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Early-Pregnancy Plasma Concentrations of Perfluoroalkyl Substances and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics?

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Associations of prenatal exposure to perfluoroalkyl substances (PFAS), ubiquitous chemicals used in stain- and water-resistant products, with adverse birth outcomes may be confounded by pregnancy hemodynamics. We measured plasma concentrations of 4 PFAS in early pregnancy (median length of gestation, 9 weeks) among 1,645 women in Project Viva, a study of a birth cohort recruited during 1999–2002 in eastern Massachusetts. We fitted multivariable models to estimate associations of PFAS with birth weight-for-gestational age z score and length of gestation, adjusting for sociodemographic confounders and 2 hemodynamic markers: 1) plasma albumin concentration, a measure of plasma volume expansion, and 2) plasma creatinine concentration, used to estimate glomerular filtration rate. Perfluorooctane sulfonate (PFOS) and perfluorononanoate (PFNA) were weakly inversely associated with birth weight-for-gestational age z scores (adjusted $\beta = -0.04$ (95% confidence interval (CI): -0.08, 0.01) and adjusted $\beta = -0.06$ (95% CI: -0.11, -0.01) per interquartile-range increase, respectively). PFOS and PFNA were also associated with higher odds of preterm birth (e.g., for highest PFOS quartile vs. lowest, adjusted odds ratio = 2.4, 95% CI: 1.3, 4.4). Adjusting for markers of pregnancy hemodynamics (glomerular filtration rate and plasma albumin), to the extent that they accurately reflect underlying pregnancy physiology, did not materially affect associations. These results suggest that pregnancy hemodynamics may not confound associations with birth outcomes when PFAS are measured early in pregnancy.

birth weight; fetal growth; gestational age; perfluoroalkyl substances; pregnancy; preterm birth

Abbreviations: CDC, Centers for Disease Control and Prevention; CI, confidence interval; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Editor's note: An invited commentary on this article appears on page 803.

Perfluoroalkyl substances (PFAS) are a family of synthetic compounds composed of a carbon-fluorine backbone. Many PFAS are resistant to oil and water and therefore useful in the manufacture of stain-resistant products (e.g., carpets and fabrics), nonstick coatings, and food packaging; they also have a broad range of other applications. These properties can also make them resistant to degradation and persistent both in the environment and in the body, with half-lives in humans of approximately 3–5 years (1).

Diet and the indoor environment are common sources of human PFAS exposure, and PFAS are universally detected at varying serum concentrations in the US population, as reported in the National Health and Nutrition Examination Survey (2, 3). PFAS can cross the placenta, and animal and human studies suggest that some PFAS may be developmental toxicants (4, 5).

A number of epidemiologic studies have found associations of prenatal exposure to 2 PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), with adverse birth outcomes, particularly reduced fetal growth (6–19), and in a recent meta-analysis, Johnson et al. (20) estimated a reduction in term birth weight of 18.9 g (95% confidence interval (CI): −29.8,

−7.9) per 1-ng/mL increase in PFOA. However, other studies have found null PFAS–birth weight associations (21–25).

There has been some concern that associations of PFAS with adverse birth outcomes are attributable to hemodynamic changes that occur during pregnancy (26, 27). Plasma volume expands at approximately 6 weeks' gestation in response to decreased mean arterial pressure, increased cardiac output, and systemic vasodilation (28); this results in serum/plasma dilution of PFAS concentrations. In addition, glomerular filtration rate (GFR) increases at 6 weeks' gestation (28), which may accelerate PFAS excretion, as demonstrated by studies showing higher PFAS concentrations in individuals with reduced GFR (29, 30). Since plasma volume expansion and changes in GFR may also be related to fetal development, including growth (31), these mechanisms could potentially induce a spurious, noncausal association between PFAS and birth outcomes.

To address these concerns, we examined associations of early-pregnancy plasma PFAS concentrations with birth weight-for-gestational length z score (fetal growth) and gestational length, adjusting for confounders, including pregnancy hemodynamics, in a large, well-characterized longitudinal cohort study of women who were pregnant during the years that coincided with peak US population exposures to PFOS and PFOA (1999–2002) (32).

METHODS

Study population

Project Viva is a prospective prebirth cohort study in which mothers were recruited between 1999 and 2002 at their first prenatal visit to one of 8 obstetrical clinics of Atrius Harvard Vanguard Medical Associates, a multispecialty group practice in eastern Massachusetts (33). Eligible mothers were fluent in English, had singleton pregnancies, had pregnancies of <22 weeks' gestation, and had no plans to move away from the study area. Of 2,128 mothers with a live birth between November 1999 and February 2003, 1,668 (78%) provided an early-pregnancy blood sample (median length of gestation, 9 weeks; range, 5–19 weeks), of which 1,645 were sufficient for quantification of PFAS. The institutional review boards of participating institutions approved all study protocols, and all participating mothers provided written informed consent. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research.

