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Abstract

Context: Per- and polyfluoroalkyl substances (PFAS) are environmental chemicals linked to weight gain and type 2 diabetes.

Objective: We examined the extent to which PFAS plasma concentrations during pregnancy were associated with postpartum anthropometry and biomarkers.

Design, Patients, Measures: We studied women recruited between 1999-2002 in the Project Viva prospective cohort with pregnancy plasma concentrations of PFAS, including perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and 2-(N-ethyl-perfluorooctane sulfonamide) acetic acid (EtFOSAA). Three-year postpartum anthropometry measurements were available from 786-801 women, blood pressure from 761 women, and blood biomarkers from 450-454 women. We used multivariable regression to evaluate the association of log_2-transformed PFAS with postpartum anthropometry, blood pressure, and blood biomarkers (leptin, adiponectin, sex hormone binding globulin [SHBG], hemoglobin A1c [HbA1c], interleukin-6 [IL-6], C-reactive protein [CRP]), adjusting for age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, parity, and breastfeeding history.

Results: Pregnancy concentrations of certain PFAS were associated with greater adiposity (e.g., 0.4 cm [95%CI: -0.1, 0.9] greater waist circumference per doubling in EtFOSAA; 0.2 cm [95%CI: -0.1, 0.5] greater mid-upper arm circumference per doubling in PFOA; 1.2 mm [95%CI: 0.1, 2.2] thicker sum of subscapular and triceps skinfolds per doubling in PFOS) and higher systolic blood pressure (e.g., 1.2 mm Hg [95%CI: 0.3, 2.2] per doubling in PFOS) at three years postpartum. Higher EtFOSAA concentrations were also associated with 10.8% higher IL-6 (95%CI: 3.3, 18.9) and 6.1% lower SHBG (95%CI: 0.7, 11.2) per doubling.

Conclusions: Pregnancy concentrations of EtFOSAA, PFOS, and PFOA were associated with adverse postpartum cardiometabolic markers.

Keywords: PFAS, anthropometry, biomarkers, pregnancy, postpartum

Introduction

Growing epidemiologic evidence suggests that pregnancy complications may indicate elevated long-term maternal cardiometabolic risk [1]. For example, gestational diabetes is strongly associated with a woman's future risk of developing type 2 diabetes [2], and excessive gestational weight gain has been linked with greater postpartum weight retention and future weight gain [3]. Indeed, metabolic disruptions in pregnancy are associated with mothers' postpartum cardiometabolic health in clinically measurable ways as soon as a few years after pregnancy. Cumulative incidence of type 2 diabetes increases most rapidly in the first 5 years after a gestational diabetes pregnancy, and the incidence of new pharmacologically-treated hypertension is highest in the first 4 years after a pregnancy complicated by preterm delivery, pre-eclampsia, or gestational diabetes [2 4]. Though research on pregnancy complications indicates that metabolic disruption in pregnancy is associated with long-term health risks, little research has examined whether exposures to other stressors in pregnancy, including chemical toxicant exposures, might be associated with postpartum cardiometabolic health.

Per- and polyfluoroalkyl substances (PFAS) are endocrine disrupting chemicals that have been manufactured in the U.S. and worldwide since the 1950s [5]. They can be used in food packaging, cooking equipment, water- and stain-resistant fabric treatments, and firefighting foams [6-8], and some have multi-year half-lives in humans [9]. U.S. adults are ubiquitously exposed to multiple PFAS, largely through consumption of contaminated food and water [10]. In pregnant populations, PFAS concentrations are positively associated with income [11-13]. Associations with race/ethnicity are inconsistent and vary across studies [11-13], possibly because drinking water contaminated by PFAS (e.g., industrial wastewater, airport runoff, military base runoff) is a major exposure source [8], and the relationship between demographic factors such as race and proximity to contaminated water varies from place to place [14]. Despite a voluntary U.S. phase-out of PFOS and PFOA beginning in 2000 [15], PFOS and PFOA continue to be widely detected in the U.S. population [16].

Exposures to several PFAS have been linked to poorer cardiometabolic health in non-pregnant adults, including weight gain, incident diabetes, and microvascular disease [17-20], potentially via activation by PFAS of PPAR-γ and ER-α [21 22]. However, prior studies inconsistently link PFAS exposure and changes in blood biomarkers associated with incident cardiometabolic outcomes, such as leptin, adiponectin, sex hormone binding globulin (SHBG), hemoglobin A1c (HbA1c), interleukin-6 (IL-6), and C-reactive protein (CRP) [23-32]. Only two studies of PFAS and biomarkers in adults evaluated associations in pregnant populations (one null study of CRP and one study of IL-6 that reported positive associations), so possible effects of PFAS in this potentially sensitive window [33] are largely unknown [34 35]. To our knowledge, no study has assessed PFAS exposure in pregnancy and maternal cardiometabolic health in the years following pregnancy, a potentially sensitive window of metabolic disruption.

