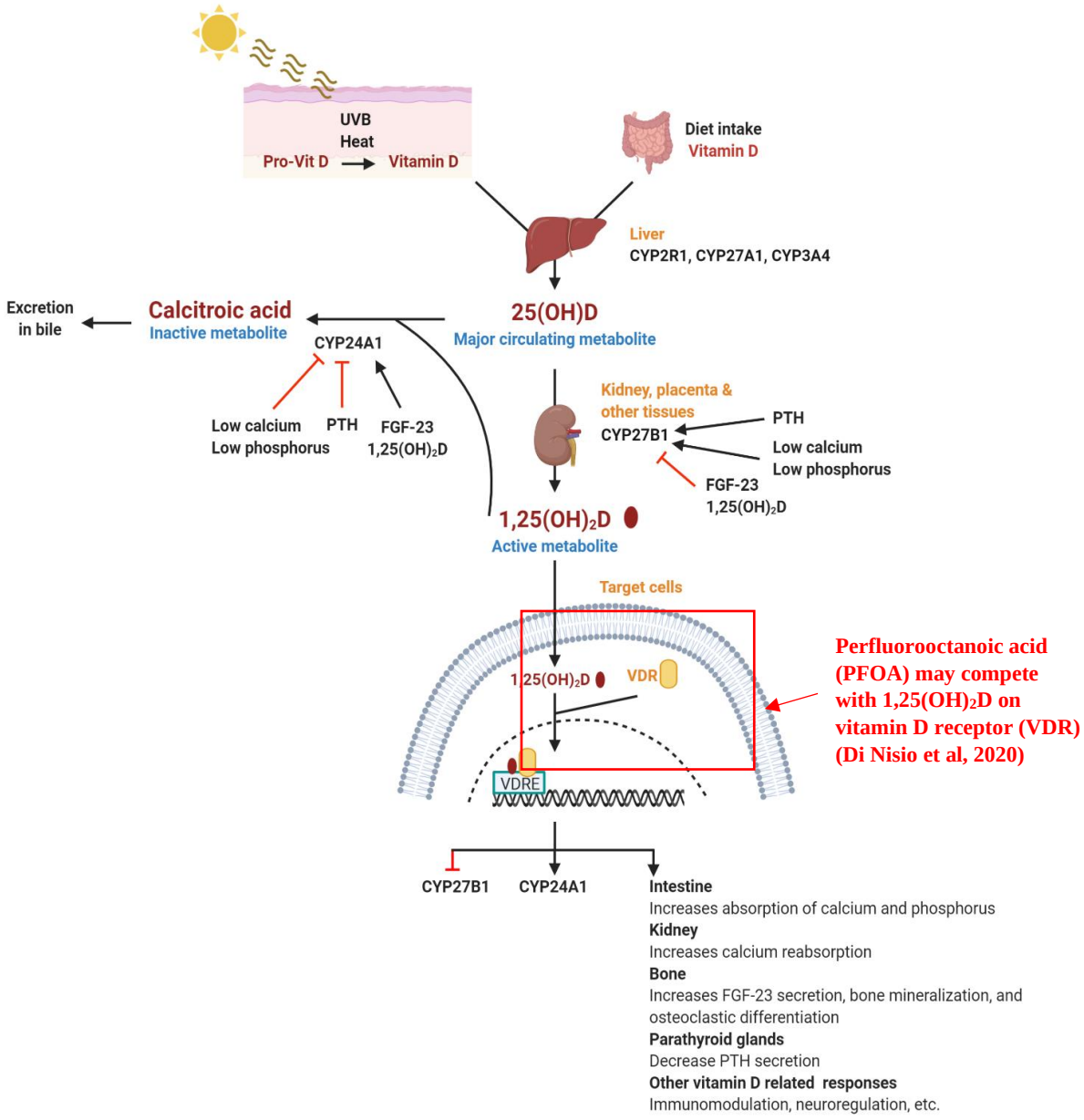

Figure S4-3. Vitamin D metaboloic pathway and potential interaction with PFOA.
Note: 25(OH)D = 25-hydroxyvitamin D; PTH = parathyroid hormone; FGF-23 = fibroblast growth factor-23; $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D; VDR = vitamin D receptor; VDRE = vitamin D response element.
This figure is adapted from Jeon & Shin (2018) and Mousavi, et al., (2019), and created with BioRender.com.

Table S4-1. Overlapped samples concentrations (ng/mL) from the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University and Wadsworth Center/New York University Laboratory Hub.

	PFHxS		PFOS		PFOA		PFNA	
Sample ID	**W**[b]	**E**	**W**[b]	**E**	**W**[b]	**E**	**W**[b]	**E**
1	1.515	1.398	3.506	3.735	1.305	1.299	0.435	0.550
2	0.710	0.587	1.719	1.021	0.492	0.425	0.868	0.811
3	1.055	1.283	1.392	1.215	0.677	0.583	0.372	0.351
4	0.679	0.565	1.733	1.967	0.228	0.376	0.091	0.214
5	0.794	0.601	1.469	1.393	0.848	1.114	0.259	0.419
6	0.999	0.606	5.145	4.509	0.734	0.900	0.197	0.357
7	0.831	0.835	2.478	2.388	0.624	0.566	0.264	0.337
8	1.534	1.645	2.325	1.670	1.013	1.133	0.285	0.401
9	1.332	0.948	2.882	2.559	1.315	1.870	0.402	0.553
10	1.830	1.264	3.965	4.430	1.289	1.845	0.632	0.876
11	1.850	1.835	2.597	2.269	1.350	2.100	0.231	0.410
Pearson correlation coefficients	**0.88**		**0.93**		**0.92**		**0.93**	
Relative percent differences, %RPD; Median (Min-Max)[a]	**4.75 (0.12-12.3)**		**3.17 (0.92-12.7)**		**5.05 (0.12-12.3)**		**8.11 (1.48-20.2)**	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.

[a] %RPD = (Lab 1 result – Lab 2 result)*100/(Lab 1 result + Lab 2 result)/2.

[b]The results from Wadsworth were used in the main analysis for these overlapped samples.

Table S4-2. Serum 25(OH)D concentrations in pregnant African American women in the Atlanta area, 2014-2018.

	n	LOD	% > LOD	Mean[a]	SD[a]	P25	P50	P75	P95	Max
Total 25(OH)D (Visit 1) (ng/mL)	436	7	92.9	19.5	8.7	13.3	19.0	25.3	34.8	56.7
Total 25(OH)D (Visit 2) (ng/mL)	337	7	94.4	23.3	11.1	15.1	22.7	29.7	42.9	77.3
Free 25(OH)D (Visit 1) (pg/mL)	439	2.4	87.2	3.7	1.4	3.38	3.62	4.32	6.20	12.2
Free 25(OH)D (Visit 2) (pg/mL)	341	2.4	90.3	4.0	1.7	3.61	3.90	4.78	7.34	12.9

Note: n = sample number; LOD = limits of detection; SD = standard deviation; P25 = the 25th percentile; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation.
[a] The values below LODs were replaced by LOD/$\sqrt{2}$.

Table S4-3. Serum PFAS concentrations (ng/mL) in pregnant African American women in the Atlanta area, 2014-2018.

	n	LOD	% > LOD	GM[a]	GSD[a]	P25	P50	P75	P95	Max
Perfluorohexane sulfonic acid (PFHxS)	442	0.02 (W); 0.2 (E)	97.1	1.00	1.93	0.75	1.10	1.52	2.31	4.80
Perfluorooctane sulfonic acid (PFOS)	442	0.02 (W); 0.05 (E)	98.6	2.03	2.08	1.44	2.19	3.23	5.36	12.4
Perfluorooctanoic acid (PFOA)	442	0.035 (W); 0.2 (E)	97.5	0.63	2.32	0.46	0.71	1.07	1.68	4.42
Perfluorononanoic acid (PFNA)	442	0.02 (W); 0.1 (E)	96.4	0.24	2.37	0.15	0.27	0.42	0.75	2.27
Perfluoropentanoic acid (PFPeA)	351	0.05 (W)	47.9	--	--	<LOD	<LOD	0.11	0.22	0.66
Perfluorodecanoic acid (PFDA)	351	0.035 (W)	48.4	--	--	<LOD	<LOD	0.13	0.27	1.06
Perfluoroundecanoic acid (PFUnDA)	351	0.02 (W)	43.3	--	--	<LOD	<LOD	0.06	0.18	0.53
N-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA)	351	0.02 (W)	49.0	--	--	<LOD	<LOD	0.07	0.26	1.46

Note: n = sample number; LOD = limits of detection; GM= geometric mean; GSD = geometric standard deviation; P25 = the 25th percentile; W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.
[a] Geometric means and standard deviations were not calculated for congeners with detection frequencies <50%; the values below LODs were replaced by LOD/$\sqrt{2}$.

Table S4-4. Pearson correlation coefficients of 25(OH)D and natural-log unit serum PFAS concentrations in pregnant African American women in the Atlanta area, 2014-2018.

		PFAS (ng/mL)								25(OH)D			
		PFHxS[a]	PFOS[a]	PFOA[a]	PFNA[a]	PFPeA[b]	PFDA[b]	PFUnDA[b]	NMe FOSAA[b]	Total[a] (ng/mL) (Visit1)	Total[a] (ng/mL) (Visit2)	Free[a] (pg/mL) (Visit1)	Free[a] (pg/mL) (Visit2)
		r (*p*)											
PFAS	**PFHxS[a]**	1.00	**0.67 (<0.01)**	**0.46 (<0.01)**	**0.35 (<0.01)**	**-0.23 (<0.01)**	**0.41 (<0.01)**	**0.12 (0.05)**	0.06 (0.29)	**0.30 (<0.01)**	**0.29 (<0.01)**	0.02 (0.69)	0.10 (0.07)
	PFOS[a]		1.00	**0.67 (<0.01)**	**0.65 (<0.01)**	**-0.29 (<0.01)**	**0.49 (<0.01)**	**0.24 (<0.01)**	0.11 (0.06)	**0.31 (<0.01)**	**0.28 (<0.01)**	0.03 (0.49)	**0.10 (0.05)**
	PFOA[a]			1.00	**0.72 (<0.01)**	**-0.13 (0.02)**	**0.34 (<0.01)**	**0.19 (0.04)**	0.06 (0.32)	**0.10 (0.04)**	**0.15 (0.04)**	0.01 (0.90)	0.09 (0.10)
	PFNA[a]				1.00	**-0.21 (<0.01)**	**0.46 (<0.01)**	**0.31 (<0.01)**	0.10 (0.08)	**0.13 (0.01)**	**0.11 (0.04)**	-0.01 (0.83)	0.02 (0.71)
	PFPeA[b]					1.00	**-0.31 (<0.01)**	0.02 (0.77)	-0.04 (0.52)	**-0.23 (<0.01)**	**-0.21 (<0.01)**	-0.09 (0.13)	-0.06 (0.38)
	PFDA[b]						1.00	0.09 (0.13)	**0.13 (0.03)**	**0.34 (<0.01)**	**0.32 (<0.01)**	**0.13 (0.02)**	**0.15 (0.02)**
	PFUnDA[b]							1.00	**-0.16 (0.01)**	-0.01 (0.91)	-0.02 (0.82)	-0.02 (0.70)	0.01 (0.93)
	NMeFOSAA[b]								1.00	**0.14 (0.01)**	**0.20 (<0.01)**	0.02 (0.74)	0.02 (0.78)
25(OH)D	**Total (Visit 1)[a]**									1.00	**0.72 (<0.01)**	**0.56 (<0.01)**	**0.43 (<0.01)**
	Total (Visit 2)[a]										1.00	**0.48 (<0.01)**	**0.62 (<0.01)**
	Free (Visit 1)[a]											1.00	**0.77 (<0.01)**
	Free (Visit 2)[a]												1.00

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; *r* = correlation coefficients; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation.

[a] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[b] The values below LODs were multiply imputed by a lognormal distribution and maximum likelihood estimation due to their lower detection frequencies (43-49%).

Table S4-5. Adjusted differences in serum 25(OH)D concentrations with per natural-log unit increase of serum PFAS concentrations (ng/mL) in pregnant African American women in the Atlanta area, 2014-2018.

25(OH)D		PFHxS[a]		PFOS[a]		PFOA[a]		PFNA[a]		PFPeA[b]		PFDA[b]		PFUnDA[b]		NMeFOSAA[b]	
		β (95% CI)	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
Total[a] Visit 1 (ng/mL)	**All[c]**	**3.44 (2.27, 4.61)**	**<0.01**	**3.17 (2.07, 4.27)**	**<0.01**	0.76 (-0.18, 1.69)	0.11	0.79 (-0.12, 1.69)	0.09	**-1.52 (-2.46, -0.57)**	**<0.01**	**1.74 (1.06, 2.42)**	**<0.01**	-0.36 (-1.02, 0.30)	0.29	**0.97 (0.38, 1.56)**	**<0.01**
	n	433		433		433		433		346		346		346		346	
Total[a] Visit 2 (ng/mL)	**All[c]**	**4.07 (2.40, 5.73)**	**<0.01**	**3.53 (2.05, 5.00)**	**<0.01**	**1.92 (0.62, 3.22)**	**<0.01**	1.28 (-0.03, 2.59)	0.06	**-2.05 (-3.38, -0.71)**	**<0.01**	**2.55 (1.44, 3.67)**	**<0.01**	-0.56 (-1.57, 0.44)	0.27	**1.59 (0.69, 2.48)**	**<0.01**
	n	336		336		336		336		261		261		261		261	
Free[a] Visit 1 (pg/mL)	**All[c]**	-0.04 (-0.25, 0.16)	0.68	-0.03 (-0.21, 0.16)	0.79	-0.01 (-0.16, 0.15)	0.93	-0.08 (-0.23, 0.08)	0.32	-0.07 (-0.23, 0.10)	0.43	0.10 (-0.03, 0.22)	0.13	-0.08 (-0.19, 0.03)	0.15	0.06 (-0.05, 0.16)	0.27
	n	436		436		436		436		348		348		348		348	
Free[a] Visit 2 (pg/mL)	**All[c]**	0.14 (-0.13, 0.40)	0.31	0.14 (-0.09, 0.37)	0.25	0.15 (-0.04, 0.35)	0.13	0.01 (-0.19, 0.21)	0.95	-0.04 (-0.27, 0.18)	0.69	0.16 (0.00, 0.33)	0.06	-0.05 (-0.21, 0.11)	0.54	0.04 (-0.09, 0.18)	0.53
	n	341		341		341		341		264		264		264		264	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; n = sample number.