Quantification of PFAS and markers of pregnancy physiology

We obtained nonfasting blood samples from women at the recruitment visit, centrifuged the samples, and stored plasma in non-PFAS-containing cryovial tubes in liquid nitrogen freezers (≤−130°C). In 2014, we thawed, aliquoted, and shipped samples to the Division of Laboratory Sciences at the CDC, where vials were stored at or below −40°C. Detailed analytical methods used for PFAS quantification have been described previously (34, 35). Briefly, CDC laboratory staff analyzed plasma by using online solid-phase extraction coupled with isotope dilution high-performance liquid chromatography–tandem mass spectrometry and reported concentrations of PFOA, PFOS, perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA). Reported concentrations for PFOS and PFOA included both linear and branched isomers. Low- and high-concentration quality control materials, prepared from a calf serum pool, were analyzed with the study samples, analytical standards, and reagent and matrix blanks to ensure the accuracy and precision of the data. Limits of detection were 0.2 ng/mL for PFOS and 0.1 ng/mL for the other 3 PFAS. The CDC did not report numerical values below the limit of detection, and we imputed these values as the limit of detection divided by the square root of 2 (36).

We sent aliquots of the samples used for PFAS measurements to the Clinical and Epidemiologic Research Laboratory at Boston Children's Hospital (Boston, Massachusetts) for analysis of markers of pregnancy hemodynamics, including plasma albumin and creatinine concentrations. These markers have been associated with plasma PFAS concentrations in Project Viva (37).

Fetal growth and length of gestation

Project Viva staff abstracted birth weight (in grams) from hospital medical records. We computed birth weight-for-gestational-age and sex z scores using a US national reference (38). We computed length of gestation by subtracting the date of the last menstrual period from the date of birth. Information on length of gestation was also available from the ultrasound at 16–20 weeks' gestation for approximately 79% of participants. For the 200 births (9%) in which gestational length derived from the date of the last menstrual period differed from that derived from the ultrasound by more than 10 days, we used the ultrasound to determine duration of gestation. Because the clinical relevance of small shifts in gestational duration is unclear, we also examined PFAS-related associations with preterm birth, categorized as birth at <37 weeks' gestation.

Statistical analysis

We estimated associations of PFOS, PFOA, PFHxS, and PFNA plasma concentrations with birth weight-for-gestational age and gestational length using multivariable linear regression models. In secondary analyses, we also estimated associations of PFAS with birth weight among term births (≥37 weeks' gestation). To examine associations with preterm birth, we computed odds ratios using logistic regression models. Data on covariates came from interviews and questionnaires administered during early pregnancy, midpregnancy, and at delivery. We used a directed acyclic graph based on a priori knowledge of relationships between variables of interest to identify potential confounders (see Web Figure 1, available at https://academic.oup.com/aje).

History of breastfeeding prior to the index pregnancy is an important variable for PFAS analyses (37), but data on this variable were not collected in Project Viva. We therefore imputed this information using parity and breastfeeding data for the index pregnancy (collected following the birth). If the mother was multiparous (regardless of the number of previous births) and breastfed following the index pregnancy, history of breastfeeding was coded as "yes," under the assumption that a mother who breastfed this child had a high likelihood of having breastfed an

older child (39). If the mother was nulliparous or did not breast-feed the current child, history of breastfeeding was coded as "no."

All multivariable models included characteristics of the mother (age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding prior to the index pregnancy, prepregnancy body mass index (weight (kg)/height (m)^2), gestational age at blood collection) and the child (sex). We also included paternal education and household income.

We examined confounding by plasma albumin concentration and GFR, both markers of pregnancy hemodynamics (26, 27). Albumin is the main binding site for PFAS, as well as a marker of plasma volume expansion during pregnancy (40). GFR is a measure of the flow rate of filtered fluid through the kidney (31). We calculated estimated GFR (eGFR) (mL/minute per 1.73 m^2) by plugging plasma creatinine values into the Cockcroft-Gault formula (Cockcroft-Gault GFR = [140 − age] × weight (kg) × 1.04/plasma creatinine (µmol/L)) (31). To examine whether markers of GFR and plasma volume were biased among subgroups of women with conditions that could influence these markers, such as diabetes or hypertension, we conducted a sensitivity analysis excluding women with these conditions.