In this study, we used a longitudinal pre-birth cohort to evaluate associations of plasma PFAS concentrations measured in pregnancy and maternal plasma biomarkers (leptin, adiponectin, SHBG, HbA1c, IL-6, CRP), blood pressure, and anthropometric measurements at three years postpartum. We hypothesized that higher pregnancy PFAS concentrations would be associated with a worse cardiometabolic profile: higher IL-6, CRP, HbA1c, and leptin; lower SHBG and adiponectin; and higher adiposity and blood pressure at three years postpartum.

Methods

Project Viva is a prospective pre-birth cohort of women recruited between 1999 and 2002 during their first prenatal visit at Atrius Harvard Vanguard Medical Associates, a multi-specialty group practice in eastern Massachusetts [36]. Eligible women spoke English, were pregnant with a single fetus, were less than 22 weeks' gestation, and planned to deliver in eastern Massachusetts. All participants provided written informed consent and the human subjects committee of Harvard Pilgrim Health Care approved all procedures. Participants completed multiple study visits, including during pregnancy, at delivery, and at three years postpartum, and completed interviews and questionnaires at each time point. Twenty-eight women participated in Project Viva for two pregnancies; we limited the

analysis to the first eligible pregnancy for each participant. We also excluded pregnancies without plasma PFAS measurements (n=472) and those with pre-pregnancy type 1 or type 2 diabetes (n=14), leaving an eligible population of 1614 participants at baseline.

We collected blood samples in early pregnancy (median 9.7 weeks; range 4.8-21.4 weeks). We stored plasma in non-PFAS-containing cryovials in liquid nitrogen freezers at ≤-130°C until shipment to the CDC laboratory in 2014. At CDC, plasma was stored at or below −40°C until analysis in 2014. CDC staff quantified PFAS by on-line solid-phase extraction coupled with isotope dilution high-performance liquid chromatography–tandem mass spectrometry as described in detail before [37]. The analytical method was the same used to analyze PFAS concentrations in the 2011–2012 Health and Nutrition Examination Survey (NHANES) cycle [38]. Repeated measurements of serum quality control pools, reflecting both inter- and intra-day variation, had coefficients of variation for the PFAS in this study of about 8-13% [38]. To ensure accuracy and reliability, the laboratory analyzed low and high-concentration quality control materials, analytical standards, and reagent and serum blanks along with the study samples; the laboratory successfully participated in external quality assessment schemes [37].The PFAS were: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), perfluorodecanoic acid (PFDA), 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (EtFOSAA), and 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA). PFOS and PFOA measures represented total PFOS and PFOA (sum of linear and branched isomers). Limits of detection (LOD) were 0.2 ng/mL for PFOS and 0.1 ng/mL for all other PFAS. All PFAS except PFDA were detected in over 98% of samples. We excluded PFDA from analysis because it was detected in only 45% of samples. For other PFAS, we imputed values below the LOD (<1% of samples) using LOD/$\sqrt{2}$ [39]. The analysis of coded samples at the CDC laboratory did not constitute engagement in human subjects research.

During in-person visits at three years postpartum, trained research assistants (RAs) measured adiposity, weight, height, and blood pressure. RAs measured subscapular skinfold thickness and triceps skinfold thickness using a Holtain caliper and recorded measurements to the nearest 0.1 mm.

We added subscapular and triceps skinfold thickness measures to create a single skinfold thickness measure (SS+TR) for analysis [40]. RAs measured waist circumference and mid-upper arm circumference using a Lefkin woven tape and recorded measurements to the nearest 0.1 cm. RAs measured weight (participants removed their shoes but were otherwise fully clothed) using a Seca scale and recorded measurements to the nearest 0.1 kg. RAs measured height using a stadiometer and recorded measurements to the nearest 0.1 cm. We used RA-measured weight and height to calculate body mass index (BMI, weight [kg]/height[m^2]).

RAs measured diastolic and systolic blood pressure five consecutive times each using the participant's right arm, with measurements 1 minute apart, using the Dinamap Pro100 or DinamapPro200 automated blood pressure recorder. We took the average of the last four measurements for analysis, discarding the first measurement [41]. For participants contributing fewer than five measurements (n=4 participants), we discarded the first measurement and calculated an average from the remaining measurements.

Trained phlebotomists collected blood samples, and we froze the blood samples in non-PFAS-containing cryovials in liquid nitrogen freezers at ≤-130°C within 24 hours of collection. The Boston Children's Hospital Clinical Chemistry Laboratory measured HbA1c, adiponectin, leptin, CRP, SHBG, and IL-6 in the collected samples. Investigators in an earlier study measured these blood biomarkers to test the effects of lactation on postpartum diabetic risk factors; we used the same biomarker measurements here [42 43]. Measurement of blood biomarkers has been previously described [42 43]. Briefly, we quantified HbA1c using the Hitachi 917 analyzer (Roche Diagnostics) and quantified leptin and adiponectin using enzyme-linked immunosorbent assays (R&D Systems). We measured CRP using an immunoturbidimetric high-sensitivity assay on a Hitachi 911 analyzer (Roche Diagnostics) and reagents and calibrators from Denka Seiken. We measured SHBG using a competitive electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyzer (Roche Diagnostics). Finally, we measured IL-6 using ultrasensitive ELISA.