[a] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[b] The values below LODs were multiply imputed by a lognormal distribution and maximum likelihood estimation due to their lower detection frequencies (43-49%).

[c] The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D.

Table S4-6. The dose-response relationships of PFAS exposure groups with serum 25(OH)D concentrations by fetal sex in pregnant African American women in the Atlanta area, 2014-2018.

				Total 25(OH)D[a] (ng/mL) Visit 1			Visit 2			Free 25(OH)D[a] (pg/mL) Visit 1			Visit 2		
		Concentration range (ng/mL)	**n**	**β (95% CI)[b]**	***p***	***p* for trend[c]**	**β (95% CI)[b]**	***p***	***p* for trend[c]**	**β (95% CI)[b]**	***p***	***p* for trend[c]**	**β (95% CI)[b]**	***p***	***p* for trend[c]**
PFHxS	**M**	Q1: <LOD-0.73	49	Ref		0.08	Ref		**0.02**	Ref		0.86	Ref		0.09
		Q2: 0.73-1.10	56	**3.04 (0.09, 5.99)**	**0.04**		4.21 (-0.13, 8.54)	0.06		0.04 (-0.48, 0.56)	0.88		0.26 (-0.42, 0.94)	0.46	
		Q3: 0.10-1.52	56	1.90 (-1.11, 4.91)	0.22		**8.66 (4.26, 13.1)**	**<0.01**		0.26 (-0.27, 0.79)	0.33		1.07 (0.39, 1.75)	<0.01	
		Q4: 1.52-4.80	49	**3.18 (0.11, 6.25)**	**0.04**		**5.85 (1.39, 10.3)**	**0.01**		-0.14 (-0.68, 0.41)	0.62		0.35 (-0.35, 1.05)	0.33	
	F	Q1: <LOD-0.73	56	Ref		**<0.01**	Ref		**<0.01**	Ref		0.26	Ref		0.65
		Q2: 0.73-1.10	56	**3.93 (1.05, 6.80)**	**0.01**		4.71 (0.43, 8.98)	0.03		-0.18 (-0.68, 0.33)	0.49		-0.13 (-0.80, 0.54)	0.70	
		Q3: 0.10-1.52	55	**7.22 (4.30, 10.1)**	**<0.01**		**10.3 (5.96, 14.7)**	**<0.01**		0.00 (-0.52, 0.51)	0.99		0.25 (-0.44, 0.93)	0.48	
		Q4: 1.52-4.80	62	**6.68 (3.83, 9.53)**	**<0.01**		**8.13 (3.96, 12.3)**	**<0.01**		-0.35 (-0.84, 0.15)	0.18		0.07 (-0.59, 0.73)	0.83	
PFOS	**M**	Q1: <LOD-1.43	51	Ref		**<0.01**	Ref		**<0.01**	Ref		0.10	Ref		**0.05**
		Q2: 1.44-2.17	58	-0.33 (-3.21, 2.55)	0.82		-1.65 (-5.88, 2.58)	0.44		0.05 (-0.47, 0.56)	0.86		-0.12 (-0.79, 0.55)	0.72	
		Q3: 2.19-3.22	56	**4.98 (2.05, 7.91)**	**<0.01**		**7.04 (2.68, 11.4)**	**<0.01**		**0.62 (0.09, 1.14)**	**0.02**		**0.68 (-0.01, 1.37)**	**0.05**	
		Q4: 3.24-12.4	45	**5.11 (2.02, 8.20)**	**<0.01**		**8.05 (3.44, 12.7)**	**<0.01**		0.25 (-0.30, 0.81)	0.37		0.47 (-0.26, 1.20)	0.21	
	F	Q1: <LOD -1.43	58	Ref		**<0.01**	Ref		**<0.01**	Ref		0.25	Ref		0.29
		Q2: 1.44-2.17	51	1.40 (-1.48, 4.29)	0.34		1.57 (-2.86, 6.00)	0.49		-0.04 (-0.55, 0.47)	0.87		-0.14 (-0.84, 0.56)	0.69	
		Q3: 2.19-3.22	55	**2.93 (0.08, 5.78)**	**0.04**		**5.27 (1.03, 9.50)**	**0.02**		-0.13 (-0.64, 0.37)	0.60		-0.24 (-0.91, 0.44)	0.49	
		Q4: 3.24-12.4	65	**6.40 (3.62, 9.19)**	**<0.01**		**7.27 (3.21, 11.3)**	**<0.01**		-0.27 (-0.76, 0.22)	0.28		-0.34 (-0.98, 0.31)	0.31	
PFOA	**M**	Q1: <LOD-0.46	58	Ref		0.77	Ref		**0.01**	Ref		0.92	Ref		0.47
		Q2: 0.46-0.71	55	-1.02 (-3.97, 1.93)	0.50		0.12 (-4.37, 4.60)	0.96		-0.41 (-0.92, 0.09)	0.11		0.13 (-0.56, 0.81)	0.72	
		Q3: 0.71-1.07	53	0.21 (-2.80, 3.22)	0.89		**5.49 (1.15, 9.82)**	**0.01**		-0.12 (-0.64, 0.39)	0.63		0.34 (-0.32, 0.99)	0.32	
		Q4: 1.08-4.42	44	0.10 (-3.06, 3.26)	0.95		4.90 (0.20, 9.59)	0.04		-0.11 (-0.65, 0.43)	0.70		0.17 (-0.55, 0.89)	0.65	
	F	Q1: <LOD-0.46	52	Ref		0.15	Ref		0.12	Ref		0.32	Ref		0.93
		Q2: 0.46-0.71	53	2.75 (-0.34, 5.83)	0.08		2.86 (-1.94, 7.65)	0.24		-0.03 (-0.56, 0.49)	0.90		0.27 (-0.47, 1.00)	0.48	
		Q3: 0.71-1.07	58	2.11 (-0.92, 5.13)	0.17		2.72 (-1.93, 7.37)	0.25		0.08 (-0.43, 0.59)	0.76		0.07 (-0.63, 0.78)	0.84	
		Q4: 1.08-4.42	66	2.63 (-0.33, 5.59)	0.08		3.77 (-0.57, 8.11)	0.09		-0.29 (-0.79, 0.21)	0.26		0.11 (-0.56, 0.77)	0.75	
PFNA	**M**	Q1: <LOD-0.16	58	Ref		0.82	Ref		0.36	Ref		0.50	Ref		0.77
		Q2: 0.16-0.27	53	**3.05 (0.08, 6.03)**	**0.04**		3.34 (-1.15, 7.83)	0.15		0.12 (-0.39, 0.63)	0.65		0.23 (-0.44, 0.91)	0.50	
		Q3: 0.27-0.42	58	0.72 (-2.17, 3.62)	0.63		0.34 (-4.03, 4.71)	0.88		-0.17 (-0.67, 0.33)	0.51		-0.12 (-0.78, 0.55)	0.73	
		Q4: 0.42-2.27	41	0.96 (-2.26, 4.19)	0.56		3.67 (-1.30, 8.63)	0.15		-0.10 (-0.65, 0.46)	0.73		0.00 (-0.75, 0.76)	0.99	
	F	Q1: <LOD-0.16	53	Ref		**0.02**	Ref		0.20	Ref		0.28	Ref		0.17
		Q2: 0.16-0.27	55	1.68 (-1.33, 4.70)	0.27		-0.32 (-5.11, 4.47)	0.90		0.13 (-0.39, 0.64)	0.63		-0.27 (-0.99, 0.45)	0.46	
		Q3: 0.27-0.42	52	2.47 (-0.60, 5.54)	0.12		-0.28 (-5.03, 4.47)	0.91		-0.06 (-0.59, 0.47)	0.83		-0.42 (-1.14, 0.31)	0.26	
		Q4: 0.42-2.27	69	**3.39 (0.48, 6.31)**	**0.02**		2.68 (-1.73, 7.09)	0.23		-0.22 (-0.72, 0.28)	0.39		-0.47 (-1.14, 0.20)	0.17	
PFPeA	**M**	<LOD	93	Ref		**<0.01**	Ref		**<0.01**	Ref		**0.05**	Ref		**0.01**
		Low: 0.05-0.11	43	**-4.94 (-7.83, -2.05)**	**<0.01**		**-7.30 (-11.7, -2.95)**	**<0.01**		-0.23 (-0.75, 0.29)	0.39		**-0.84 (-1.53, -0.16)**	**0.02**	
		High: 0.11-0.66	33	**-5.57 (-8.77, -2.37)**	**<0.01**		**-7.74 (-12.7, -2.76)**	**<0.01**		**-0.58 (-1.16, 0.00)**	**0.05**		**-0.81 (-1.61, 0.00)**	**0.05**	
	F	<LOD	90	Ref		0.19	Ref		0.36	Ref		0.60	Ref		0.10
		Low: 0.05-0.11	41	-0.90 (-3.89, 2.10)	0.56		-3.77 (-8.65, 1.12)	0.13		-0.06 (-0.59, 0.48)	0.84		0.15 (-0.64, 0.93)	0.71	
		High: 0.11-0.66	51	-1.82 (-4.57, 0.93)	0.20		-1.52 (-5.78, 2.74)	0.49		0.15 (-0.35, 0.64)	0.56		0.59 (-0.09, 1.28)	0.09	

				Total 25(OH)D[a] (ng/mL) Visit 1			Visit 2			Free 25(OH)D[a] (pg/mL) Visit 1			Visit 2		
		Concentration range (ng/mL)	**n**	**β (95% CI)[b]**	***p***	***p* for trend[c]**	**β (95% CI)[b]**	***p***	***p* for trend[c]**	**β (95% CI)[b]**	***p***	***p* for trend[c]**	**β (95% CI)[b]**	***p***	***p* for trend[c]**
PFDA	**M**	<LOD	91	Ref		**<0.01**	Ref		**<0.01**	Ref		**0.03**	Ref		**<0.01**
		Low: 0.04-0.13	45	**3.77 (0.93, 6.61)**	**0.01**		**6.71 (2.47, 10.9)**	**<0.01**		0.40 (-0.13, 0.93)	0.14		**0.99 (0.30, 1.69)**	**0.01**	
		High: 0.13-1.06	33	**5.35 (2.27, 8.43)**	**<0.01**		**8.81 (4.22, 13.4)**	**<0.01**		**0.59 (0.02, 1.17)**	**0.04**		**0.92 (0.17, 1.68)**	**0.02**	
	F	<LOD	90	Ref		**<0.01**	Ref		<0.01	Ref		0.41	Ref		0.67
		Low: 0.04-0.13	40	**4.21 (1.23, 7.19)**	**0.01**		**5.67 (1.29, 10.1)**	**0.01**		0.25 (-0.30, 0.79)	0.38		0.16 (-0.56, 0.89)	0.66	
		High: 0.13-1.06	52	**7.01 (4.25, 9.76)**	**<0.01**		**8.54 (4.35, 12.7)**	**<0.01**		0.19 (-0.32, 0.70)	0.46		0.14 (-0.55, 0.84)	0.69	
PFUnDA	**M**	<LOD	99	Ref		**<0.01**	Ref		**0.03**	Ref		0.07	Ref		0.33
		Low: 0.02-0.07	34	-2.49 (-5.66, 0.67)	0.12		-1.83 (-6.61, 2.95)	0.45		-0.10 (-0.67, 0.47)	0.72		-0.24 (-1.00, 0.52)	0.54	
		High: 0.07-0.53	36	**-4.35 (-7.48, -1.22)**	**0.01**		**-5.58 (-10.5, -0.62)**	**0.03**		-0.53 (-1.08, 0.03)	0.06		-0.35 (-1.14, 0.43)	0.38	
	F	<LOD	100	Ref		0.82	Ref		0.81	Ref		0.66	Ref		0.97
		Low: 0.02-0.07	43	-0.05 (-2.95, 2.84)	0.97		-1.61 (-5.97, 2.76)	0.47		0.22 (-0.30, 0.73)	0.41		0.24 (-0.46, 0.93)	0.50	
		High: 0.07-0.53	39	0.41 (-2.65, 3.48)	0.79		-0.15 (-5.06, 4.75)	0.95		-0.20 (-0.74, 0.34)	0.47		-0.06 (-0.84, 0.72)	0.87	
NMeFOSAA	**M**	<LOD	92	Ref		**<0.01**	Ref		**<0.01**	Ref		0.37	Ref		0.81
		Low: 0.02-0.07	35	3.55 (0.44, 6.66)	0.03		**6.08 (0.98, 11.2)**	**0.02**		0.21 (-0.35, 0.77)	0.46		0.18 (-0.65, 1.00)	0.67	
		High: 0.07-1.46	42	**3.88 (0.85, 6.91)**	**0.01**		**6.08 (1.66, 10.5)**	**0.01**		0.23 (-0.32, 0.79)	0.41		0.07 (-0.64, 0.78)	0.84	
	F	<LOD	87	Ref		**0.02**	Ref		0.06	Ref		0.54	Ref		0.86
		Low: 0.02-0.07	50	1.81 (-1.06, 4.67)	0.22		3.26 (-1.23, 7.74)	0.16		0.22 (-0.29, 0.73)	0.39		0.28 (-0.44, 1.01)	0.44	
		High: 0.07-1.46	45	**3.26 (0.40, 6.12)**	**0.03**		4.06 (-0.22, 8.34)	0.06		0.13 (-0.39, 0.65)	0.62		0.03 (-0.66, 0.72)	0.93	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; n = sample number; CI = confidence interval; *p* = *p*-value; M = male fetus; F = female fetus; <LOD = below limits of detection; Ref = reference group.

[a] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[b] The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D, and PFAS × fetal sex interaction term. The effect estimates (β) by fetal sex were derived from the model with the interaction term.