We examined linearity of PFAS-outcome associations by fitting generalized additive models with a penalized spline term and also by analyzing PFAS plasma concentrations as quartiles. We examined sex differences in associations of PFAS with fetal and infant outcomes by including a term for interaction between sex and PFAS in the multivariable model. To account for missing covariate data, we used chained equations to impute missing values, generating 50 imputed data sets and combining multivariable model results using PROC MI ANALYZE in SAS (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Characteristics of the 1,645 live births with prenatal maternal plasma PFAS measurements are shown in Table 1. Mothers were predominantly white (69%), and at enrollment many had high educational attainment (65% had a college or graduate degree). Most were married or cohabiting (91%), and many had a high household income (>$70,000/year for 58%) and had never smoked (68%). Table 1 also shows that mothers who were older, were white, had higher educational attainment, were married or cohabiting, had higher partner educational attainment, had a higher household income, did not smoke during pregnancy, were multiparous, and had a prepregnancy body mass index in the overweight (≥25) or obese (≥30) range had infants with higher birth weight for gestational age. These patterns were more or less consistent for length of gestation, with the exception of partner education and parity, where we did not observe meaningful differences.

Median plasma concentrations of prenatal PFOS, PFOA, PFHxS, and PFNA are reported in Table 2. PFAS levels were moderately correlated with each other, with Spearman correlation coefficients as high as 0.72 for PFOS and PFOA. Table 2 also shows that PFAS were moderately correlated with hemodynamic indicators measured in samples collected at the same time as those used to quantify PFAS, including positive correlations with plasma albumin (Spearman correlation coefficients

ranged from 0.14 to 0.24), consistent with serum dilution due to blood volume expansion (higher albumin indicates less dilution, leading to higher plasma PFAS concentrations). PFAS were also negatively correlated with eGFR (values ranged from −0.15 to −0.27), consistent with an increased flow rate during pregnancy (a higher flow rate results in more PFAS excretion and therefore lower plasma PFAS concentrations). PFOS, PFOA, and PFNA were weakly inversely correlated with birth weight for gestational age and were not correlated with length of gestation (Table 2).

Fetal growth

Table 3 shows associations of PFAS with birth weight-for-gestational length z scores. Adjusting for traditional socio-demographic covariates attenuated estimates to some degree, indicating the presence of positive confounding; this attenuation occurred primarily after adjusting for parity (data not shown). Adjusted models show that PFOS and PFNA were associated with small decrements in birth weight-for-gestational age z score (β = −0.04 (95% CI: −0.08, 0.01) and β = −0.06 (95% CI: −0.11, −0.01) per interquartile-range increase, respectively). In secondary analyses (Web Table 1), we also found reductions in term birth weight per interquartile-range increase in PFOS (β = −17.9, 95% CI: −40.9, 5.1), PFOA (β = −18.5, 95% CI: −45.4, 8.3), and PFNA (β = −28.2, 95% CI: −52.0, −4.4). While patterns were not strictly monotonic, we observed overall decrements in fetal growth across quartiles of PFOS, PFOA, and PFNA (Table 3 and Web Table 1). Fetal growth associations with PFHxS were null.

Gestational length

Associations of PFAS with gestational length followed the same pattern (Table 4) as those of fetal growth, with the strongest associations being seen for PFOS and PFNA. When we examined odds ratios for preterm birth (dichotomized at birth <37 weeks vs. ≥37 weeks), we estimated over 2-fold odds of preterm birth among women with concentrations in the highest quartile of PFOS versus those with concentrations in the lowest quartile (odds ratio = 2.4, 95% CI: 1.3, 4.4). Odds of preterm birth were weaker for PFNA and null for PFOA and PFHxS (Table 4).

Confounding by pregnancy physiology

Adjusting for eGFR only slightly attenuated associations of PFAS with birth outcomes (e.g., PFOS–birth weight-for-gestational age associations were attenuated from −0.04 to −0.03) (Tables 3 and 4). Estimates were unchanged after adjustment for plasma albumin (Tables 3 and 4). Excluding women with conditions that could bias markers of GFR and plasma volume, including hypertension or diabetes (n = 39 women), did not influence effect estimates (data not shown).

Sex differences

We did not observe strong evidence for sex differences in PFAS–birth weight-for-gestational age associations (Figure 1A). For gestational length, associations with PFOS were stronger among males (per interquartile-range increase in PFOS, β =

Table 1.   Characteristics of Parents and Infants With Perfluoroalkyl Substance Data in Project Viva and Fetal Growth and Gestational Length According to These Characteristics, Eastern Massachusetts, 1999–2002