Covariate data collection

At recruitment, participants reported race/ethnicity, age, education, parity, marital status, household income, and smoking status. To calculate pre-pregnancy BMI, we used self-reported pre-pregnancy weight and height. We estimated history of breastfeeding using parity and information about breastfeeding after the index pregnancy: we classified nulliparous women and women who did not breastfeed after the index pregnancy as having no history of breastfeeding, and we classified parous women who breastfed after the index pregnancy as having a history of breastfeeding [13]. We selected confounders using directed acyclic graphs and previous literature [11-13] and adjusted all models for age, continuous pre-pregnancy BMI, marital status (married/cohabitating versus not), race/ethnicity (black, white, or other), education (college graduate or more versus less than college graduate), household income (>$70,000 versus ≤$70,000), smoking (current, former, or never), parity (0, 1, or >1), and history of breastfeeding (ever versus never).

Statistical analysis

We $\log_2$-transformed PFAS plasma concentrations; effect estimates are presented per doubling of PFAS concentrations. We natural-log transformed blood biomarker levels measured in postpartum; effect estimates for these models are reported as percent difference in biomarker per doubling in PFAS. We used chained equations in SAS PROC MI to multiply impute missing baseline covariate values (12% of participants were missing any covariate) and breastfeeding in fifty datasets, using data from exposures, outcomes, and additional covariates (a full list of covariates used in imputations can be found in the Supplemental Material [44]). We chose to generate fifty imputations to ensure reproducibility of the estimates [36]. We combined the results from the fifty imputed datasets in the analysis.

We evaluated the association between plasma PFAS concentrations and each 3-year postpartum biomarker and anthropometry measure using multivariable linear regression models. We excluded participants who were pregnant within the last 6 months prior to the 3-year postpartum visit

(n=192). Because of small amounts of missing data for some outcomes, sample sizes in fully adjusted models ranged from n=786 to n=801 for 3-year anthropometry models; we excluded women with pre-pregnancy hypertension (n=73) from blood pressure models resulting in a sample size of n=761 for fully adjusted blood pressure models. Because only a subset of participants elected to give blood samples at three years postpartum, sample sizes ranged from n=450 to n=454 for blood biomarker models. To correct for non-random loss to follow-up associated with a post-baseline variable (gestational weight gain) resulting in potential selection bias, we used stabilized inverse probability of censoring weights (IPCW) [45], which upweighted individuals remaining in the population to take the place of those who were lost to follow-up, based on demographics and gestational weight gain. We calculated separate IPCW for each PFAS in each imputed dataset, and calculated denominators of the weights using all baseline confounders as well as total gestational weight gain (predictors of loss to follow-up). Analyses were performed using SAS 9.4 (SAS Institute Inc.).

Sensitivity analyses

To ensure that our results were not due to confounding by correlations among the PFAS, we additionally repeated all analyses including all PFAS in a single model to control for PFAS-PFAS confounding. To ensure that our results were not affected by length of time since the most recent pregnancy, we repeated the analysis excluding women who became pregnant again after the index pregnancy and before the time of the health outcome measurements. To test whether findings were modified by maternal age at baseline, we additionally stratified all models by maternal age (<35 years versus ≥ 35 years at pregnancy).

Results

Compared to women with PFAS measurements, women without PFAS measurements were slightly more likely to have a history of breastfeeding (45% versus 41%) and have a normal pre-pregnancy BMI (67% versus 61%), were more likely to be a race other than white (40% versus 32%), and were more likely to have enrolled in the study in 2001 or 2002 (instead of 1999 or 2000) (54% versus 38%). They did not substantially differ from included women by age, parity, education, marital status, smoking status, or household income. Most participants included in our current analyses were aged 30 or older at pregnancy, were white, had graduated from college, and had a household income greater than $70,000/year (Table 1, Supplemental Table S1 [44]). Imputations did not affect the distribution of covariates in the study population (Supplemental Table S2 [44]).

Compared to those without anthropometry, blood pressure, or blood biomarker measurements at three years postpartum, the subset included in the analysis was more often over age 35 at pregnancy, white, and college graduates; and less often smoked in pregnancy. Spearman correlation coefficients between the six PFAS ranged from 0.18 to 0.74; most correlations were between 0.2 and 0.5 (Supplemental Table S3 [44]).

Anthropometry

In the anthropometry sample, median BMI at three years postpartum was 24.9 kg/m^2 (interquartile range: 22.0-28.7); approximately 50% of women had BMI ≥ 25 kg/m^2 and approximately 20% of women had BMI ≥ 30kg/m^2. Mean waist circumference was 87.0 cm (standard deviation (SD)= 12.8 cm).