[c] Test for trend was performed by modeling the exposure categories as ordinal variables.

Table S4-7. Adjusted odds ratios for natural-log unit PFAS concentrations (ng/mL) and vitamin D deficiency (≤ 20 total 25(OH)D ng/mL) by fetal sex in pregnant African American women in the Atlanta area, 2014-2018.

25(OH)D		PFHxS[a]		PFOS[a]		PFOA[a]		PFNA[a]		PFPeA[b]		PFDA[b]		PFUnDA[b]		NMeFOSAA[b]	
		OR (95% CI)	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Vitamin D deficiency Visit 1	**All**[c]	**0.44 (0.30, 0.64)**	**<0.01**	**0.46 (0.32, 0.66)**	**<0.01**	0.84 (0.65, 1.09)	0.20	0.79 (0.61, 1.02)	0.07	**1.49 (1.11, 2.01)**	**0.01**	**0.61 (0.51, 0.74)**	**<0.01**	1.08 (0.89, 1.31)	0.42	**0.82 (0.70, 0.96)**	**0.02**
	M[d]	0.65 (0.38, 1.10)	0.11	**0.59 (0.37, 0.95)**	**0.03**	1.09 (0.77, 1.52)	0.63	0.97 (0.70, 1.35)	0.87	**1.58 (1.00, 2.48)**	**0.05**	**0.67 (0.51, 0.89)**	**0.01**	1.24 (0.97, 1.60)	0.09	**0.76 (0.60, 0.98)**	**0.04**
	F[d]	**0.32 (0.19, 0.54)**	**<0.01**	**0.35 (0.20, 0.59)**	**<0.01**	**0.61 (0.41, 0.91)**	**0.02**	**0.59 (0.39, 0.89)**	**0.01**	1.42 (0.98, 2.08)	0.07	**0.56 (0.41, 0.75)**	**<0.01**	0.95 (0.72, 1.26)	0.72	0.87 (0.70, 1.09)	0.23
	p_{int}	**0.06**		0.14		**0.03**		**0.06**		0.73		0.39		0.15		0.45	
	n	433		433		433		433		346		346		346		346	
Vitamin D deficiency Visit 2	**All**[c]	**0.50 (0.34, 0.73)**	**<0.01**	**0.51 (0.36, 0.74)**	**<0.01**	**0.75 (0.57, 0.99)**	**0.04**	0.78 (0.59, 1.02)	0.07	**1.39 (1.02, 1.89)**	**0.04**	**0.54 (0.40, 0.72)**	**<0.01**	1.21 (0.98, 1.51)	0.08	**0.71 (0.57, 0.87)**	**<0.01**
	M[d]	**0.42 (0.23, 0.74)**	**<0.01**	**0.40 (0.24, 0.69)**	**<0.01**	**0.54 (0.36, 0.80)**	**<0.01**	**0.67 (0.46, 0.97)**	**0.03**	**2.06 (1.24, 3.44)**	**0.01**	**0.48 (0.32, 0.74)**	**<0.01**	**1.37 (1.01, 1.87)**	**0.05**	**0.73 (0.53, 1.00)**	**0.05**
	F[d]	**0.58 (0.35, 0.96)**	**0.03**	0.64 (0.40, 1.03)	0.07	1.11 (0.74, 1.68)	0.61	0.94 (0.62, 1.42)	0.75	1.04 (0.68, 1.59)	0.85	**0.58 (0.41, 0.84)**	**<0.01**	1.08 (0.80, 1.45)	0.61	**0.69 (0.51, 0.92)**	**0.01**
	p_{int}	0.38		0.20		**0.01**		0.24		**0.05**		0.49		0.26		0.80	
	n	336		336		336		336		261		261		261		261	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; OR = odds ratio; CI = confidence interval; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; p_{int} = *p*-value for interaction term; n = sample number; M = male fetus; F = female fetus.

[a] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[b] The values below LODs were multiply imputed by a lognormal distribution and maximum likelihood estimation due to their lower detection frequencies (43-49%).

[c] The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D.

[d] The models were adjusted for the covariates in [c], and additionally included PFAS × fetal sex interaction term. The ORs by fetal sex were derived from the model with the interaction term.

Table S4-8. Associations between serum PFAS mixture concentrations and 25(OH)D concentrations based on weighted quantile sum (WQS) regression analyses: comparison between multiple imputation and single imputation for PFAS values below limits of detection in pregnant African American women in the Atlanta area, 2014-2018.

25(OH)D		WQS β (95 CI%)[a]	*p*	Weights							
				PFHxS	PFOS	PFOA	PFNA	PFPeA	PFDA	PFUnDA	NMeFOSAA
Total Visit 1 (ng/mL) Positive n=346	MI[b]	**2.88 (1.14, 4.59)**	**<0.01**	0.03	0.08	0.02	0.02	0.08	0.41	0.00	0.36
	SI[c]	**4.78 (0.79, 8.76)**	**0.02**	0.02	0.03	0.01	0.01	0.09	0.36	0.00	0.48
Total Visit 2 (ng/mL) Positive n=261	MI[b]	**5.68 (3.31, 8.04)**	**<0.01**	0.01	0.24	0.08	0.01	0.01	0.17	0.09	0.38
	SI[c]	**7.52 (4.62, 10.4)**	**<0.01**	0.02	0.18	0.11	0.01	0.04	0.13	0.05	0.46
Free Visit 1 (pg/mL) Negative n=348	MI[b]	0.001 (-0.302, 0.305)	0.99	0.01	0.04	0.34	0.03	0.16	0.00	0.41	0.02
	SI[c]	-0.17 (-0.70, 0.36)	0.54	0.00	0.01	0.16	0.01	0.15	0.00	0.67	0.00
Free Visit 1 (pg/mL) Positive n=348	MI[b]	-0.026 (-0.400, 0.347)	0.89	0.20	0.01	0.00	0.00	0.19	0.28	0.00	0.30
	SI[c]	-0.34 (-1.02, 0.34)	0.33	0.14	0.00	0.00	0.00	0.16	0.31	0.00	0.39
Free Visit 2 (pg/mL) Negative n=264	MI[b]	0.001 (-0.307, 0.310)	0.99	0.16	0.34	0.01	0.00	0.41	0.02	0.03	0.04
	SI[c]	0.20 (-0.65, 1.06)	0.64	0.08	0.14	0.02	0.09	0.45	0.00	0.12	0.11
Free Visit 2 (pg/mL) Positive n=264	MI[b]	0.118 (-0.329, 0.564)	0.61	0.06	0.01	0.06	0.00	0.22	0.41	0.06	0.17
	SI[c]	0.24 (-0.30, 0.77)	0.39	0.06	0.01	0.09	0.01	0.10	0.49	0.09	0.16

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; n = sample number.

[a] The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D.

[b] The values of PFPeA, PFDA, PFUnDA, and NMeFOSAA (detection frequencies between 43-49%) below limits of detection (LODs) were multiply imputed by a lognormal distribution and maximum likelihood estimation; the values of PFHxS, PFOS, PFOA, PFNA, and 25(OH)D below LODs were replaced by LOD/$\sqrt{2}$.

[c] The values below LODs were all replaced by LOD/$\sqrt{2}$.

Table S4-9. Adjusted differences in serum 25(OH)D concentrations with per natural-log unit increase of serum PFAS concentrations (ng/mL) by fetal sex: the comparison between the main models and the models additionally adjusted for fish and vitamin D supplement intake in pregnant African American women in the Atlanta area, 2014-2018.

25(OH)D		PFHxS[a]		PFOS[a]		PFOA[a]		PFNA[a]		PFPeA[b]		PFDA[b]		PFUnDA[b]		NMeFOSAA[b]	
		β (95%CI)	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***
Total[a] Visit 1 (ng/mL)	**M1[c]**	**3.46 (2.07, 4.84)**	**<0.01**	**2.97 (1.59, 4.35)**	**<0.01**	0.98 (-0.24, 2.21)	0.06	0.74 (-0.40, 1.89)	0.18	-1.13 (-2.33, 0.07)	0.07	**1.93 (1.01, 2.86)**	**<0.01**	-0.56 (-1.39, 0.28)	0.19	**1.26 (0.51, 2.01)**	**<0.01**
	M2[d]	**3.48 (2.11, 4.85)**	**<0.01**	**3.00 (1.64, 4.36)**	**<0.01**	1.18 (-0.03, 2.39)	0.12	0.78 (-0.36, 1.91)	0.20	-0.94 (-2.13, 0.25)	0.12	**1.88 (0.98, 2.79)**	**<0.01**	-0.53 (-1.37, 0.30)	0.21	**1.23 (0.49, 1.98)**	**<0.01**
	n	285		285		285		285		220		220		220		220	
	M3[e]	**3.66 (2.01, 5.32)**	**<0.01**	**2.95 (1.33, 4.58)**	**<0.01**	**1.70 (0.12, 3.28)**	**0.04**	0.51 (-0.93, 1.96)	0.49	-0.29 (-1.72, 1.14)	0.69	**1.99 (1.01, 2.98)**	**<0.01**	-0.47 (-1.51, 0.58)	0.39	**1.14 (0.20, 2.08)**	**0.02**
	n	157		157		157		157		127		127		127		127	
Total[a] Visit 2 (ng/mL)	**M1[c]**	**3.93 (1.96, 5.89)**	**<0.01**	**3.39 (1.59, 5.18)**	**<0.01**	**2.42 (0.73, 4.11)**	**0.01**	1.42 (-0.22, 3.06)	0.06	**-2.20 (-3.94, -0.46)**	**0.02**	**2.81 (1.47, 4.15)**	**<0.01**	-0.91 (-2.16, 0.34)	0.16	**2.14 (1.03, 3.26)**	**<0.01**
	M2[d]	**3.86 (1.91, 5.80)**	**<0.01**	**3.32 (1.53, 5.10)**	**<0.01**	**2.41 (0.73, 4.08)**	**0.01**	1.58 (-0.05, 3.21)	0.09	**-2.17 (-3.94, -0.40)**	**0.02**	**2.80 (1.45, 4.16)**	**<0.01**	-0.87 (-2.14, 0.39)	0.18	**2.21 (1.10, 3.32)**	**<0.01**
	n	238		238		238		238		179		179		179		179	
	M3[e]	**3.47 (0.91, 6.33)**	**0.02**	**2.93 (0.47, 5.40)**	**0.02**	1.57 (-0.91, 4.06)	0.22	1.43 (-0.97, 3.84)	0.25	-1.51 (-4.11, 1.10)	0.26	**2.62 (0.79, 4.44)**	**<0.01**	-0.84 (-2.73, 1.05)	0.39	**2.64 (1.16, 4.13)**	**<0.01**
	n	129		129		129		129		90		90		90		90	
Free[a] Visit 1 (pg/mL)	**M1[c]**	-0.07 (-0.31, 0.17)	0.60	-0.03 (-0.25, 0.19)	0.86	-0.07 (-0.27, 0.13)	0.66	-0.19 (-0.37, 0.00)	0.06	-0.10 (-0.30, 0.10)	0.34	0.13 (-0.02, 0.29)	0.10	-0.13 (-0.27, 0.00)	0.06	0.08 (-0.04, 0.21)	0.20
	M2[d]	-0.06 (-0.30, 0.17)	0.57	-0.02 (-0.24, 0.20)	0.81	-0.04 (-0.25, 0.16)	0.48	-0.18 (-0.37, 0.01)	0.06	-0.07 (-0.27, 0.13)	0.50	0.12 (-0.03, 0.27)	0.13	-0.13 (-0.27, 0.01)	0.06	0.08 (-0.05, 0.21)	0.21
	n	289		289		289		289		223		223		223		223	
	M3[e]	0.00 (-0.28, 0.29)	0.97	0.05 (-0.22, 0.33)	0.71	0.06 (-0.20, 0.32)	0.65	-0.09 (-0.32, 0.14)	0.46	0.05 (-0.18, 0.28)	0.66	**0.17 (0.01, 0.33)**	**0.04**	-0.15 (-0.31, 0.01)	0.07	0.09 (-0.06, 0.24)	0.24
	n	160		160		160		160		129		129		129		129	

25(OH)D		PFHxS[a]		PFOS[a]		PFOA[a]		PFNA[a]		PFPeA[b]		PFDA[b]		PFUnDA[b]		NMeFOSAA[b]	
		β (95%CI)	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***
Free[a] Visit 2 (pg/mL)	**M1**[c]	0.11 (-0.19, 0.41)	0.52	0.12 (-0.16, 0.39)	0.46	0.12 (-0.14, 0.37)	0.39	-0.12 (-0.37, 0.13)	0.40	-0.16 (-0.45, 0.12)	0.27	**0.20 (0.00, 0.39)**	**0.05**	-0.13 (-0.33, 0.06)	0.19	0.13 (-0.03, 0.30)	0.12
	M2[d]	0.10 (-0.20, 0.40)	0.48	0.10 (-0.17, 0.38)	0.39	0.11 (-0.14, 0.36)	0.37	-0.11 (-0.35, 0.14)	0.34	-0.14 (-0.43, 0.15)	0.34	0.19 (-0.01, 0.39)	0.06	-0.12 (-0.32, 0.08)	0.23	0.15 (-0.02, 0.31)	0.08
	n	243		243		243		243		182		182		182		182	
	M3[e]	-0.12 (-0.50, 0.27)	0.56	0.04 (-0.29, 0.37)	0.82	0.05 (-0.28, 0.37)	0.78	-0.17 (-0.49, 0.15)	0.29	-0.16 (-0.52, 0.19)	0.37	0.07 (-0.18, 0.32)	0.59	-0.05 (-0.30, 0.20)	0.71	**0.22 (0.00, 0.44)**	**0.05**
	n	131		131		131		131		91		91		91		91	

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; CI = confidence interval; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; M1 = model 1; M2 = model 2; M3 = model 3; n = sample number.

[a] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[b] The values below LODs were multiply imputed by a lognormal distribution and maximum likelihood estimation due to their lower detection frequencies (43-49%).

[c] Model 1 was adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D.

[d] Model 2 included the covariates in [c] plus fish and vitamin D supplement intake.

[e] Model 3 included the covariates in [c] plus fish intake; the analyses were restricted to the participants not taking vitamin D supplement.