Participant Characteristic	No. of Women	%	BW-for-GA z Score[a] (n = 1,644)		Gestational Length, weeks (n = 1,645)	
			Mean (SD)	P Value[b]	Mean (SD)	P Value[b]
Maternal age at enrollment, years				<0.0001		0.01
<20	55	3.3	−0.43 (0.80)		38.6 (2.1)	
20–34	1,133	68.9	0.18 (0.95)		39.5 (2.0)	
≥35	457	27.8	0.30 (0.98)		39.5 (1.9)	
Maternal race/ethnicity				<0.0001		<0.0001
White	1,126	68.5	0.30 (0.95)		39.6 (1.7)	
Black	254	15.4	−0.06 (0.93)		39.1 (2.2)	
Hispanic	120	7.3	−0.05 (0.98)		39.1 (2.1)	
Other	145	8.8	0.04 (0.99)		38.9 (2.6)	
Maternal education at enrollment				<0.0001		0.003
Less than college degree	583	35.5	0.05 (0.96)		39.3 (2.0)	
College degree	601	36.5	0.31 (0.96)		39.4 (2.1)	
Graduate degree	461	28.0	0.23 (0.96)		39.7 (1.7)	
Married or cohabitating at enrollment				0.0002		0.03
Yes	1,499	91.1	0.22 (0.96)		39.5 (1.9)	
No	146	8.9	−0.10 (0.93)		39.1 (2.5)	
Partner's education at enrollment				0.001		0.28
Less than college degree	652	39.6	0.08 (0.98)		39.3 (2.0)	
College degree	553	33.6	0.28 (0.97)		39.5 (1.9)	
Graduate degree	440	26.8	0.25 (0.92)		39.5 (2.0)	
Annual household income at enrollment				<0.0001		0.01
<$40,000	297	18.1	−0.06 (1.02)		39.1 (2.2)	
$40,000–$70,000	399	24.2	0.20 (0.93)		39.6 (1.7)	
>$70,000	949	57.7	0.27 (0.95)		39.5 (1.9)	
Prenatal smoking				0.003		0.01
Never smoker	1,118	68.0	0.19 (0.95)		39.3 (2.1)	
Former smoker	310	18.8	0.32 (0.92)		39.7 (1.6)	
Smoked during pregnancy	217	13.2	0.04 (1.07)		39.5 (1.8)	
Maternal parity				<0.0001		0.91
0	800	48.6	0.02 (0.93)		39.4 (2.1)	
≥1	845	51.4	0.36 (0.97)		39.4 (1.7)	
Prepregnancy body mass index[c]				<0.0001		0.05
<18.5 (underweight)	56	3.4	−0.16 (0.81)		39.6 (1.2)	
18.5–24.9 (normal)	954	58.0	0.11 (0.95)		39.5 (1.9)	
25–29.9 (overweight)	368	22.3	0.38 (0.98)		39.5 (1.9)	
≥30 (obese)	267	16.2	0.30 (0.98)		39.1 (2.2)	

Abbreviations: BW, birth weight; GA, gestational age; SD, standard deviation.
[a] Calculated using a US national reference standard (38).
[b] P value for the unadjusted covariate-outcome association from analysis of variance.
[c] Weight (kg)/height (m)^2.

−0.19, 95% CI: −0.33, −0.05) than among females (β = 0.01, 95% CI: −0.11, 0.14; P-interaction = 0.09) (Figure 1B). We found similar patterns for PFNA in males (β = −0.19, 95% CI: −0.33, −0.06) and females (β = 0.03, 95% CI: −0.11, 0.16; P-interaction = 0.01). We did not find evidence for sex differences in any other studied associations.

Table 2.   Correlations of Plasma Concentrations of Perfluoroalkyl Substances With Hemodynamic Indicators and Birth Outcomes Among Participants in Project Viva (*n* = 1,645), Eastern Massachusetts, 1999–2002

Measure	Median (IQR)	PFOS	PFOA	PFHxS	PFNA	eGFR-CG[a]	Albumin	GA at Blood Draw	BW-for-GA z Score[b]	Gestational Length
PFOS, ng/mL	25.7 (16.0)	1.00	0.72	0.50	0.61	−0.19	0.14	−0.13	−0.08	−0.05
PFOA, ng/mL	5.8 (3.8)		1.00	0.52	0.52	−0.15	0.17	−0.12	−0.07	0.02
PFHxS, ng/mL	2.4 (2.2)			1.00	0.42	−0.15	0.14	−0.06	0.01	0.01
PFNA, ng/mL	0.7 (0.4)				1.00	−0.27	0.24	−0.12	−0.08	−0.01
eGFR-CG[a], mL/minute per 1.73 m^2	101.4 (44.1)					1.00	−0.01	0.16	0.15	0.02
Albumin concentration, g/dL	8.3 (2.4)						1.00	−0.06	0.03	0.02
GA at blood draw, weeks	9.7 (2.1)							1.00	0.02	0.06
BW-for-GA z score (*n* = 1,644)[b]	0.2 (1.4)								1.00	0.19
Length of gestation, weeks	39.7 (1.7)									1.00

Abbreviations: BW, birth weight; eGFR-CG, estimated glomerular filtration rate computed with the Cockcroft-Gault formula; GA, gestational age; IQR, interquartile range; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a] $[140 - \text{age}] \times \text{weight (kg)} \times 1.04/\text{plasma creatinine (μmol/L)}$ (31).

[b] Calculated using a US national reference standard (38).