Certain PFAS were associated with greater adiposity at three years postpartum, though some confidence intervals crossed the null (Figure 1; Supplemental Table S4 [44]). Participants had, on average, 0.3 kg/m^2 (95% confidence interval [CI]: 0.0, 0.6) higher 3-year postpartum BMI per doubling in pregnancy PFOA concentrations, and 0.4 cm (95%CI: -0.1, 0.9) greater 3-year postpartum

waist circumference per doubling in pregnancy EtFOSAA concentrations, in fully adjusted models (which include pre-pregnancy BMI as a covariate). Applying these findings to the observed range of PFOA and EtFOSAA, those at the 75th percentile of PFOA concentrations in pregnancy (7.6 ng/mL) would have on average 0.3 kg/m^2 higher BMI at three years postpartum than those at the 25th percentile of pregnancy concentrations (4.0 ng/mL), and those at the 75th percentile of pregnancy EtFOSAA concentrations (1.9 ng/mL) would have on average 0.6 cm larger waist circumference at three years postpartum than those at the 25th percentile of EtFOSAA pregnancy concentrations (0.7 ng/mL). PFOA, PFOS, and EtFOSAA were also associated with greater average mid-upper arm circumference, though confidence intervals crossed the null (0.2 cm [95%CI: -0.1, 0.4] higher per doubling in PFOS, 0.2 cm [95%CI: -0.1, 0.5] higher per doubling in PFOA, and 0.1 cm [95%CI: -0.0, 0.3] higher per doubling in EtFOSAA); and greater SS+TR skinfold thicknesses (1.2 [95%CI: 0.1, 2.2] mm higher per doubling in PFOS, 0.9 mm [95%CI: -0.3, 2.1] higher per doubling in PFOA, and 0.6 mm [95%CI: -0.1, 1.4] higher per doubling in EtFOSAA), though confidence intervals crossed the null (Figure 1; Supplemental Table S4 [44]). PFHxS, PFNA, and MeFOSAA were not associated with measures of adiposity.

Blood pressure

In the blood pressure sample, mean systolic blood pressure was 108 mm Hg (SD = 11 mm Hg), and mean diastolic blood pressure was 66 mm Hg (SD= 8 mm Hg). Six women (0.8%) had incident hypertension (defined as systolic blood pressure >140 or diastolic blood pressure >90 mmHg and no hypertension before the index pregnancy). Pregnancy plasma concentrations of PFOS, PFOA, and MeFOSAA were associated with greater systolic blood pressure at three years postpartum, though confidence intervals contained the null in some cases (Figure 2; Supplemental Table S4 [44]). Systolic blood pressure was 1.2 mm Hg (95%CI: 0.3, 2.2) higher per doubling of PFOS and 0.8 mm Hg (95%CI: -0.3, 1.8) higher per doubling of PFOA. Applying these findings to the observed range of PFOS, those at the 75th percentile of PFOS concentrations during pregnancy (33.6 ng/mL) would have

on average 1.1 mm Hg higher systolic blood pressure at three years postpartum than those at the 25th percentile of PFOS pregnancy concentrations (18.2 ng/mL). No PFAS were associated with diastolic blood pressure. PFHxS and PFNA were not strongly associated with blood pressure at three years postpartum (Figure 2; Supplemental Table S4 [44]).

Blood biomarkers (leptin, adiponectin, SHBG, HbA1c, IL-6, CRP)

Certain PFAS were associated with concentrations of multiple blood biomarkers at three years postpartum (Figure 3; Supplemental Table S5 [44]). Pregnancy plasma concentrations of EtFOSAA and MeFOSAA were associated with greater 3-year postpartum IL-6 (10.8% [95%CI: 3.3, 18.9] higher and 14.5% [95%CI: 5.7, 24.1] higher IL-6 per doubling in EtFOSAA and MeFOSAA, respectively). Each doubling in pregnancy PFHxS concentration was associated with 8.0% (95%CI: 2.2, 14.0) greater SHBG, while each doubling in pregnancy EtFOSAA was associated with 6.1% (95%CI: 0.7, 11.2) lower SHBG at three years postpartum. Participants had on average 16.1% (95%: 3.8, 26.9) lower 3-year postpartum CRP per doubling in pregnancy MeFOSAA and 5.3% (95%CI: 0.3, 10.4) higher 3-year postpartum adiponectin per doubling in pregnancy PFHxS. No pregnancy PFAS concentrations were associated with leptin at three years postpartum. PFOS, PFOA, and PFNA were generally weakly associated with blood biomarkers of cardiometabolic disruption at three years postpartum (Figure 3; Supplemental Table S5 [44]).

Secondary and sensitivity analyses

When we repeated analyses with all PFAS in a single model, associations with each PFAS were generally consistent in both strength and direction with results from individual PFAS models (Supplemental Table S4, S5 [44]). Models that excluded all women who became pregnant after the index pregnancy were also in general similar to results from the main models (which excluded women pregnant within the last 6 months) (Supplemental Table S4, S5 [44]). We did not find strong evidence for effect modification by maternal age in age-stratified models, though the associations of PFOS and

PFOA with systolic blood pressure were stronger among women aged 35 years or older at pregnancy as compared to women younger than 35 (Supplemental Table S6, S7 [44]).