Table S4-10. Adjusted differences in serum total 25(OH)D concentrations (ng/mL) with per natural-log unit increase in serum PFAS concentrations (ng/mL) in the NHANES 2011-2014 survey cycles stratified by race, sex, and age: the comparison between the models with and without serum albumin adjustment.

Population	n		PFHxS DF = 97-99%[a]	PFOS DF = 99-100%[a]	PFOA DF = 98-100%[a]	PFNA DF = 98-99%[a]	PFDA DF = 67-85%[a]	PFUnDA DF = 45-68%[a]	NMeFOSAA DF = 37-52%[a]
			β (95% CI)						
All[b]	3460	M1	**0.86 (0.50, 1.22)**	0.24 (-0.14, 0.61)	**0.94 (0.47, 1.42)**	0.37 (-0.08, 0.83)	0.29 (-0.12, 0.71)	-0.10 (-0.51, 0.31)	-0.13 (-0.51, 0.25)
		M2[e]	**0.80 (0.43, 1.16)**	0.13 (-0.25, 0.51)	**0.83 (0.35, 1.32)**	0.26 (-0.21, 0.72)	0.23 (-0.18, 0.65)	-0.13 (-0.54, 0.28)	-0.17 (-0.54, 0.21)
Female reproductive age[c]	697	M1	**1.03 (0.25, 1.82)**	0.12 (-0.72, 0.96)	0.91 (-0.15, 1.96)	0.11 (-0.83, 1.06)	0.06 (-0.81, 0.93)	-0.67 (-1.55, 0.21)	0.38 (-0.51, 1.28)
		M2[e]	**1.16 (0.38, 1.95)**	0.03 (-0.81, 0.87)	1.05 (-0.01, 2.10)	0.24 (-0.70, 1.19)	0.10 (-0.76, 0.96)	-0.55 (-1.42, 0.32)	0.40 (-0.48, 1.29)
Female NHB[d]	392	M1	**1.15 (0.09, 2.21)**	-0.09 (-1.10, 0.92)	**1.54 (0.22, 2.85)**	0.38 (-0.87, 1.62)	-0.44 (-1.73, 0.84)	-0.12 (-1.30, 1.05)	0.02 (-1.06, 1.10)
		M2[e]	**1.14 (0.04, 2.23)**	-0.14 (-1.16, 0.88)	**1.57 (0.18, 2.96)**	0.30 (-0.98, 1.59)	-0.48 (-1.76, 0.81)	-0.13 (-1.31, 1.04)	-0.01 (-1.10, 1.08)
Female NHB reproductive age[d]	156	M1	-0.22 (-1.72, 1.27)	-1.23 (-2.69, 0.23)	0.97 (-0.92, 2.86)	-0.01 (-1.73, 1.71)	0.14 (-1.49, 1.76)	0.41 (-1.20, 2.01)	-0.88 (-2.41, 0.65)
		M2[e]	-0.15 (-1.69, 1.40)	-1.20 (-2.69, 0.29)	1.20 (-0.77, 3.18)	0.10 (-1.67, 1.87)	0.17 (-1.47, 1.80)	0.39 (-1.22, 2.00)	-0.86 (-2.40, 0.68)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acid; DF = detection frequency; n = sample number; CI = confidence interval; NHB = non-Hispanic Black; reproductive age = 18-40 years old (matching the age range of the population in this study).

[a] The values below limits of detection (LODs) were replaced by LOD/$\sqrt{2}$.

[b] Multicovariate linear regressions were conducted to assess the associations between total 25(OH)D concentrations and natural log-transformed PFAS concentrations adjusting for education levels (less than high school/high school/college/college and above), age (continuous, year), 6-month examination time period (during November 1st to April 30th/May 1st to October 31st), urine cotinine (continuous, ng/mL), BMI (<18.5/18.5-25/25-30/≥30, kg/m^2), and vitamin D supplement intake in the past 30 days (yes/no), race/ethnicity (Mexican American/other Hispanic/non-Hispanic White/non-Hispanic Black/other), and sex (male/female).

[c] Same as b, not adjusted for sex.

[d] Same as b, not adjusted for race/ethnicity or sex.

[e] Additionaly adjusted for albumin (continuous, g/dL).

Table S4-11. Adjusted differences in serum 25(OH)D concentrations with per natural-log unit increase of serum PFAS concentrations (ng/mL) by fetal sex: comparison between multiple imputation and single imputation for PFAS values below limits of detection in pregnant African American women in the Atlanta area, 2014-2018.

			PFPeA		PFDA		PFUnDA		NMeFOSAA	
25(OH)D			**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***	**β (95%CI)**	***p***
Total Visit 1 (ng/mL) n=346	**All**[a]	MI[c]	**-1.52 (-2.46, -0.57)**	**<0.01**	**1.74 (1.06, 2.42)**	**<0.01**	-0.36 (-1.02, 0.30)	0.29	**0.97 (0.38, 1.56)**	**<0.01**
		SI[d]	**-2.14 (-3.38, -0.91)**	**<0.01**	**2.83 (1.91, 3.74)**	**<0.01**	-0.65 (-1.63, 0.33)	0.29	**1.37 (0.56, 2.19)**	**<0.01**
	M[b]	MI[c]	**-2.23 (-3.50, -0.95)**	**<0.01**	**1.59 (0.62, 2.57)**	**<0.01**	**-1.00 (-1.92, -0.08)**	**0.03**	**1.21 (0.34, 2.08)**	**0.01**
		SI[d]	**-3.52 (-5.40, -1.64)**	**<0.01**	**2.54 (1.24, 3.83)**	**<0.01**	**-1.83 (-3.22, -0.44)**	**0.01**	**1.61 (0.40, 2.82)**	**0.01**
	F[b]	MI[c]	-0.88 (-2.21, 0.45)	0.20	**1.88 (0.95, 2.81)**	**<0.01**	0.25 (-0.65, 1.16)	0.58	0.77 (-0.09, 1.62)	0.08
		SI[d]	-1.14 (-2.75, 0.47)	0.17	**3.11 (1.84, 4.38)**	**<0.01**	0.40 (-0.91, 1.71)	0.55	**1.19 (0.12, 0.47)**	**0.03**
	p_{int}	MI[c]	0.14		0.67		**0.05**		0.49	
		SI[d]	**0.06**		0.53		**0.02**		0.61	
Total Visit 2 (ng/mL) n=261	**All**[a]	MI[c]	**-2.05 (-3.38, -0.71)**	**<0.01**	**2.55 (1.44, 3.67)**	**<0.01**	-0.56 (-1.57, 0.44)	0.27	**1.59 (0.69, 2.48)**	**<0.01**
		SI[d]	**-2.77 (-4.66, -0.89)**	**<0.01**	**3.95 (2.60, 5.31)**	**<0.01**	-1.07 (-2.57, 0.43)	0.27	**2.32 (1.15, 3.50)**	**<0.01**
	M[b]	MI[c]	**-3.53 (-5.68, -1.38)**	**<0.01**	**2.59 (1.12, 4.06)**	**<0.01**	-1.17 (-2.56, 0.23)	0.10	**1.90 (0.60, 3.20)**	**0.01**
		SI[d]	**-5.56 (-8.45, -2.68)**	**<0.01**	**3.95 (2.03, 5.87)**	**<0.01**	**-2.27 (-4.43, -0.11)**	**0.04**	**2.76 (0.99, 4.52)**	**<0.01**
	F[b]	MI[c]	-0.77 (-2.70, 1.16)	0.44	**2.52 (1.06, 3.99)**	**<0.01**	0.02 (-1.36, 1.40)	0.98	**1.31 (0.03, 2.60)**	**0.05**
		SI[d]	-0.77 (-3.21, 1.68)	0.54	**3.95 (2.02, 5.89)**	**<0.01**	-0.06 (-2.05, 1.94)	0.96	**1.99 (0.44, 3.54)**	**0.01**
	p_{int}	MI[c]	0.08		0.95		0.22		0.54	
		SI[d]	**0.01**		1.00		0.13		0.52	
Free Visit 1 (pg/mL) n=348	**All**[a]	MI[c]	-0.07 (-0.23, 0.10)	0.43	0.10 (-0.03, 0.22)	0.13	-0.08 (-0.19, 0.03)	0.15	0.06 (-0.05, 0.16)	0.27
		SI[d]	-0.08 (-0.30, 0.15)	0.43	0.15 (-0.02, 0.32)	0.13	-0.18 (-0.35, 0.00)	0.15	0.08 (-0.07, 0.22)	0.27
	M[b]	MI[c]	-0.21 (-0.47, 0.06)	0.13	0.14 (-0.05, 0.34)	0.14	-0.14 (-0.31, 0.04)	0.12	0.08 (-0.07, 0.23)	0.29
		SI[d]	-0.30 (-0.64, 0.04)	0.08	0.21 (-0.03, 0.45)	0.09	**-0.26 (-0.51, -0.02)**	**0.04**	0.11 (-0.11, 0.33)	0.35
	F[b]	MI[c]	0.06 (-0.16, 0.28)	0.60	0.05 (-0.12, 0.22)	0.57	-0.03 (-0.18, 0.12)	0.70	0.04 (-0.11, 0.19)	0.59
		SI[d]	0.09 (-0.20, 0.38)	0.56	0.09 (-0.15, 0.32)	0.48	-0.10 (-0.33, 0.13)	0.39	0.05 (-0.14, 0.25)	0.59
	p_{int}	MI[c]	0.14		0.48		0.36		0.70	
		SI[d]	0.09		0.46		0.33		0.72	
Free Visit 2 (pg/mL) n=264	**All**[a]	MI[c]	-0.04 (-0.27, 0.18)	0.69	0.16 (0.00, 0.33)	0.06	-0.05 (-0.21, 0.11)	0.54	0.04 (-0.09, 0.18)	0.53
		SI[d]	-0.05 (-0.35, 0.26)	0.69	0.24 (0.01, 0.46)	0.06	-0.12 (-0.35, 0.12)	0.54	0.05 (-0.14, 0.24)	0.53
	M[b]	MI[c]	**-0.37 (-0.72, -0.02)**	**0.04**	**0.28 (0.05, 0.52)**	**0.02**	-0.12 (-0.36, 0.13)	0.36	0.05 (-0.15, 0.25)	0.62
		SI[d]	**-0.60 (-1.06, -0.14)**	**0.01**	**0.43 (0.11, 0.74)**	**0.01**	-0.20 (-0.54, 0.14)	0.25	0.08 (-0.21, 0.36)	0.60
	F[b]	MI[c]	0.23 (-0.05, 0.52)	0.11	0.04 (-0.18, 0.26)	0.72	0.01 (-0.20, 0.22)	0.93	0.04 (-0.15, 0.22)	0.71
		SI[d]	0.35 (-0.04, 0.74)	0.08	0.05 (-0.27, 0.37)	0.77	-0.05 (-0.36, 0.27)	0.76	0.03 (-0.22, 0.29)	0.79
	p_{int}	MI[c]	0.01		0.15		0.45		0.91	
		SI[d]	<0.01		**0.10**		0.52		0.82	

Note: PFPeA = perfluoropentanoic acid; PFDA = perfluorodecanoic acid; PFUnDA: perfluoroundecanoic acid; NMeFOSAA = n-methyl perfluorooctane sulfonamido acetic acidCI = confidence interval; *p* = *p*-value; Visit 1 = 25(OH)D collected at 8-14 weeks of gestation; Visit 2 = 25(OH)D collected at 24-30 weeks of gestation; n = sample number; M = male fetus; F = female fetus; p_{int} = *p*-value for interaction term; MI = multiple imputation; SI = single imputation.

[a] The models were adjusted for maternal age, education, BMI, parity, fetal sex, tobacco use, marijuana use, and season of sample collection for 25(OH)D.

[b] The models were adjusted for the covariates in [a], and additionally included PFAS × fetal sex interaction term.

[c] The values below limits of detection (LODs) were multiply imputed by a lognormal distribution and maximum likelihood estimation.

[d] The values below LODs were replaced by LOD/$\sqrt{2}$.

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Chapter 5. PFAS, Metabolome, and Fetal Growth

Per- and Polyfluoroalkyl substance (PFAS) Exposure, Maternal Metabolomic Perturbation, and Fetal Growth in African American Women: A Meet-in-the-Middle Approach

Chang, C. J., Barr, D. B., Ryan, P. B., Panuwet, P., Smarr, M. M., Kannan, K., Yakimavets, V., Tan, Y., Ly, V., Marsit, C. J., Jones, D. P., Corwin, E. J., Dunlop, A. L., & Liang, D. (2021). Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: a meet-in-the-middle approach.

Abstract

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. 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. Each $\log_2$-unit increase in serum PFNA was significantly associated with higher odds of SGA birth (OR = 1.32, 95% CI: 1.07, 1.63); similar associations were found with PFOA and PFNA concentrations and fetal growth endpoints. 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 sulfate 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. 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.

Keywords: high-resolution metabolomics, PFAS, fetal growth, biomarkers

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, 2001), 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 of 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), 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.

Materials and Methods

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 the participants provided informed consent at enrollment. This study was reviewed and approved by the Institutional Review Board of Emory University (approval reference number 68441).

Data and sample collection

The collection of blood samples, clinical, and questionnaire data, and clinical data from medical records post-delivery 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 8-14 weeks gestation and categorized according to accepted definitions (obesity $\geq$ 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 < 10^{th} 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).

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). Serum samples were quantified by high-performance liquid chromatography interfaced with tandem mass spectrometry (HPLC-MS/MS) for four PFAS – perfluorohexane sulfonic acid (PFHxS), PFOS, perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA). The analytical methods used in Wadsworth/NYU (Honda et al., 2018) and LEADER (Chang et al., 2020) 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 S5-1).