DISCUSSION

In this large prospective study, we measured plasma PFAS concentrations in pregnant women before the US phaseout of PFOS (41) and PFOA (42). As a result, plasma concentrations of these PFAS (37), and of PFOS in particular, were considerably higher in this cohort than those reported in more recent US national surveys (43, 44) but were similar to concentrations in a nationally representative survey with measurements taken during the same time period (3).

In this context, we observed modest reductions in fetal growth with higher concentrations of PFOS, PFOA, and PFNA. Higher PFOS and PFNA concentrations were also associated with shorter gestational length, though primarily among males. Associations of PFHxS with all birth outcomes were null. Notably, we did not observe confounding by measures of pregnancy hemodynamics, including eGFR and plasma albumin level.

Gestational exposure to some PFAS has been linked with poorer fetal growth and development in animal models (45, 46). Though a direct mechanism for the impact of PFAS exposure on fetal growth has not been characterized, structural homology of PFAS with fatty acids may induce disruption of lipid metabolism (47), which could interfere with fetal growth. PFAS can also alter thyroid hormone levels (48) and activate peroxisome proliferator-activated receptors (49), pathways that may influence fetal growth.

Two meta-analyses, one of 9 published studies (20) and another of 7 studies (all of which were included in the 9-study meta-analysis) (50), found 18.9-g (95% CI: −29.8, −7.9) and 14.7-g (95% CI: −21.76, −7.8) reductions in birth weight per 1-ng/mL increase in serum PFOA and plasma PFOA, respectively. These associations were considerably stronger than those we detected in the current study: Converting PFOA estimates per interquartile-range increase (3.8 ng/mL) in Web Table 1 to estimates per 1-ng/mL increase, we found a reduction in term birth weight of 4.9 g (95% CI: −11.9, 2.2). In addition, in the 7-study meta-analysis, Verner et al. (50) reported

a 5.0-g (95% CI: −8.9, −1.1) reduction in birth weight per 1-ng/mL increase in serum or plasma PFOS, as compared with only a 1.1-g (95% CI: −2.6, 0.3) reduction for plasma PFOS in the current study (computed using estimates in Web Table 1).

We detected associations of PFNA with birth outcomes in the current study; however, given the low plasma concentrations of PFNA in Project Viva compared with other, more commonly studied PFAS, such as PFOS and PFOA, these results should be interpreted with caution. Only a few other studies have examined associations of PFNA with birth outcomes, presumably because of the relatively low PFNA concentrations, with mixed findings (9, 13, 51).

A primary objective of the current study was to evaluate whether adjusting for pregnancy hemodynamics affected PFAS–birth outcome associations. Adjusting for albumin and eGFR did not materially alter effect estimates, suggesting that there was little or no confounding by these hemodynamic markers. Only 1 previous study, the Norwegian Mother and Child Cohort Study, adjusted for plasma albumin when examining associations of PFOA and PFOS measured in early gestation (17 weeks) with birth outcomes (19). This analysis also showed no confounding by albumin. We are not aware of any previous studies of PFAS and birth outcomes that adjusted for GFR. However, confounding by GFR was examined by Verner et al. (50), who used simulated data on maternal and umbilical cord plasma PFOA and PFOS concentrations. In contrast to the current study, Verner et al. observed considerable confounding of the PFAS–term birth weight association by GFR, with strong attenuation of both PFOA- and PFOS-related associations with term birth weight after adjustment for GFR (50). One explanation for these conflicting findings is that plasma samples in Project Viva were drawn early in pregnancy, when pregnancy hemodynamic changes are just beginning. We speculate that this confounding may therefore only be present in studies in which blood is drawn later in pregnancy. This is further supported by the previous literature, where studies with the strongest

Table 3. Associations of Prenatal Plasma Concentrations of Perfluoroalkyl Substances With Birth-Weight-for-Gestational Age z Score[a] Among Participants in Project Viva (n = 1,644), Eastern Massachusetts, 1999–2002