Discussion

We observed that plasma concentrations of several PFAS during early pregnancy were associated with poorer anthropometry, blood pressure, and blood biomarkers (leptin, adiponectin, SHBG, HbA1c, IL-6, CRP) measured three years postpartum, suggesting that greater PFAS exposures were associated with higher cardiometabolic risk. Specifically, EtFOSAA was associated with a higher-risk profile across both anthropometric measures (greater waist circumference, mid-upper arm circumference, and SS+TR skinfold thickness) and biomarkers (higher IL-6, HbA1c, and lower SHBG), though not all associations were statistically significant. PFOA was linked to higher-risk anthropometry such as greater mid-upper arm circumference and BMI, and both PFOS and PFOA were associated with greater SS+TR skinfold thickness and systolic blood pressure, though not all associations were statistically significant. To our knowledge, this is the first study to report that PFAS concentrations in pregnancy are associated with postpartum anthropometry, blood pressure, and blood biomarkers of cardiometabolic risk.

Our findings linking certain PFAS to higher postpartum adiposity are consistent with a variety of previous studies in non-pregnant populations. For example, PFOS and PFOA have been associated with adiposity, weight gain [18 20] and type 2 diabetes [46]. Similarly, EtFOSAA was linked to prevalent microvascular disease in a non-pregnant U.S. population in the Diabetes Prevention Program Outcomes Study [17]. In prior work in this cohort, we also found that EtFOSAA is associated with higher gestational weight gain [47]. PFAS may be causing increased adiposity by activating PPARγ [21 22], which is expressed in adipose tissue and regulates adipocyte differentiation as well as fatty acid storage [48]. In addition to epidemiologic findings, evidence from *in vitro* studies, showing that PFAS exposure increases adipocyte cell number while reducing cell size and causes less lipid accumulation in differentiating adipocytes, supports this potential biological mechanism [49 50].

On the other hand, as far as we are aware, this is the first prospective study to report a positive association between PFAS plasma concentrations and systolic blood pressure. Some prior cross-sectional studies have suggested a positive association of PFAS and blood pressure [20 51], though not all reported positive findings [52]. Several prospective studies in substantially older, non-pregnant populations reported null associations of PFAS and hypertension [46 53], though one of these studies reported positive associations of cumulative PFOA exposure and hypertension among women aged 20-39 only (similar to the demographics of the Viva population) [53]. One recent longitudinal trial in a population at risk of type 2 diabetes reported inverse associations of MeFOSAA and PFOS with systolic blood pressure in a subset of the population undergoing a lifestyle intervention [54]. The biological mechanism by which PFAS exposure may affect blood pressure is not well understood, but evidence in animals [55] and *in vitro* in human cell lines [56 57] suggests that PFAS exposure may increase oxidative stress, which may in turn lead to elevated blood pressure [58]. Though additional studies are needed, our results suggest that plasma concentrations of certain PFAS in pregnancy are associated with a shift towards a higher-risk profile across multiple distinct measures of anthropometry, blood pressure, and biomarkers in postpartum.

Interestingly, we found protective associations between PFHxS and several biomarkers of metabolism. Specifically, higher plasma concentrations of PFHxS were statistically significantly associated with higher adiponectin and SHBG, and higher PFHxS was statistically significantly associated with smaller SS+TR skinfold thickness in models containing all PFAS. A few previous studies of other outcomes have reported protective effects of PFHxS, including inverse associations of PFHxS with low-density lipoprotein cholesterol levels [59] and risk of breast cancer [60 61]. In vitro, PFHxS and PFOA have similar affinity to human PPARγ ligand binding domain [62], so it is not clear why PFHxS might act in the opposite direction of other PFAS.

Our biomarker results add to an inconsistent and largely cross-sectional previous literature that has reported positive, inverse, and null results for all six biomarkers investigated in this study. Our findings do align with some prior literature. For example, the significant positive association of PFHxS and adiponectin in our study is similar to findings from the C8 cohort [29] (though three other

studies reported mixed findings [30-32]); similarly, the non-significantly positive association of EtFOSAA and HbA1c is broadly consistent with findings from two cross-sectional studies (though two other studies reported null or inverse findings [20 32 63 64]) and aligns with our findings that PFAS concentrations are associated with increased adiposity. Additionally, we reported statistically significant positive associations of EtFOSAA and MeFOSAA and IL-6; this is in line with a previous prospective study in pregnant and postpartum women that found a positive association with PFOS and PFOA and IL-6 [35]. IL-6 may be upregulated in response to higher oxidative stress [65] that may result from PFAS exposure [56 57]. However, in other cases our results conflicted with prior literature. For example, we reported null associations of PFAS and leptin, but prior prospective and cross-sectional studies have linked PFAS concentrations with higher leptin [20 29 30], and we expected to find a positive association given the positive associations we reported with PFAS and measures of adiposity. We also reported a statistically significant positive association of PFHxS and SHBG, and a statistically significant inverse association of EtFOSAA and SHBG; most previous studies of this association were restricted to men, and six of eight studies reported null findings (two studies, both in men, reported positive associations for PFOS and PFOA) [66-73]. Finally, we reported statistically significant inverse associations of MeFOSAA and CRP, but three cross-sectional studies (including one in pregnant women) have reported null associations of PFAS and CRP [31 34 74]. Additional work is needed to understand the complex potential associations of PFAS and blood biomarkers.