High-resolution metabolomics

Untargeted high-resolution metabolomics profiling was conducted at Emory Clinical Biomarker Laboratory using established protocol (Liu et al., 2020). 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. Extractants were then analyzed in triplicate by liquid-chromatography high-resolution mass spectrometry (LC-HRMS) (Dionex Ultimate 3000, Thermo Scientific Q-Exactive HF). Two analytical columns – the hydrophilic interaction liquid chromatography (HILIC) column for positive electrospray ionization (ESI) analysis and the C18 hydrophobic reversed-phase chromatography column for negative ESI analysis – were applied to maximize the coverage of metabolic features for each sample (Johnson et al., 2010). LC-HRMS was operated in scan mode at 120k resolution and cover the range of mass-to-charge ratio (m/z) from 85 to 1,275. 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 (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). Additional quality control measures were performed before statistical analyses. 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 and $\log_2$-transformed to normalize the datafor future statistical analyses.

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 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 S5-1).

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. We used the following models to evaluate the effects of PFAS exposure and the potential predictors of fetal growth endpoints:

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

$$Birth\ weight = \beta_0 + \beta_1 log_2(Intensity) + \beta_2 Age + \beta_3 Education + \beta_4 Parity + \beta_5 BMI + \beta_6 Tobacco\ use + \beta_7 Marijuana\ use + \beta_8 Sex + \epsilon \text{ (restricted to term births)} \quad (2)$$

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

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).

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 $M^{[1+]}$, $M+H^{[1+]}$, $M\text{-}H2O+H^{[1+]}$, $M+Na^{[1+]}$, $M+K^{[1+]}$, $M+2H^{[2+]}$, and $M(C_{13})+2H^{[2+]}$ were considered. For C18 column, the adducts $M\text{-}H^{[1-]}$, $M+Cl^{[1-]}$, $M+ACN\text{-}H^{[1-]}$, $M+HCOO^{[1-]}$, $M(C_{13})\text{-}H^{[1-]}$, $M\text{-}H2O\text{-}H^{[1-]}$, 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 S5-2). The analyses were separately conducted for four PFAS, birth weight (restricting to term births), and SGA birth by two different analytical columns. We created a heat map to show the enriched metabolic pathways associated with both PFAS and fetal growth endpoints, and shaded each cell based on the strength of the associations.

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 (Yu & Jones, 2014). The features passing the examination were annotated and confirmed using the Metabolomics Standards Initiative criteria described below. 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 (Sumner et al., 2007). 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., 2016; Uppal et al., 2017). We presented the features with level 2 confidence when the features were further confirmed by either experimental or *in silico* predicted fragmentation patterns from previous literature or library spectra (i.e., HMDB) at a retention time corresponding to the primary adduct (Sumner et al., 2007).

Meet-in-the-middle (MITM) approach

Figure 5-1 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 used to explore the potential biological mechanisms and intermediate biomarkers linking PFAS concentrations to fetal growth endpoints.

Sensitivity analysis

While birth weight is an accepted proxy measure of fetal growth, a newborn with a low birth weight may not be growth restricted and a newborn with normal weight may be growth restricted (Wilcox, 2001).

Additionally, a previous study in the U.S found that disparities in birth weight and birth weight z-scores among race/ethnicities remained following adjustment for various socioeconomic factors. Shorter length of gestation was shown to be the strongest predictors of birth weight differences among African American infants (Morisaki et al., 2017). Thus, it is important to account for length of gestation in the analysis. Different methods separating the effect of gestational length 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 for the significance in the pathway enrichment analyses, we performed sensitivity analyses using p-value < 0.005, < 0.01, and < 0.05.

Results

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 in this study 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 5-1) (Ruggles et al., 2021). In total, 56 participants (18%) delivered their infants preterm, and the average birth weight was 3,050 grams (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 5-1. The characteristics among these 313 participants with metabolomics data were similar to the larger population (n=426) in this study (Table S5-3). The four PFAS were detected with high frequencies (97-98%) among the subsets with metabolomics data. The GMs are 1.00 (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 S5-4). Pearson correlation coefficients between these four PFAS ranged from 0.35 to 0.75 (all *p*-values < 0.05) (Table S5-5).

Associations between serum PFAS concentrations and fetal growth endpoints

Table5-2 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 grams [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 S5-6; the effect estimates did not materially change.

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

After ensuring data quality, 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 associated with either birth weight or SGA birth were 274 and 229 in HILIC and C18 column, respectively.

Overlapping enriched pathways associated with serum PFAS and fetal growth endpoints

MWAS results were used to perform pathway analyses. The enriched metabolic pathways associated with both serum PFAS and fetal growth endpoints were summarized in Figure 5-2. Similar enriched pathways were shown when using different cut-offs for significance (i.e., p-values < 0.005, < 0.01, and < 0.05). 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 overlap to pathway metabolite size ranged from 30% to 86%. The results of pathway enrichment analyses associated with PFAS or fetal growth endpoints are presented in Figures S5-2 and S5-3, 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.

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 in HILIC and C18 columns were excluded. As shown in Table 5-3, 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.

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, deoxygcholylglycine, or ursodeoxycholylglycine). The results of sensitivity analyses between term and all births of the birth weight models are presented in Table S5-7, where the directionalities of coefficients were consistent across the two models.

Discussion

Maternal serum PFAS associated to reduced fetal growth

We found that serum 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 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 levels 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 the heterogeneity of study designs, choice of fetal growth endpoints, study populations, sample sizes, or different exposure ranges.

We recognize that associations observed in the present 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. It is for these reasons that we also assessed continuous birth weight in grams as a fetal growth endpoint instead of birth weight for gestational age z-scores.

Amino acid metabolism contributing to PFAS-fetal growth relationship

A series of amino acid pathways were associated with serum PFAS and fetal growth endpoints, including four essential amino acid metabolic pathways (i.e., histidine, tryptophan, lysine, and methionine and cysteine) and four non-essential amino acid metabolic pathways (i.e., arginine and proline, alanine and aspartate, glutamate, and aspartate and asparagine). Previous human observational studies have discovered similar perturbed amino acid pathways; however, these studies focused on the untargeted metabolomics approach and the exact molecular mechanisms still need to be further validated (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; Peng et al., 2013; Salihovic et al., 2019; Yu et al., 2016). A mouse study indicated that PFOS exposure can reduce expression levels of the system-A amino acid transporter on the placenta, leading to decreased concentrations of amino acids in the placentas and fetal livers (Wan et al., 2020). A decreased amino acid concentration in the placentas and fetuses might suggest an increased concentration in maternal serum.

Previous studies have discovered that 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). Despite lack of information of fetal metabolomics, we found consistent evidence in maternal metabolites, where PFNA concentration was associated with increased serum glycine and taurine intensities. Since amino acids are vital nutrients for fetal growth and development, the dysfunction of placental transport function induced by PFAS exposure may, in part, lead to reduced fetal growth.

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. In the current study, we identified several pathways of lipid and fatty acid metabolism (i.e., linoleate metabolism, *de novo* fatty acid biosynthesis, fatty acid activation, glycerophospholipid metabolism), glycosphingolipid biosynthesis and metabolism, butanoate metabolism (a metabolic pathway for short-chain fatty acids and alcohols), and unsaturated fatty acids C18:1 associated with both serum 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 metabolism and fatty acid metabolism were regulated by several nuclear receptors such as peroxisome proliferator-activated receptor subtypes (e.g., PPARα, PPARβ, and PPARγ), which are also substantially involved in many 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 in the previous studies (Bjork et al., 2011; Blake & Fenton, 2020; Jacobsen et al., 2018). For example, PFOA, a potential PPARγ ligand, can promote fatty acid accumulation and influence adipocyte differentiation (Yamamoto et al., 2015). Additionally, PFAS exposure may influence gene expressions related to mitochondrial β-oxidation, which breaks down fatty acid and produces acetyl-CoA

in the energy generation process, leading to concentration changes in fatty acids, triglycerides (Jacobsen et al., 2018; Wan et al., 2012), and carnitine-related metabolites (Chen et al., 2020; Lu et al., 2019).

Different maternal lipid and fatty acid profiles were observed among the women with adverse pregnancy and birth outcomes (Heazell et al., 2012; Horgan et al., 2011; Liu et al., 2017; Paules et al., 2020; Starling et al., 2014). Changes in sphingolipid, phospholipids, carnitine, and fatty acid were found associated with SGA or preterm delivery in three different matrixes – human cord plasma, human maternal peripheral plasma at 14-16 weeks gestation, as well as an animal model with reduced uterine perfusion restriction (Horgan et al., 2011). Another study showed perturbation in maternal circulating free fatty acids, glycerophospholipids, and sphingolipids at the third trimester among mothers with poor birth outcomes (i.e., SGA birth, preterm, and admission to neonatal intensive care) (Heazell et al., 2012). Although the actual biological mechanisms linking changes in maternal lipid metabolism to fetal growth need to be further elucidated, 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). It is also possible that the alteration of lipid metabolism may subsequently lead to preeclampsia via endothelial damage or oxidative stress (Starling et al., 2014), and then cause reduced 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.

Bile acid metabolism contributing to PFAS-fetal growth relationship

Exposure to PFAS can downregulate the expression of 7-alpha-hydroxylase (CYP7A1), which plays a crucial role in catalyzing the synthesis of bile acid from cholesterol. Decreased bile acid synthesis can lead to increased reabsorption of bile acids from the intestine into liver (Beggs et al., 2016; Behr et al., 2020; Salihovic et al., 2019). Accordingly, PFOA and PFOS levels were associated with altered bile acid profiles and changed bile canalicular morphology, suggesting the potential for cholestasis (Behr et al., 2020). Moreover, bile acid conjugation with glycine and taurine, a process of detoxification before excretion into

the intestines, was also downregulated by PFAS exposure (Behr et al., 2020). This inhibitory effect of conjugation could explain the associations of some serum PFAS with elevated parent bile acids, decreased bile acid conjugates, and perhaps with increased glycine and taurine observed in the present study.

Gestational cholestasis has been associated with increased risks of several pregnancy complications and adverse birth outcomes such as preeclampsia (Raz et al., 2015), gestational diabetes mellitus (Martineau et al., 2014), 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 circulating bile acids can stimulate inflammatory response (Li et al., 2017; Shao et al., 2017), induce oxidative stress and apoptosis (Monte et al., 2009), inhibited miRNA expressions on the placentas (Krattinger et al., 2016), higher bile acid levels may lead to reduced fetal growth (Amarilyo et al., 2011; Chen et al., 2019). In this study, we found that parent bile acids were associated with increased odds of SGA birth, and bile acid conjugates (less toxic bile acids) were associated with higher birth weight, 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 are also important mediating pathways for many PFAS-outcome relationships; thus, the interrelationship between PFAS exposure, bile acids, and the related metabolic pathways deserved further investigation.

Androgenic hormones disruption contributing to the PFAS-fetal growth relationship

Several androgenic hormone sulfate conjugates – dehydroepiandrosterone sulfate (DHEA-S), testosterone sulfate, and androsterone sulfate – were found associated with both PFNA and lower birth weight in this population. Serum PFNA was associated with higher androgenic hormone sulfate conjugates, and higher intensities of conjugates predicted lower birth weights. Dehydroepiandrosterone (DHEA) and DHEA-S are both main precursors of sex hormones and can be transformed to androsterone, testosterone, and the other sex steroid hormones. Previous mechanistic studies have shown multiple mode of actions of PFAS exposure on the 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). Few epidemiologic studies have focused on the associations between serum PFAS and androgenic hormones in females. For testosterone, no association with PFAS was found in female adults in all age groups in 2011-2012 (Lewis et al., 2015), but positive associations with PFOA and PFHxS were observed among postmenopausal women in 2013-2016 in the U.S. population (Wang et al., 2021). Moreover, previous studies have reported inverse associations of testosterone with PFOS among girls at 6-9 years of age from the C8 Health Project (Lopez-Espinosa et al., 2016), and with PFOA and PFOS 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). Conflicting results were found across existing human studies, which may be explained by the heterogeneity of study population, age stratifications, and exposure concentration ranges; thus, further studies are warranted to clarify the mechanisms of PFAS on androgenic hormones.

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). Similar findings were presented in human observational studies as well – elevated maternal serum testosterone levels measured at mid- and late-pregnancy were found associated with lower birth weight and shorter length; however, no association was found for DHEAS or androstenedione (Carlsen et al., 2006). Several biological mechanisms underlying the association between prenatal testosterone and fetal growth were reported in the previous studies. Maternal testosterone levels can modify maternal energy metabolism (Carlsen et al., 2006), decrease the expression of amino acid transporter proteins 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 to their fetuses. Alternatively, androgenic hormones can also 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 only focused on testosterone but not DHEA, androsterone, or their conjugates. Although the evidence of androgenic hormones disruption was presented, the results of sulfate conjugates were less comparable to the existing findings.

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

Uric acid, a final product of purine metabolism with both prooxidant and antioxidant properties (Kang & Ha, 2014), is positively associated with serum PFAS and inversely associated with birth weight in our analyses. The associations between PFAS exposure and increased uric acid concentrations were published previously among the populations exposed to either occupational or environmental background levels (Geiger et al., 2013; Gleason et al., 2015; Mitro et al., 2021; Salihovic et al., 2019; Shankar et al., 2011; Steenland et al., 2010). Although the mechanism linking PFAS exposure to higher uric acid remains unclear, two possibilities were discussed in the literature. First, PFAS exposure could induce oxidative stress primarily through dysregulation of PPAR and upregulation of NF-E2-related factor 2 (Nrf2), which could subsequently lead to 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 (i.e., Organic Ion Transporter 1 and 3) in tubular secretion (Johnson et al., 2018; Stanifer et al., 2018), an increase in PFAS concentrations can lead to a decrease in uric acid secretion, and in turn, elevated serum uric acid might be observed. However, this finding should be interpreted with caution due to the possibility of reverse causation for the outcome (Steenland et al., 2010).

Additionally, elevated maternal uric acid has been a predictor and/or a pathogenic factor for several adverse pregnancy and birth outcomes such as gestational hypertension, preeclampsia, and restricted fetal growth (Akahori et al., 2012; Hawkins et al., 2012; Laughon et al., 2009, 2011; Ryu et al., 2019; Wu et al., 2012). For example, increased maternal uric acid could 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). These biological responses could further influence the development and function of the placentas leading to a detrimental effect on oxygen and nutrient supply from pregnant women to their fetus. Collectively, we found that uric acid might reflect inflammation, oxidative stress, or placental

dysfunction partially induced by PFAS exposure in early pregnancy and serve as a predictor for reduced birth weight.