| PFAS | Range, ng/mL | No. of Women | Change in Birth-Weight-for-Gestational Age z Score | | | | | | | |
| | | | Unadjusted | | Adjusted[b] | | Adjusted[b] + eGFR-CG[c] | | Adjusted[b] + Albumin | |
			β	95% CI	β	95% CI	β	95% CI	β	95% CI
PFOS										
Continuous IQR[d]		1,644	−0.07	−0.11, −0.02	−0.04	−0.08, 0.01	−0.03	−0.08, 0.02	−0.04	−0.09, 0.00
Quartile 1	0.1–18.8	411	0	Referent	0	Referent	0	Referent	0	Referent
Quartile 2	18.9–25.6	409	−0.13	−0.26, 0.01	−0.09	−0.22, 0.04	−0.08	−0.21, 0.05	−0.09	−0.22, 0.03
Quartile 3	25.7–34.8	411	−0.14	−0.27, 0.00	−0.09	−0.22, 0.04	−0.07	−0.20, 0.06	−0.09	−0.22, 0.04
Quartile 4	34.9–185.0	413	−0.20	−0.33, −0.07	−0.13	−0.26, 0.00	−0.10	−0.24, 0.03	−0.14	−0.27, 0.00
PFOA										
Continuous IQR[d]		1,644	−0.05	−0.10, 0.00	−0.02	−0.08, 0.03	−0.01	−0.07, 0.04	−0.02	−0.08, 0.03
Quartile 1	0.9–4.1	412	0	Referent	0	Referent	0	Referent	0	Referent
Quartile 2	4.2–5.8	410	−0.09	−0.22, 0.04	−0.04	−0.17, 0.09	−0.03	−0.16, 0.10	−0.04	−0.17, 0.09
Quartile 3	5.9–7.9	416	−0.20	−0.33, −0.07	−0.12	−0.25, 0.02	−0.10	−0.24, 0.03	−0.12	−0.26, 0.01
Quartile 4	8.0–49.3	406	−0.16	−0.29, −0.03	−0.07	−0.21, 0.07	−0.05	−0.19, 0.09	−0.08	−0.22, 0.06
PFHxS										
Continuous IQR[d]		1,644	−0.01	−0.04, 0.02	0.00	−0.03, 0.02	0.00	−0.03, 0.02	0.00	−0.03, 0.02
Quartile 1	0.1–1.6	414	0	Referent	0	Referent	0	Referent	0	Referent
Quartile 2	1.7–2.4	409	−0.06	−0.20, 0.07	−0.10	−0.23, 0.03	−0.08	−0.21, 0.05	−0.10	−0.23, 0.03
Quartile 3	2.5–3.7	404	0.06	−0.07, 0.19	0.04	−0.09, 0.17	0.06	−0.07, 0.19	0.04	−0.09, 0.17
Quartile 4	3.8–74.5	417	0.00	−0.13, 0.13	0.00	−0.14, 0.13	0.02	−0.12, 0.15	−0.01	−0.14, 0.12
PFNA										
Continuous IQR[d]		1,644	−0.08	−0.12, −0.03	−0.06	−0.11, −0.01	−0.05	−0.10, −0.01	−0.06	−0.11, −0.02
Quartile 1	0.1–0.4	331	0	Referent	0	Referent	0	Referent	0	Referent
Quartile 2	0.5–0.6	462	−0.02	−0.16, 0.12	−0.03	−0.16, 0.10	−0.02	−0.15, 0.12	−0.03	−0.17, 0.10
Quartile 3	0.7–0.9	470	−0.19	−0.33, −0.06	−0.19	−0.32, −0.06	−0.17	−0.31, −0.04	−0.20	−0.33, −0.06
Quartile 4	1.0–6.0	381	−0.18	−0.32, −0.04	−0.15	−0.30, −0.01	−0.13	−0.28, 0.02	−0.17	−0.31, −0.02

Abbreviations: CI, confidence interval; eGFR-CG, estimated glomerular filtration rate computed with the Cockcroft-Gault formula; IQR, interquartile range; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a] Calculated using a US national reference standard (38).

[b] Adjusted for maternal age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, prepregnancy body mass index, paternal education, household income, child's sex, and gestational age at blood draw (see Table 1 for variable categories).

[c] $[140 - \text{age}] \times \text{weight (kg)} \times 1.04/\text{plasma creatinine (μmol/L)}$ (38).

[d] IQRs for PFAS were: PFOS, 16.0 ng/mL; PFOA, 3.8 ng/mL; PFHxS, 2.2 ng/mL; PFNA, 0.4 ng/mL.

Table 4. Associations of Prenatal Plasma Concentrations of Perfluoroalkyl Substances With Length of Gestation and Preterm Birth (Birth <37 Weeks' Gestation vs. ≥37 Weeks) Among Participants in Project Viva (*n* = 1,645), Eastern Massachusetts, 1999–2002