Pregnancy is a well-known sensitive window for fetal development, but few studies have tested pregnancy as a sensitive window for long-term maternal health. As an exception, some recent breast cancer research has identified pregnancy as a window of susceptibility for environmental exposures, likely because breast tissue develops and changes during pregnancy [75]. The maternal cardiovascular system also changes rapidly during pregnancy [76], and cardiometabolic health may be affected by environmental exposures similar to breast tissue. Indeed, our findings suggest that higher PFAS concentrations in pregnancy are linked with worse cardiometabolic health in postpartum even

after adjustment for pre-pregnancy risk factors such as BMI, supporting the idea of pregnancy as a sensitive window for maternal health.

Our work is subject to limitations. Between pregnancy and three years postpartum, the cohort had substantial loss to follow-up; we corrected for potential selection bias due to this loss to follow up using IPCW. Breastfeeding may be an important predictor of postpartum cardiometabolic health; however, breastfeeding after the index pregnancy may lie on the causal pathway between PFAS and the outcomes of interest and therefore would be inappropriate to adjust for in multivariate models [77 78]. However, we adjusted for history of breastfeeding. The effects of PFAS on cardiometabolic outcomes could be modified by kidney function; however, in our population of relatively young, healthy women, only 3.9% had inadequate kidney function based on a single measurement of estimated glomerular filtration rate calculated using the Cockroft-Gault formula [79 80], so we were not able to evaluate this possible effect modifier. We examined multiple correlated outcomes, but existing composite cardiovascular risk scores were not meaningful to summarize our findings because Project Viva is comprised of young, relatively healthy women. Additionally, we did not adjust our results for multiple testing, so individual statistically significant associations should be interpreted with caution. However, the pattern of results across multiple markers paints a clear picture of associations of higher concentrations of PFAS and worse cardiometabolic health. Finally, we report generally small effect sizes with wide confidence intervals. We cannot rule out false negatives due to lack of power to detect subtle effects; the direction of associations (including non-significant associations) is largely consistent with our hypotheses. The cohort was generally young and healthy at pregnancy; longer follow-up time may produce larger effects as participants age.

Our study also had many strengths. We were able to follow a large cohort of participants from early pregnancy to three years postpartum, so our study was prospective and captured the sensitive window of pregnancy. Trained RAs collected outcome measurements without knowledge of participants' PFAS concentrations; because these outcomes are not highly correlated, they each contribute to a broad picture of cardiometabolic risk. We also used biomonitoring (e.g., PFAS in plasma), which is the gold standard for assessing PFAS body burden. Because PFAS plasma

concentrations reported here are similar to those reported nationally in the same time frame [13], our findings may be generalizable to an American population with typical exposures.

Conclusions

In Project Viva, we found that pregnancy plasma concentrations of EtFOSAA, PFOS, and PFOA were associated with a shift towards a higher-risk profile at three years postpartum across several measures of anthropometry, blood pressure, and biomarkers of cardiometabolic health. Our results reinforce the status of pregnancy as a sensitive window for maternal health, though further research is needed to fully elucidate the mechanism connecting pregnancy PFAS concentrations and changes in postpartum health. Future research should evaluate postpartum maternal health effects of chemical exposures in pregnancy, using animal models and additional epidemiological cohort studies. Studies should additionally incorporate longer follow-up time to test duration of effects. Intervention studies during and before pregnancy that aim to reduce these exposures could also help elucidate causal pathways. If additional evidence supports our findings, incorporating PFAS exposure reduction strategies into clinical care for women planning pregnancy could improve maternal cardiometabolic health in the years following pregnancy.

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Table 1. Baseline demographics based on multiply imputed data (n=812 women)[1]

Characteristic	Population with anthropometry measurements[3] n (%)
Age at enrollment	
Mean (SD) age (years)	32.7 (5.2)
<25 years	58 (7.1)
25 - <30 years	143 (17.6)
30 - <35 years	324 (39.9)
≥35 years	287 (35.3)
Year of enrollment	
1999	204 (25.1)
2000	294 (36.2)
2001	288 (35.5)
2002	26 (3.2)
Pre-pregnancy body mass index (BMI)	
Median (IQR) BMI (kg/m^2)	23.7 (21.3, 27.3)
<25.0 kg/m^2	496 (61.1)
25.0 - <30.0 kg/m^2	194 (23.9)
≥30.0 kg/m^2	122 (15.0)
Race/ethnicity	