Other overlapping metabolic pathways and metabolites

We found two cofactor and vitamin metabolic pathways, including vitamin B_3 and D_3, associated with both serum PFAS concentrations and fetal growth endpoints. PFAS exposure has shown the ability to interfere with vitamin D metabolism in both epidemiological and mechanistic studies (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). Therefore, vitamin D deficiency can lead 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 chemicals on the list of overlapping metabolites. These two metabolites may be correlated to both serum PFAS concentrations and fetal growth endpoints but not 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, would be due to the dietary preference as PFAS exposure was usually linked to more fish and meat consumptions (Papadopoulou et al., 2019; Tittlemier et al., 2007). Accordingly, the positive association between ferulic acid and risk of SGA birth could be attributed to the preference of plant-based food consumption during pregnancy, which has been linked to SGA birth (Kesary et al., 2020). 2-hexyl-3-phenyl-2-propenal, one of the most important fragrance and flavoring agent in many consumer products (Kim et al., 2018), was positively associated with PFHxS and PFOS. The association might be explained by the behaviors of using consumer products since serum PFAS concentrations have been linked to the use of various consumer products (Chang et al., 2020; Kotthoff et al., 2015). The positive association between 2-hexyl-3-phenyl-2-propenal and odds of SGA birth might be confounded by the factors correlated to the use of consumer products through co-exposure to other chemicals.

Difference of metabolic profiles using different approaches to control for length of gestation

Although similar results were found when using two different approaches to control for length of gestation in the sensitivity analyses, the enriched pathways were somewhat distinct (Figure S5-3). It is possible that the metabolic pathways associated with birth weight might differ between women with preterm and term births. In addition, prematurity might be more influential in the models including all births, whereas the models restricting to the women with term deliveries might capture 'purer' effects associated with only reduced fetal growth.

Strength and limitations

The strengths of our study include the use of untargeted metabolomics techniques to explore global metabolic changes, a relatively large sample size in human metabolomic studies affording adequate statistical power, and a novel MITM approach to identify potential biological mechanisms and intermediate biomarkers linking exposure to outcome. Moreover, 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 levels and metabolomic features, it is difficult to derive causal relationships. Second, it is important to know that dietary and some lifestyle variables were not considered in this study. Adjusting for more variables might introduce unknown biasing paths and cause issues of overadjustment. Thus, we only included a basic set of covariates in the MWAS analyses. Third, we only included four PFAS concentrations in the present studies. It is possible that the other PFAS or other co-exposed chemicals may also yield similar results. Another potential limitation in the assessment of fetal growth is the selection of fetal growth indicators. Clinically, the late assessment of growth restriction as measured by birth weight and SGA may introduce bias into the effect estimates; when feasible, repeated ultrasound measurements of fetal anthropometrics is the 'gold' standard for fetal growth assessment (Smarr et al., 2013). Still, birth weight and several variables derived from birth weight, including SGA, are commonly utilized proxies of fetal growth in epidemiological assessment of environmental exposures, allowing for comparison of results in

our cohort with those previously reported in birth cohorts. Finally, the results from this pregnant African American women cohort might reduce our generalizability to a broader population. Nevertheless, we observed consistent perturbations in similar metabolic pathways previously reported in other population.

Conclusions

To our knowledge, this is the first study to investigate the interrelationship between serum PFAS concentrations, maternal metabolomic perturbation, and fetal growth using the MITM approach. 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. Moreover, uric acid was identified as a potential intermediate biomarker representing the early responses of PFAS exposure and predicting the reduced fetal growth. These biological mechanisms were substantially 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 both public health and clinical settings (Kingsley et al., 2019; Alderete et al., 2019; Chen et al., 2020).

Figures and Tables

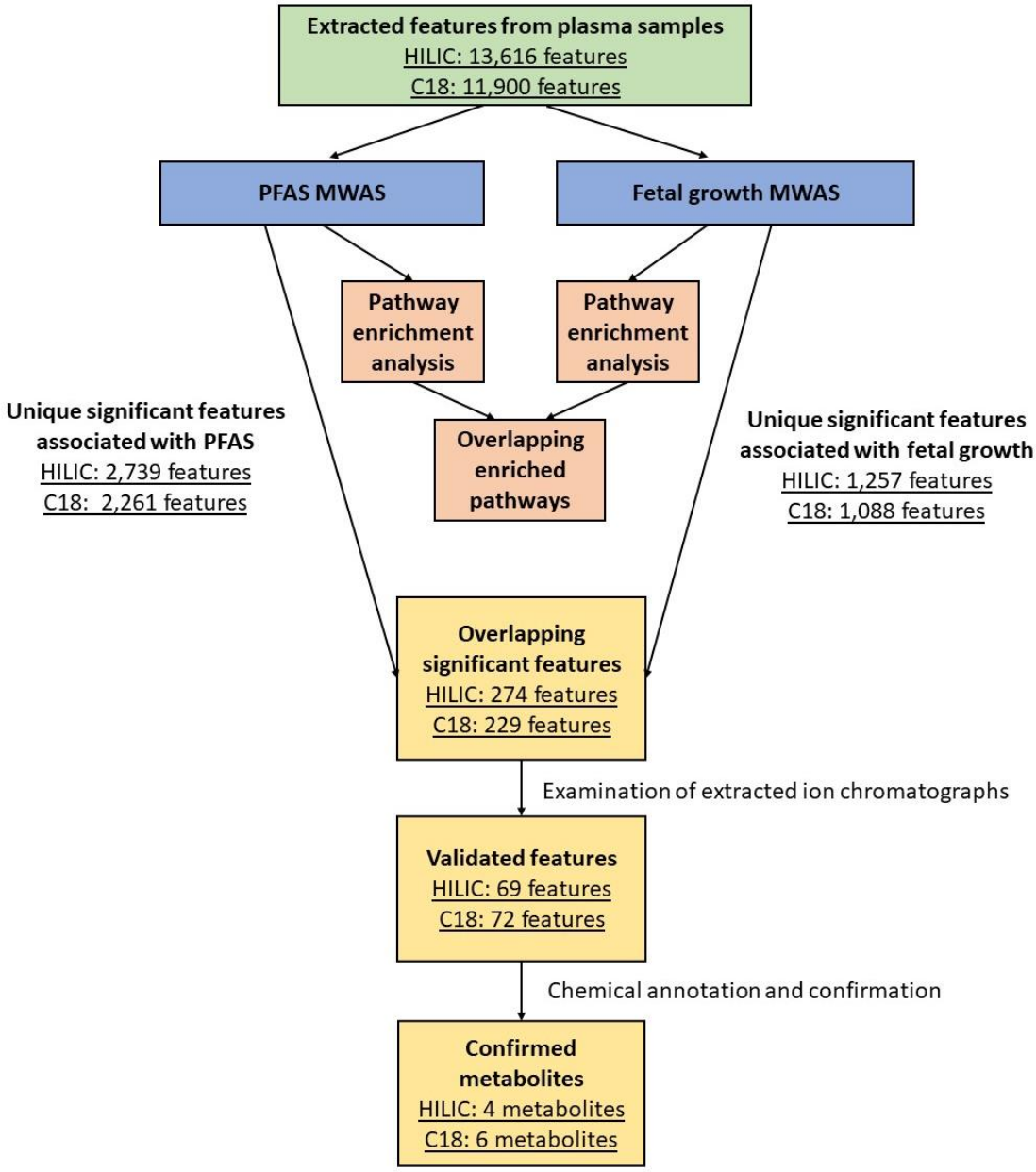


Figure 5-1. Workflow of the meet-in-the-middle (MITM) approach and the number of extracted or significant metabolic features in each analytical step.

Overlapping significant features are the metabolic features associated with both PFAS and fetal growth endpoints. Validated features are the overlapping feature passed examination of extracted ion chromatographs. Confirmed metabolites are the validated features successfully annotated and confirmed with chemical identities. (Note: HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column; MWAS = metabolome-wide association study)

(a)

Pathway	Overlap size	Pathway size	%	HILIC PFHxS	HILIC PFOS	HILIC PFOA	HILIC PFNA	HILIC BW	HILIC SGA	C18 PFHxS	C18 PFOS	C18 PFOA	C18 PFNA	C18 BW	C18 SGA
Associated with ≥2 PFAS, BW, and SGA															
Linoleate metabolism	11	20	55%												
Arginine and proline metabolism	16	39	41%												
Histidine metabolism	8	24	35%												
Nitrogen metabolism	3	4	86%												
Alanine and aspartate metabolism	8	21	37%												
Pyrimidine metabolism	20	59	34%												
Tryptophan metabolism	25	67	38%												
Vitamin B_3 metabolism	9	24	37%												
Associated with ≥2 PFAS and BW															
De novo fatty acid biosynthesis	16	31	50%												
Fatty acid activation	9	29	32%												
Purine metabolism	20	66	30%												
Vitamin D_3 (cholecalciferol) metabolism	5	10	46%												
Associated with ≥2 PFAS and SGA															
Glutamate metabolism	7	12	54%												
Glycerophospholipid metabolism	19	46	41%												
Glycosphingolipid metabolism	14	34	42%												
Keratan sulfate degradation	5	8	59%												
Lysine metabolism	13	27	46%												
Methionine and cysteine metabolism	18	54	34%												
Aspartate and asparagine metabolism	27	69	39%												
Butanoate metabolism	11	26	44%												
Glycosphingolipid biosynthesis - ganglioseries	7	17	42%												

***p*-values** 0 0.025 0.05 1

(b)

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

(c)

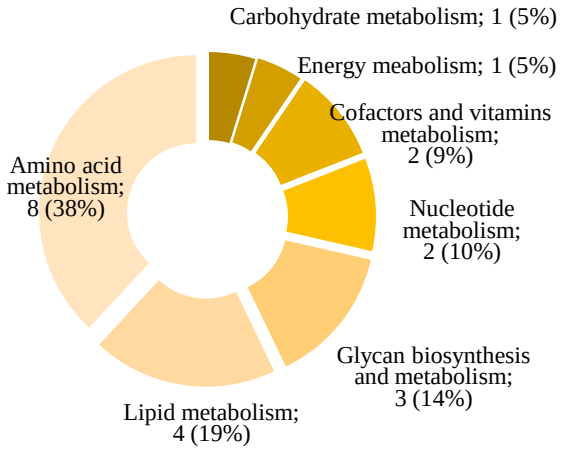


Figure 5-2. The enriched metabolic pathways significantly associated with ≥ 2 PFAS levels and fetal growth in pregnant African American women in the Atlanta area, 2014-2018 (n=313).

(a) Heat map showing the *p*-value levels. 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 metabolic features (*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 class of enriched metabolic pathways. (c) The percentages of each class among 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)

Table 5-1. Selected population characteristics in pregnant African American women in the Atlanta area, 2014-2018 (n=313).

Characteristics	n (%)[a]	Characteristics	n (%)[a]
Age (years)		**Pre-pregnancy BMI (kg/m^2)**	
Mean ± SD	24.9 ± 4.73	< 18.5	9 (3%)
18-25	166 (53%)	18.5-24.9	121 (39%)
25-30	81 (26%)	25-29.9	70 (22%)
30-35	54 (17%)	≥ 30	113 (36%)
≥ 35	12 (4%)	**Infant's sex**	
Education [b]		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 (%)[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 grams)**	
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] The sample numbers do not be summed up to the total sample size due to missingness in some cases.

[b] 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)

Table 5-2. 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, pre-pregnancy 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, pre-pregnancy 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.

Table 5-3. Associations of serum PFAS with birth weight and small-for-gestational age (SGA) in pregnant African American women in the Atlanta area, 2014-2018 (n = 313).

							β (95% CI)					OR (95%CI)
m/z	**RT**	**Metabolites**	**HMDB ID**	**Column**	**Adduct**	**Class**	**PFHxS[a]**	**PFOS[a]**	**PFOA[a]**	**PFNA[a]**	**BW[a,b]**	**SGA[c]**
Biomarkers with level 1 confidence												
120.0025	60.2	Glycine	HMDB00123	HILIC	M+2Na -H	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)***
Biomarkers with level 2 confidence												
217.1587	21.2	2-Hexyl-3-phenyl-2-propenal	HMDB31736	HILIC	M+H	Cinnamaldehyde	**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)

							β (95% CI)					OR (95%CI)
m/z	**RT**	**Metabolites**	**HMDB ID**	**Column**	**Adduct**	**Class**	**PFHxS[a]**	**PFOS[a]**	**PFOA[a]**	**PFNA[a]**	**BW[a,b]**	**SGA[c]**
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)
		Deoxygcholylglycine	HMDB00631	C18	M+Cl	Bile acid						
		Ursodeoxycholylglycine	HMDB00708	C18	M+Cl	Bile acid						

Note: m/z = mass to charge ratio; RT = retention time; 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.

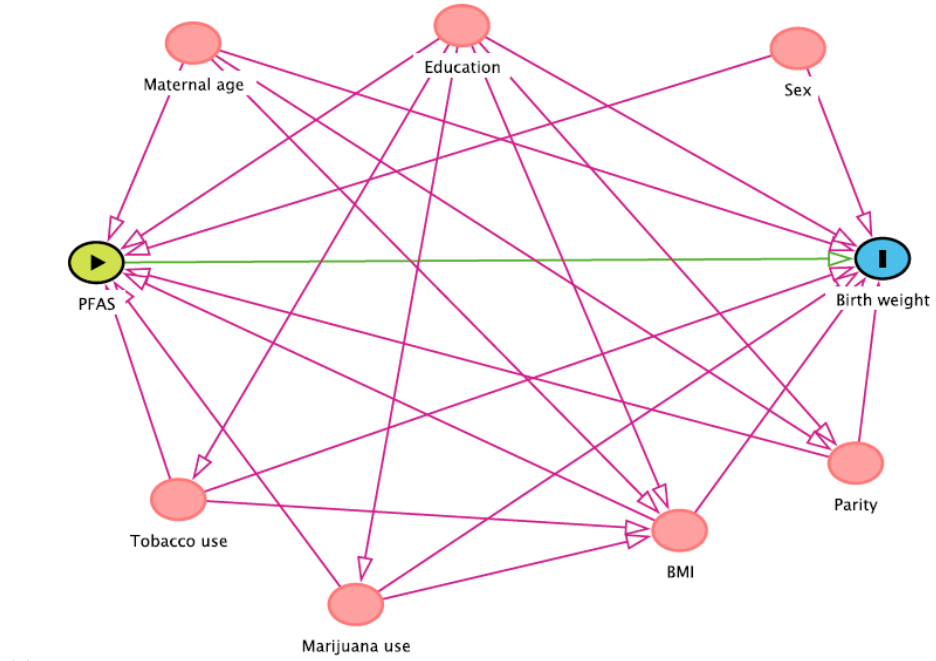
[a] Adjusted for maternal age, education, pre-pregnancy BMI, parity, tobacco use, marijuana use, and infant's sex.