PFAS	Range, ng/mL	No. of Women	Unadjusted		Adjusted[a]		Adjusted[a] + eGFR-CG[b]		Adjusted[a] + Albumin		No. of Preterm Births	Adjusted Odds Ratio[a]	95% CI
			β	95% CI	β	95% CI	β	95% CI	β	95% CI			
PFOS													
Continuous IQR[c]		1,645	−0.10	−0.19, 0.00	−0.08	−0.17, 0.02	−0.06	−0.16, 0.03	−0.08	−0.17, 0.01	120	1.1	1.0, 1.3
Quartile 1	0.1–18.8	411	0	Referent	0	Referent	0	Referent	0	Referent	16	1	Referent
Quartile 2	18.9–25.6	410	−0.24	−0.51, 0.03	−0.20	−0.47, 0.06	−0.17	−0.44, 0.09	−0.20	−0.47, 0.06	31	2.0	1.1, 3.7
Quartile 3	25.7–34.8	411	−0.15	−0.42, 0.11	−0.08	−0.35, 0.19	−0.05	−0.32, 0.23	−0.09	−0.36, 0.18	33	2.0	1.1, 3.7
Quartile 4	34.9–185.0	413	−0.43	−0.69, −0.16	−0.36	−0.64, −0.09	−0.31	−0.59, −0.03	−0.37	−0.65, −0.10	40	2.4	1.3, 4.4
PFOA													
Continuous IQR[c]		1,645	−0.04	−0.69, −0.16	−0.05	−0.16, 0.06	−0.03	−0.14, 0.08	−0.05	−0.16, 0.06	120	1.0	0.9, 1.3
Quartile 1	0.3–4.1	413	0	Referent	0	Referent	0	Referent	0	Referent	26	1	Referent
Quartile 2	4.2–5.8	410	0.07	−0.20, 0.34	0.05	−0.22, 0.32	0.07	−0.20, 0.34	0.05	−0.22, 0.32	30	1.1	0.6, 2.0
Quartile 3	5.9–7.9	416	0.02	−0.25, 0.28	0.00	−0.28, 0.28	0.05	−0.24, 0.33	0.00	−0.28, 0.28	30	1.1	0.6, 1.9
Quartile 4	8.0–49.3	406	−0.03	−0.29, 0.24	−0.04	−0.33, 0.24	0.01	−0.28, 0.30	−0.05	−0.33, 0.24	34	1.2	0.7, 2.2
PFHxS													
Continuous IQR[c]		1,645	0.03	−0.03, 0.08	0.02	−0.04, 0.07	0.02	−0.03, 0.08	0.02	−0.04, 0.07	120	1.0	0.9, 1.1
Quartile 1	0.1–1.6	415	0	Referent	0	Referent	0	Referent	0	Referent	26	1	Referent
Quartile 2	1.7–2.4	409	0.12	−0.15, 0.38	0.08	−0.18, 0.35	0.11	−0.16, 0.38	0.08	−0.19, 0.35	23	0.9	0.5, 1.7
Quartile 3	2.5–3.7	404	−0.13	−0.39, 0.14	−0.18	−0.45, 0.09	−0.15	−0.42, 0.12	−0.19	−0.46, 0.08	41	1.8	1.1, 3.1
Quartile 4	3.8–74.5	417	0.00	−0.27, 0.26	−0.10	−0.37, 0.17	−0.06	−0.33, 0.22	−0.10	−0.38, 0.17	30	1.3	0.7, 2.2
PFNA													
Continuous IQR[c]		1,645	−0.08	−0.18, 0.01	−0.07	−0.17, 0.02	−0.06	−0.15, 0.04	−0.08	−0.17, 0.02	120	1.2	1.0, 1.4
Quartile 1	0.1–0.4	331	0	Referent	0	Referent	0	Referent	0	Referent	20	1	Referent
Quartile 2	0.5–0.6	463	0.12	−0.15, 0.40	0.10	−0.17, 0.38	0.14	−0.14, 0.42	0.10	−0.18, 0.38	23	0.8	0.5, 1.6
Quartile 3	0.7–0.9	470	0.01	−0.27, 0.28	−0.02	−0.30, 0.26	0.03	−0.25, 0.31	−0.03	−0.31, 0.25	39	1.4	0.8, 2.6
Quartile 4	1.0–6.0	381	−0.09	−0.38, 0.20	−0.07	−0.37, 0.22	−0.01	−0.31, 0.30	−0.08	−0.38, 0.22	38	1.7	0.9, 3.0

Abbreviations: CI, confidence interval; eGFR-CG, estimated glomerular filtration rate computed with the Cockcroft-Gault formula; IQR, interquartile range; PFAS, perfluoroalkyl substance(s); PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a] Adjusted for maternal age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, prepregnancy body mass index, paternal education, household income, child's sex, and gestational age at blood draw (see Table 1 for variable categories).

[b] [140 − age] × weight (kg) × 1.04/plasma creatinine (μmol/L) (38).