White	577 (71.1)
Black	103 (12.7)
Other	132 (16.2)
Parity	
0	353 (43.5)
1	309 (38.1)
>1	150 (18.5)
Education	
< College graduate	252 (31.0)
College graduate or more	560 (69.0)
Married or cohabitating	
No	67 (8.2)
Yes	745 (91.8)
Smoking status	
Smoked during pregnancy	93 (11.5)
Former	164 (20.3)
Never	554 (68.3)
Annual household income	
≤$70,000/year	322 (39.6)
>$70,000/year	490 (60.4)
History of breastfeeding	
No	424 (52.3)
Yes	388 (47.7)
Gestational weight gain[2]	
Inadequate	98 (12.2)
Adequate	232 (28.9)
Excessive	474 (59.0)

1-year postpartum weight retention	
<5 kg	529 (86.6)
≥5 kg	82 (13.4)

[1]*Calculated from all imputations. Ns are rounded to the nearest integer; values may not sum to 812. Gestational weight gain and postpartum weight retention are not imputed.*

[2]*According to Institute of Medicine (2009) guidelines*

[3]*Three participants have measurements of blood pressure and not anthropometry, and three participants have biomarkers measurements and not anthropometry. Otherwise the blood pressure and biomarker populations are subsets of the anthropometry population. Further study population details are provided in the Supplemental Material Table S1 [44].*

Figure legends

Figure 1. Associations between PFAS plasma concentrations in pregnancy and anthropometry at 3 years postpartum. Effect estimates are per doubling in each PFAS, based on parameter estimates from regression models (adjusted for age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, and parity). Plotted values are listed in Supplemental Table S4 [44].

Figure 2. Associations between PFAS plasma concentrations in pregnancy and blood pressure (BP) at 3 years postpartum. Effect estimates are per doubling in each PFAS, based on parameter estimates from regression models (adjusted for age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, and parity). Plotted values are listed in Supplemental Table S4 [44].

Figure 3. Associations between PFAS plasma concentrations in pregnancy and anthropometry and blood pressure at 3 years postpartum. Effect estimates are percent change in each biomarker per doubling in each PFAS, based on parameter estimates from regression models (adjusted for age, pre-pregnancy BMI, marital status, race/ethnicity, education, income, smoking, and parity). Plotted values are listed in Supplemental Table S5 [44].