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

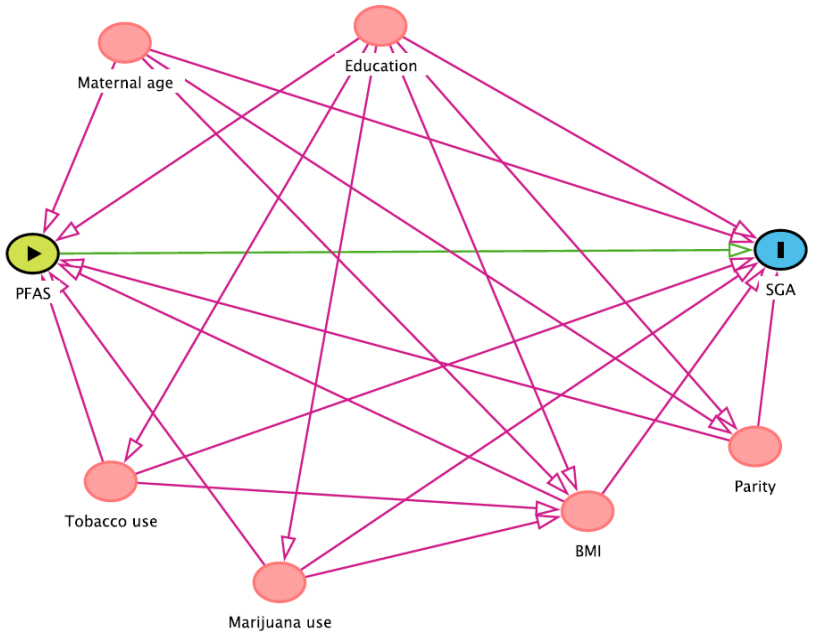
[c] Adjusted for maternal age, education, pre-pregnancy BMI, parity, tobacco use, marijuana use.

* *p*-value < 0.05.

Supplementary



(a)



(b)

Figure S5-1. Directed acyclic graph (DAG) showing the association of PFAS exposure with (a) birth weight (restricted to term births) and (b) small-for-gestational age (SGA).
Green lines represent causal paths and red lines were biasing paths. Minimal sufficient adjustments for estimating the association between PFAS and birth weight include maternal age, education, pre-pregnancy BMI, parity, tobacco use, marijuana use, and infant's sex. Same adjustment except for infant's sex is required for estimating the association between PFAS and SGA.

Pathway	Overlap size	Pathway size	%	HILIC				C18			
				PFHxS	PFOS	PFOA	PFNA	PFHxS	PFOS	PFOA	PFNA
Alanine and aspartate metabolism	10	21	45%								
Aminosugars metabolism	15	36	42%								
Arginine and proline metabolism	17	39	44%								
Aspartate and asparagine metabolism	27	69	40%								
Benzoate degradation via CoA ligation	2	2	100%								
Beta-alanine metabolism	6	14	43%								
Biopterin metabolism	10	19	49%								
Blood group biosynthesis	5	6	83%								
Butanoate metabolism	11	26	42%								
De novo fatty acid biosynthesis	19	27	69%								
Electron transport chain	3	3	100%								
Fatty acid activation	12	28	41%								
Fatty acid metabolism	9	17	50%								
Glutamate metabolism	6	12	52%								
Glycerophospholipid metabolism	20	45	44%								
Glycosphingolipid biosynthesis - ganglioseries	7	17	41%								
Glycosphingolipid biosynthesis - globoseries	6	9	69%								
Glycosphingolipid biosynthesis - lactoseries	4	6	71%								
Glycosphingolipid biosynthesis - neolactoseries	5	6	83%								
Glycosphingolipid metabolism	15	33	46%								
Histidine metabolism	9	24	38%								
Hyaluronan metabolism	3	4	82%								
Keratan sulfate degradation	5	8	68%								
Leukotriene metabolism	18	50	36%								
Linoleate metabolism	12	20	60%								
Lysine metabolism	12	27	46%								
Methionine and cysteine metabolism	18	54	33%								
N-Glycan degradation	5	7	64%								
Nitrogen metabolism	4	4	90%								
O-Glycan biosynthesis	5	5	96%								
Omega-6 fatty acid metabolism	6	13	44%								
Prostaglandin formation from arachidonate	19	62	30%								
Purine metabolism	23	66	34%								
Pyrimidine metabolism	24	59	41%								
Saturated fatty acids beta-oxidation	4	17	20%								
Tryptophan metabolism	29	67	43%								
Tyrosine metabolism	41	97	42%								
Ubiquinone biosynthesis	4	6	63%								
Valine, leucine and isoleucine degradation	14	29	48%								

Pathway	Overlap size	Pathway size	%	HILIC				C18			
				PFHxS	PFOS	PFOA	PFNA	PFHxS	PFOS	PFOA	PFNA
Vitamin B_3 metabolism	10	24	41%								
Vitamin B_6 (pyridoxine) metabolism	4	8	50%								
Vitamin D_3 (cholecalciferol) metabolism	6	10	53%								

Figure S5-2. The enriched metabolic pathways significantly associated with ≥ 2 serum PFAS concentrations in pregnant African American women in the Atlanta area, 2014-2018 (n=313).

Each cell was colored by the *p*-value of the association between each metabolic pathway and serum PFAS concentrations. Overlap size represents the average number of significant metabolic features (*p*-value <0.05) that were associated with serum PFAS 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. (Note: HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid)

Pathway	Overlap size	Pathway size	%	HILIC BW (All)	HILIC BW (Term)	C18 BW (All)	C18 BW (Term)
Arginine and proline metabolism	15	40	36%				
Alanine and aspartate metabolism	7	21	34%				
Butanoate metabolism	11	26	42%				
Glycosphingolipid biosynthesis - ganglioseries	7	16	44%				
Histidine metabolism	8	24	32%				
Keratan sulfate degradation	3	9	39%				
Linoleate metabolism	9	21	42%				
Nitrogen metabolism	3	4	75%				
Pyrimidine metabolism	20	59	33%				
Tryptophan metabolism	25	68	37%				
Vitamin B_3 metabolism	8	24	35%				
Vitamin D_3 (cholecalciferol) metabolism	5	10	44%				
Aspartate and asparagine metabolism	29	69	42%				
Glutamate metabolism	7	12	54%				
Glycerophospholipid metabolism	19	46	40%				
Glycosphingolipid metabolism	14	35	39%				
Lysine metabolism	13	28	45%				
Methionine and cysteine metabolism	20	53	38%				
Purine metabolism	18	65	28%				
De novo fatty acid biosynthesis	6	35	17%				
Fatty acid activation	5	29	17%				

Figure S5-3. The enriched metabolic pathways significantly associated with birth weight (BW) using different approaches to control for length of gestation in pregnant African American women in the Atlanta area, 2014-2018 (n=313).
Each cell was colored by the *p*-value of the association between each metabolic pathway and fetal growth endpoints. Overlap size represents the average number of significant metabolic features (*p*-value < 0.05) that were associated with serum PFAS concentrations 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. (Note: HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column; BW = birth weight; All = including all births; Term = restricted to term births)

Table S5-1. Overlapped samples concentrations (ng/mL) from the Laboratory of Exposure Assessment and Development for Environmental Health Research at Emory University and Wadsworth Center/New York University Laboratory Hub.

	PFHxS		PFOS		PFOA		PFNA	
Sample ID	**W[b]**	**E**	**W**	**E**	**W**	**E**	**W**	**E**
1	1.515	1.398	3.506	3.735	1.305	1.299	0.435	0.550
2	0.710	0.587	1.719	1.021	0.492	0.425	0.868	0.811
3	1.055	1.283	1.392	1.215	0.677	0.583	0.372	0.351
4	0.679	0.565	1.733	1.967	0.228	0.376	0.091	0.214
5	0.794	0.601	1.469	1.393	0.848	1.114	0.259	0.419
6	0.999	0.606	5.145	4.509	0.734	0.900	0.197	0.357
7	0.831	0.835	2.478	2.388	0.624	0.566	0.264	0.337
8	1.534	1.645	2.325	1.670	1.013	1.133	0.285	0.401
9	1.332	0.948	2.882	2.559	1.315	1.870	0.402	0.553
10	1.830	1.264	3.965	4.430	1.289	1.845	0.632	0.876
11	1.850	1.835	2.597	2.269	1.350	2.100	0.231	0.410
Pearson correlation coefficients	**0.88**		**0.93**		**0.92**		**0.93**	
Relative percent differences, %RPD; Median (Min-Max)[a]	**4.75 (0.12-12.3)**		**3.17 (0.92-12.7)**		**5.05 (0.12-12.3)**		**8.11 (1.48-20.2)**	

Note: W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.

[a] %RPD = (Lab 1 result – Lab 2 result)*100/(Lab 1 result + Lab 2 result)/2.

[b] The results from Wadsworth were used in the main analysis for these overlapped samples.

Table S5-2. Numbers of significant metabolic features in metabolome-wide association study (MWAS) when using different thresholds in pregnant African American women in the Atlanta area, 2014-2018 (n=313).

	HILIC			C18		
	FDR at 5%	**FDR at 20%**	**Raw *p*-value < 0.05**	**FDR at 5%**	**FDR at 20%**	**Raw *p*-value < 0.05**
PFHxS	1	12	816	27	67	797
PFOS	4	16	974	28	82	803
PFOA	1	4	922	17	26	709
PFNA	1	27	1126	24	42	899
Birth weight	0	0	693	0	0	586
SGA	0	0	742	0	0	673

Note: HILIC = hydrophilic interaction liquid chromatography column; C18 = C18 column; FDR = false discovery rate for correlation of multiple comparison; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; SGA = small-for-gestational age.

Table S5-3. Selected population characteristics in data subsets in pregnant African American women in the Atlanta area, 2014-2018.

Data availability	Serum PFAS & birth outcome (n=426)[a]	Serum PFAS, birth outcome & metabolomic data (n=313)[a]
Age (years)		
Mean ± SD	24.7 ± 4.66	24.9 ± 4.73
18-25	239 (56%)	166 (53%)
25-30	108 (25%)	81 (26%)
30-35	62 (15%)	54 (17%)
≥35	17 (4%)	12 (4%)
Education		
Less than high school	66 (16%)	49 (16%)
High school	161 (38%)	118 (38%)
Some college	131 (31%)	97 (31%)
College and above	68 (16%)	49 (16%)
Income-to-poverty ratio (%)		
< 100	180 (42%)	134 (43%)
100-150	67 (16%)	48 (15%)
150-300	68 (16%)	48 (15%)
≥ 300	52 (12%)	38 (12%)
Married or cohabiting		
Yes	199 (47%)	147 (47%)
No	227 (53%)	166 (53%)
Insurance		
Private	94 (22%)	65 (21%)
Medicaid	332 (78%)	248 (79%)
Hospital		
Emory	172 (40%)	123 (39%)
Grady	254 (60%)	190 (61%)
Parity (#)		
0	201 (47%)	137 (44%)
1	120 (28%)	88 (28%)
≥ 2	105 (25%)	88 (28%)
Pre-pregnancy BMI (kg/m^2)		
< 18.5	14 (3%)	9 (3%)
18.5-24.9	170 (40%)	121 (39%)
25-29.9	90 (21%)	70 (22%)
≥ 30	152 (36%)	113 (36%)
Infant's sex		
Male	206 (48%)	157 (50%)
Female	220 (52%)	156 (50%)
Marijuana use		
Not during pregnancy	331 (78%)	240 (77%)
During pregnancy	95 (22%)	73 (23%)
Tobacco use		
Not during pregnancy	370 (87%)	267 (85%)
During pregnancy	56 (13%)	46 (15%)
Birth weight (grams)		
Mean ± SD	3040 (558)	3050 (611)
Low birth weight (LBW) (< 2,500 grams)		
No	376 (88%)	274 (88%)
Yes	50 (12%)	39 (13%)
Birth weight percentile for gestational age		

Data availability	Serum PFAS & birth outcome (n=426)[a]	Serum PFAS, birth outcome & metabolomic data (n=313)[a]
Mean ± SD	32.7 ± 26.2	35.9 ± 27.3
Small-for-gestational age (SGA) (<10th percentiles)		
No	313 (74%)	236 (75%)
Yes	113 (27%)	77 (25%)
Delivery gestational week (weeks)		
Mean ± SD	38.6 ± 2.32	38.5 ± 2.64
Preterm birth (< 37 gestational weeks)		
No	370 (87%)	257 (82%)
Yes	56 (13%)	56 (18%)

Note: SD = standard deviation.

[a] The sample numbers do not be summed up to the total sample size due to missingness in some cases.

Table S5-4. Serum PFAS concentrations (ng/mL) in different data subsets in pregnant African American women, 2014-2018 (n=313).