[c] IQRs for PFAS were: PFOS, 16.0 ng/mL; PFOA, 3.8 ng/mL; PFHxS, 2.2 ng/mL; PFNA, 0.4 ng/mL.
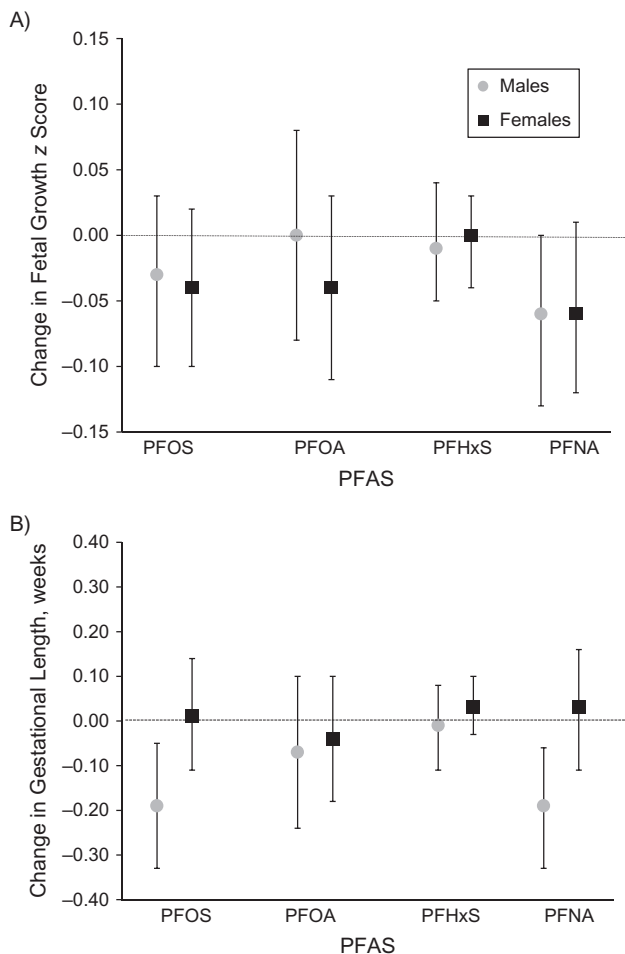
Figure 1. Associations of prenatal plasma concentrations of perfluoroalkyl substances (PFAS) with fetal growth (birth weight-for-gestational age) z score (n = 1,644) (A) and length of gestation (weeks) (n = 1,645) (B), by sex, among participants in Project Viva, eastern Massachusetts, 1999–2002. Results were adjusted for maternal age at enrollment, race/ethnicity, education, prenatal smoking, parity, history of breastfeeding, prepregnancy body mass index, paternal education, household income, and gestational age at blood draw. PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate. Bars, 95% confidence intervals.

reported PFOA–birth weight associations measured PFOA in umbilical cord serum at birth (7, 52), while studies with weaker birth weight associations measured PFOA in early pregnancy (first trimester or early second trimester) (6, 8, 19, 21). The suggestion that confounding by eGFR has less influence when PFOS and PFOA concentrations are measured in early pregnancy is also supported by the simulated data (50).

Although a cohort with blood samples drawn late in pregnancy (second or third trimester) might best reveal the extent of potential confounding by plasma volume expansion and GFR, our results do suggest that studies that examine associations of birth outcomes with PFAS in serum/plasma drawn early in pregnancy are unlikely to be substantially confounded by pregnancy hemodynamics.

When we compared our birth weight results (Web Table 1) with those of 4 previous studies that measured PFAS in early pregnancy (9–17 weeks) (6, 8, 19, 21)—as these are likely to be the associations that are least confounded by pregnancy hemodynamics—we found some consistency across individual PFAS. For example, the 4 studies found that a 1-ng/mL increase in PFOA was associated with a reduction in birth weight of 11–34 g, whereas in our study we found a 19-g reduction in birth weight (Web Table 1). However, the previous studies observed null associations of PFOS with birth weight, while we observed associations for PFOS that were comparable to our PFOA associations. In one previous study, Maisonet et al. (8) reported lower birth weight with exposure to PFHxS, though our study and another previous study (21) found null associations.

A limitation of our analysis examining confounding by pregnancy hemodynamics is that we used markers measured in early-pregnancy plasma: creatinine for GFR and albumin for plasma volume expansion. Whether these markers adequately represent pregnancy hemodynamics is unclear. Confounder measurement error would have resulted in residual confounding when controlling for these variables in multivariable models. However, given that we observed minimal attenuation when we included these markers as covariates, we would not expect residual confounding to completely account for our observed associations.

This study had a number of strengths. We had a large sample size with participants recruited before the voluntary phase-out of PFOS and PFOA, and thus during the time of likely peak exposure to these PFAS in the United States (32). We also adjusted for key confounders, including parity, sociodemographic factors, and pregnancy hemodynamics.

In conclusion, concentrations of early-pregnancy PFOS, PFOA, and PFNA were inversely associated, albeit modestly, with fetal growth and length of gestation in Project Viva. These findings, in a population enrolled when exposures to PFOS and PFOA were likely at their peak in the United States, are consistent with other studies showing weak inverse associations of PFOS and PFOA measured early in pregnancy with fetal growth. Measurement of PFAS in early pregnancy in future studies may avoid confounding by pregnancy hemodynamics. However, in studies that measure PFAS in later pregnancy, researchers should consider adjusting for markers of pregnancy hemodynamics.

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