Figure 1

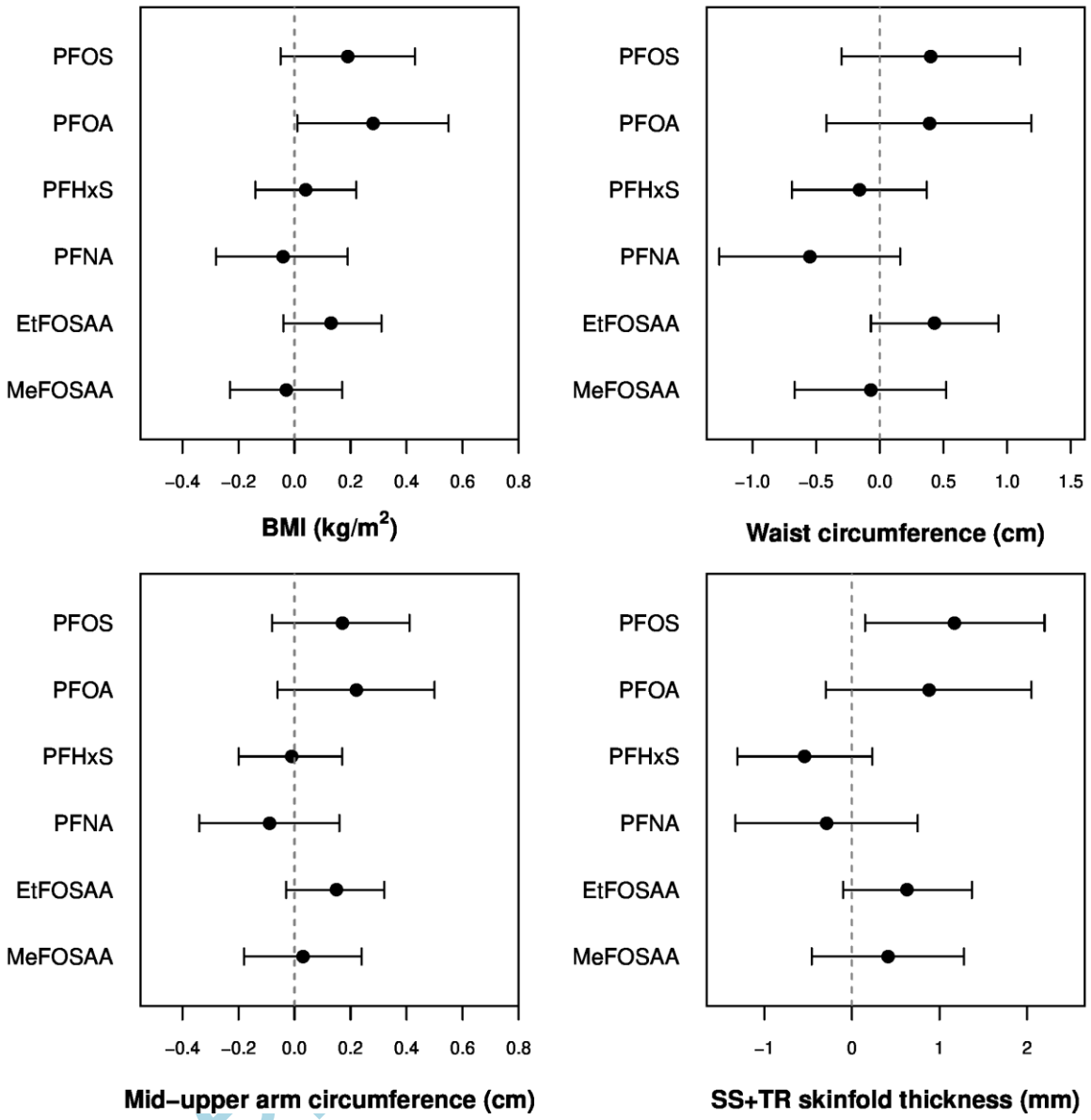

Figure 2

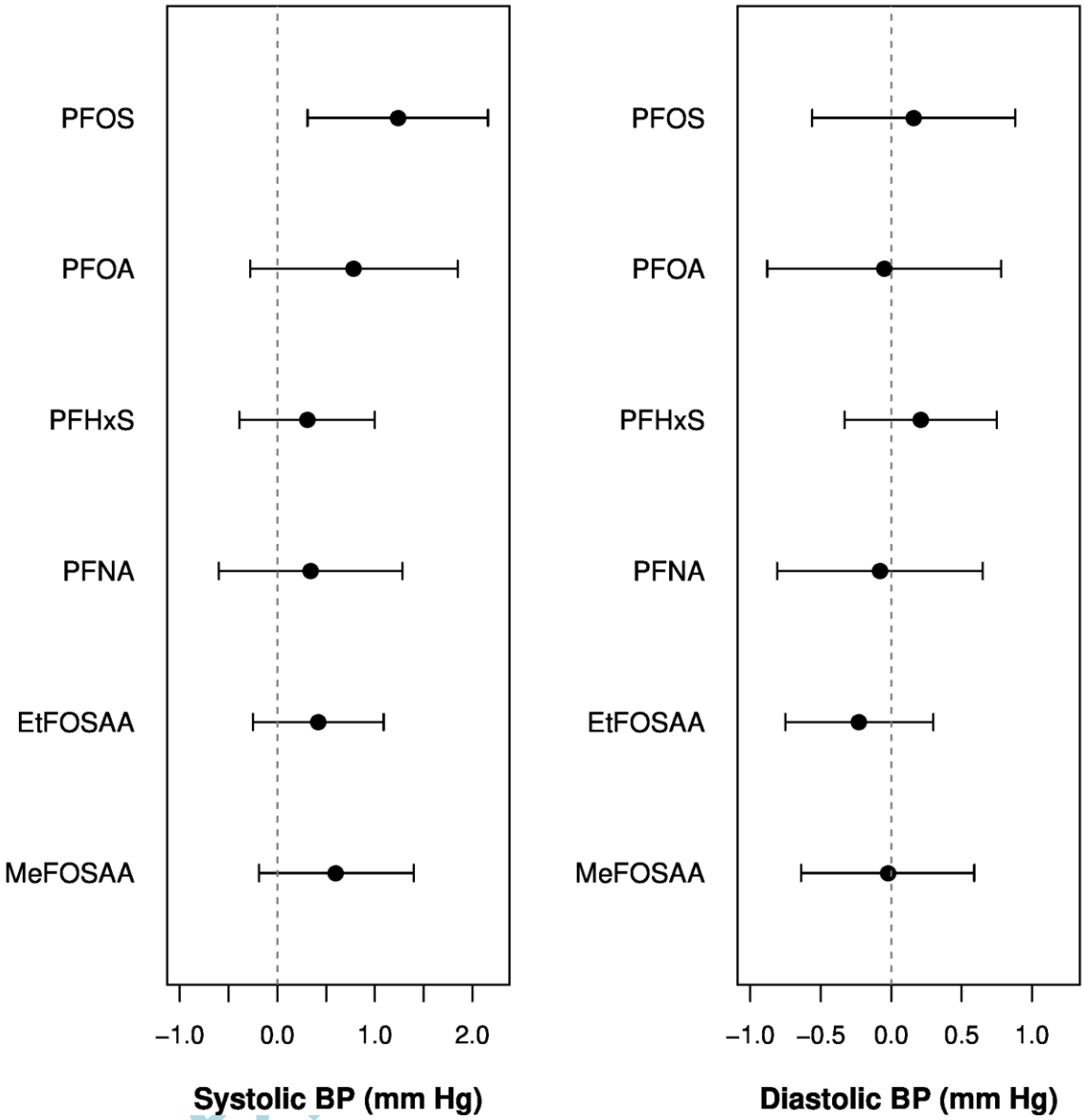

Figure 3