	LOD	% > LOD	GM[a]	GSD[a]	P25	P50	P75	Max
Serum PFAS & birth outcome (n=426)								
PFHxS	0.02 (W); 0.2 (E)	97	1.02	1.90	0.76	1.10	1.53	4.80
PFOS	0.02 (W); 0.05 (E)	99	2.05	2.05	1.45	2.19	3.24	12.4
PFOA	0.035 (W); 0.2 (E)	97	0.63	2.34	0.45	0.71	1.07	4.42
PFNA	0.02 (W); 0.1 (E)	97	0.24	2.30	0.16	0.27	0.42	2.27
Serum PFAS, birth outcome & metabolomic data (n=313)								
PFHxS	0.02 (W); 0.2 (E)	97	1.00	1.91	0.76	1.09	1.52	4.80
PFOS	0.02 (W); 0.05 (E)	98	1.97	2.13	1.42	2.10	3.16	12.4
PFOA	0.035 (W); 0.2 (E)	97	0.62	2.35	0.45	0.72	1.07	4.42
PFNA	0.02 (W); 0.1 (E)	97	0.23	2.34	0.15	0.27	0.40	2.27

Note: LOD = limits of detection; GM = geometric mean; GSD = geometric standard deviation; P25 = the 25th percentile; PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; W = Wadsworth Center/New York University Laboratory Hub; E = the Laboratory of Exposure Assessment and Development for Environmental Health Research (LEADER) at Emory University.

[a] The values below LODs were replaced by LOD/$\sqrt{2}$.

Table S5-5. Pearson correlation coefficients of log_2-transformed serum PFAS concentrations in pregnant African American women in the Atlanta area, 2014-2018 (n=313).

	PFHxS[a]	PFOS[a]	PFOA[a]	PFNA[a]
PFHxS	1	**0.69***	**0.46***	**0.35***
PFOS		1	**0.69***	**0.67***
PFOA			1	**0.75***
PFNA				1

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid.

[a] The values below LODs were replaced by LOD/$\sqrt{2}$.

* p-value < 0.05.

Table S5-6. Sensitivity analyses: associations of serum PFAS with birth weight using different approaches to control for length of gestation in pregnant African American women in the Atlanta area, 2014-2018.

	Birth weight (continuous; grams) β (95%CI)	
	All[a,b] (n=426)	**Term[a,c] (n=370)**
PFHxS (ng/mL)		
Q1: < LOD-0.75	0 (Ref)	0 (Ref)
Q2: 0.75-1.10	-20 (-118, 78)	-36 (-154, 83)
Q3: 1.10-1.53	-32 (-132, 68)	5 (-112, 123)
Q4: 1.53-4.80	-60 (-161, 41)	-54 (-173, 66)
p for trend[d]	0.24	0.50
Per log_2-unit	-15 (-54, 23)	-14 (-58, 31)
PFOS (ng/mL)		
Q1: < LOD-1.44	0 (Ref)	0 (Ref)
Q2: 1.44-2.19	48 (-51, 146)	78 (-40, 196)
Q3: 2.19-3.24	-5 (-105, 96)	20 (-98, 138)
Q4: 3.24-12.40	-13 (-115, 88)	-16 (-136, 105)
p for trend[d]	0.53	0.48
Per log_2-unit	-10 (-44, 25)	-7 (-48, 34)
PFOA (ng/mL)		
Q1: < LOD-0.45	0 (Ref)	0 (Ref)
Q2: 0.45-0.71	-83 (-180, 15)	**-126 (-241, -10)***
Q3: 0.71-1.07	-45 (-143, 53)	-44 (-162, 73)
Q4: 1.07-4.42	-86 (-186, 14)	-107 (-227, 13)
p for trend[d]	0.19	0.23
Per log_2-unit	-13 (-42, 16)	-14 (-49, 21)
PFNA (ng/mL)		
Q1: < LOD-0.16	0 (Ref)	0 (Ref)
Q2: 0.16-0.27	-42 (-139, 56)	-41 (-159, 77)
Q3: 0.27-0.42	-22 (-120, 77)	-48 (-165, 69)
Q4: 0.42-2.27	-99 (-200, 2)	-106 (-227, 14)
p for trend[d]	0.07	0.09
Per log_2-unit	-27 (-56, 3)	-32 (-67, 3)

Note: PFHxS = perfluorohexane sulfonic acid; PFOS = perfluorooctane sulfonic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid.

[a] Adjusted for maternal age, education, pre-pregnancy BMI, parity, tobacco use, marijuana use, and infant's sex.

[b] Additionally adjusted for gestational week at delivery.

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

[d] Median serum PFAS concentrations of each quartile group were used as a continuous exposure variable.

* *p*-value < 0.05.

Table S5-7. Sensitivity analyses: associations between confirmed overlapping biomarkers and birth weight using different approaches to control for length of gestation in pregnant African American women in the Atlanta area, 2014-2018 (n=313).

m/z	RT	Metabolites	HMDB ID	Column	Adduct	Class	β (95% CI)	
							All[a,b]	Term[a,c]
Biomarker with level 1 confidence								
120.0025	60.2	Glycine	HMDB00123	HILIC	M+2Na-H	Amino acid	**-160 (-287, -33)***	**-171 (-314, -28)***
126.0220	57.7	Taurine	HMDB00251	HILIC	M+H	Amino acid	**-96 (-185, -7)***	**-84 (-188, -3)***
167.0208	18.8	Uric acid	HMDB00289	C18	M-H	Purine derivative	**-116 (-219, -12)***	**-161 (-291, -31)***
195.0661	23.7	Ferulic acid	HMDB00954	C18	M-H	Hydroxycinnamic acids	-12 (-40, 16)	-8 (-41, 26)
Biomarker with level 2 confidence								
217.1587	21.2	2-Hexyl-3-phenyl-2-propenal	HMDB31736	HILIC	M+H	Cinnamaldehydes	-50 (-129, 29)	-57 (-142, 27)
283.2634	181.6	Elaidic acid	HMDB00573	HILIC	M+H	Long-chain fatty acids	25 (-12, 63)	38 (-4, 79)
		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	**-46 (-90, -2)***	**-27 (-54, -1)***
		Testosterone sulfate	HMDB02833	C18	M-H	Steroid hormone		
369.1742	23.4	Androsterone sulfate	HMDB02759	C18	M-H	Steroid hormone	**-57 (-97, -18)***	**-22 (-42, -2)***
391.2878	260.9	Chenodeoxycholic acid (CDCA)	HMDB00518	C18	M-H	Bile acid	-4 (-46, 38)	-24 (-77, 29)
		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	27 (-2, 57)	**50 (12, 87)***
		Deoxygcholylglycine	HMDB00631	C18	M+Cl	Bile acid		
		Ursodeoxycholylglycine	HMDB00708	C18	M+Cl	Bile acid		

Note: m/z = mass to charge ratio; RT = retention time; HMDB ID = Human Metabolome Database ID; HILIC = HILIC column; C18 = C18 column.
[a] Adjusted for maternal age, education, pre-pregnancy BMI, parity, tobacco use, marijuana use, and infant's sex.
[b] Additionally adjusted for gestational week at delivery.
[c] Restricted to term births (> 37 gestational weeks and 0 day).
* *p*-value < 0.05.

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Chapter 6. Conclusions

Although per and poly-fluoroalkyl substances (PFAS) are frequently detected in environmental and biological samples, exposure in certain populations is particularly of concerns, such as pregnant women and their developing fetuses, because environmental insults during the fetal development period can not only impact the health at the time but result in later health risk. African American women often disproportionately experience higher exposures to environmental pollutants and higher adverse birth outcome rates than white populations (Calafat, Kuklenyik, et al., 2007; Calafat, Wong, et al., 2007; Giscombé & Lobel, 2005; Jain, 2014; Martin et al., 2017; Nelson et al., 2012; Park et al., 2019), and were also underserved and underrepresented in the previous research and national surveys. Thus, in this dissertation, we examined PFAS exposures and theirs impact on fetal growth in an African American birth cohort in Atlanta, Georgia (**Aim 1&2**; Chapter 2&3). Furthermore, since the mechanisms of how PFAS exposures influence fetal growth remain unclear, we explored underlying biological mechanisms through investigating the associations of serum PFAS with targeted biomarkers (i.e., vitamin D) (**Aim 3**; Chapter 4) and with untargeted high-resolution metabolomics (**Aim 4**; Chapter 5).

In **Aim 1** (Chapter 2), we found that PFAS exposures are ubiquitous in this pregnant African American population. Perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were detected in > 95% of the serum samples with PFOS having the highest concentrations. Perfluoropentanoic acid (PFPeA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and n-methyl perfluorooctane sulfonamido acetic acid (NMeFOSAA) were detected in 40-50% of the samples, whereas the other six PFAS were below 15%. The serum concentrations of PFOS, PFOA, and PFNA were similar in this cohort and also the participants in the National Health and Nutrition Examination Survey (NHANES) when matching sex, race, and age with this cohort. Higher PFHxS concentrations were found in this cohort than NHANES (geometric mean 0.99 ng/mL in this study; 0.63 and 0.40 ng/mL in the 2014-2015 and 2016-2017 NHANES cycle). A decline in concentrations over the study period was found for most PFAS but not PFPeA, suggesting a possibility of

substitution of legacy PFAS with short-chain PFAS. Furthermore, education, sampling year, parity, BMI, tobacco and marijuana use, age of house, drinking water source, and cosmetic use were found to be significant predictors associated with serum PFAS concentrations.

In **Aim 2** (Chapter 3), we investigated the associations between serum PFAS concentrations and fetal growth outcomes. We found that serum PFNA and PFOA concentrations were associated with higher odds of small-for-gestational age (SGA) birth. Moreover, marginal or significant associations with ponderal index were observed in PFOS, PFNA, and PFDA. However, similar directionality of results across multiple fetal growth outcomes were only observed for PFNA and PFOA; the results of PFOS and PFDA were relatively inconclusive; no association of PFHxS, PFPeA, PFUnDA, and NMeFOSAA with fetal growth outcome was found. In this aim, we found suggestive results that maternal serum PFNA and PFOA concentrations were associated with reduced fetal growth in this African American birth cohort.

Several important biological mechanisms were then identified through examining the associations of serum PFAS concentrations with vitamin D biomarkers and with high-resolution metabolomics. We found suggestive results that the biological mechanisms including vitamin D, amino acid, lipid and fatty acid, bile acid, uric acid, and androgenic hormone metabolism may contribute to this PFAS-fetal growth association. In **Aim 3** (Chapter 4), we examined the associations of serum PFAS concentrations with serum total and free 25-hydroxyvitamin D (25(OH)D) through a cross-sectional (both 25(OH)D and PFAS measured in early pregnancy) and a prospective study design (25(OH)D measured in late pregnancy and PFAS in early pregnancy). Generally, total 25(OH)D levels were positively associated with serum PFHxS, PFOS, PFDA, and NMeFOSAA concentrations, and negatively associated with PFPeA. For free 25(OH)D, positive associations were observed with PFHxS, PFOS, PFOA, and PFDA, and a negative association with PFPeA among the women with male fetuses under the prospective study design. PFAS mixture effect was also examined utilizing weighted quantile sum (WQS) regressions − positive associations of the PFAS mixture with total 25(OH)D concentrations measured in early and late pregnancy were observed. Since vitamin D is crucial to fetal growth and maternal and fetal health maintenance during pregnancy (Luk et al., 2012;

Ponsonby et al., 2010; Shin et al., 2010; Wagner & Hollis, 2018), changes in concentrations of 25(OH)D in relation to PFAS exposure suggest that vitamin D metabolism could be a potential underlying biological pathway linking PFAS exposure to reduced fetal growth.

In **Aim 4** (Chapter 5), 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. Among 25,516 metabolic features extracted from the serum samples, we successfully annotated and confirmed 10 metabolites and 21 metabolic pathways associated with both serum PFAS and fetal growth endpoints. Collectively, these overlapping metabolites and pathways were closely related to amino acid, lipid and fatty acid, bile acid, and androgenic hormone metabolism perturbations; additionally, uric acid was shown to be a potential intermediate biomarker.

Scientific Contributions and Public Health Implications

Overall, our studies demonstrated that PFAS exposures are ubiquitous and may contribute to health disparities, especially reduced fetal growth, in this African American women birth cohort. The observed exposure pathways provide opportunities for individuals and public sectors to mitigate PFAS exposure in the pregnant African American women population. Furthermore, the identified biological pathways show potentials for future studies to develop early detection and intervention for PFAS-induced effect as well as strengthen the biological plausibility of the association between PFAS and reduced fetal growth through elucidating several mechanisms.

It is noteworthy that the results from this racial minority cohort in the Atlanta area may not be fully generalizable to another population or even to the larger U.S. African American population given the lower socioeconomic statuses among the study participants. However, studies focusing specifically on certain minority population or in certain regions are still crucial (St. Sauver et al., 2012) because 1) limited representation of minority has been recognized in the previous studies in the U.S., and 2) environmental exposures and their pathways to human have not yet been characterized in pregnant women in many areas

in the U.S., especially the Southeast. Thus, we believe that this dissertation research offers a unique opportunity to address environmental exposure and related health outcomes of a large African American minority population in a major metropolitan area in the U.S.

Future Directions

Although some PFAS have been phased-out by major manufacturers in the U.S., it is noted that these chemicals may still be produced internationally and imported into the U.S in various consumer products. A recent study under the Stockholm Convention found that atmospheric PFAS concentrations, including PFHxS, PFOS, PFNA, PFDA, and the other PFAS, are increasing from 2009 to 2015 (about 10-15 years after the phase-out) at 21 sites around the globe, suggesting constant and/or new emissions of these chemicals worldwide (Rauert et al., 2018). Furthermore, previous studies have only focused on a small subset of PFAS and few of them have considered the mixture effects. It is highly possible that current PFAS exposure and the related health risks are being underestimated, especially when many substitutions with similar toxicities such as short-chain PFAS and GenX have been already identified (Ateia et al., 2019; Sun et al., 2016; Valsecchi et al., 2017). Future studies are needed to continue monitor legacy PFAS in different populations, to investigate exposure of their alternatives and related health risks, and to consider the mixture effect of this large family of fluorinated compounds.

Lastly, we want to address the importance of examining underlying biological mechanisms in epidemiological studies. Information on biological mechanisms in response to environmental exposures can help us avoid potential biases from physiological conditions and further assess the causal plausibility, and offer opportunities to develop public health strategies for early detection and intervention, and ultimately, mitigate health risks across the lifespan. Thus, it is suggested for future epidemiological studies to go beyond simple exposure-outcome associations to complex biological responses or perturbations in order to close the gap between association and causation in environmental epidemiological studies.

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