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Prenatal Exposure to Perfluoroalkyl Substances and Cardiometabolic Risk in Children from the Spanish INMA Birth Cohort Study

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Background: Perfluoroalkyl substances (PFAS) may affect body mass index (BMI) and other components of cardiometabolic (CM) risk during childhood, but evidence is scarce and inconsistent.

Objectives: We estimated associations between prenatal PFAS exposures and outcomes relevant to cardiometabolic risk, including a composite CM-risk score.

Methods: We measured perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) in maternal plasma (first trimester). We assessed weight gain from birth until 6 mo. At 4 and 7 y, we calculated the age- and sex-specific z-scores for BMI, waist circumference (WC), and blood pressure (BP) ($n \approx 1{,}000$). At age 4, we calculated the age-, sex-, and region-specific z-scores for cholesterol, triglycerides (TGs), high-density (HDL-C), and low-density lipoprotein cholesterol (LDL-C) ($n = 627$). At age 4, we calculated a CM-risk score ($n = 386$) as the sum of the individual age-, sex-, and region-specific z-scores for WC, BP, HDL-C, and TGs. We used the average between the negative of HDL-C z-score and TGs z-score to give similar weight to lipids and the other components in the score. A higher score indicates a higher cardiometabolic risk at age 4.

Results: PFOS and PFOA were the most abundant PFAS (geometric mean: 5.80 and 2.32 ng/mL, respectively). In general, prenatal PFAS concentrations were not associated with individual outcomes or the combined CM-risk score. Exceptions were positive associations between prenatal PFHxS and TGs z-score [for a doubling of exposure, $\beta = 0.11$; 95% confidence interval (CI): 0.01, 0.21], and between PFNA and the CM-risk score ($\beta = 0.60$; 95% CI: 0.04, 1.16). There was not clear or consistent evidence of modification by sex.

Conclusions: We observed little or no evidence of associations between low prenatal PFAS exposures and outcomes related to cardiometabolic risk in a cohort of Spanish children followed from birth until 7 y. https://doi.org/10.1289/EHP1330

Introduction

Childhood obesity has been steadily increasing during the past four decades (de Onis et al. 2010). Overweight children are more likely to present obesity, hypertension, dyslipidemia, and cardiovascular disease in adulthood than normal-weight children (Deshmukh-Taskar et al. 2006; Janssen et al. 2005). Obesity is known to be caused by a mismatch between caloric intake and energy expenditure, but early-life exposure to obesogens, including perfluoroalkyl substances (PFAS), may play a role (Holtcamp 2012; La Merrill and Birnbaum 2012). PFAS are synthetic chemicals widely used in industrial and commercial applications, including nonstick cookware, consumer products, textiles, and food packaging (Buck et al. 2011; Casals-Casas and Desvergne 2011; Stahl et al. 2011). PFAS have been detected in cord blood samples, suggesting that PFAS exposure starts prenatally (Inoue et al. 2004; Manzano-Salgado et al. 2015).

Rodents prenatally exposed to perfluorooctanoic acid (PFOA) showed higher weight gain, body fat accumulation, and cardiovascular disease (Hines et al. 2009; Lv et al. 2013; Tan et al. 2013). Epidemiological studies of the potential effects of PFAS on cardiometabolic outcomes in children have focused primarily on the two most common PFAS, perfluorooctanesulfonic acid (PFOS) and PFOA (reviewed by Vrijheid et al. 2016). Prenatal exposure to PFOS and PFOA was associated with lower weight at 5–12 mo of life (Andersen et al. 2010) among >1,000 children from the Danish National Birth Cohort (DNBC) study, but not with body mass index (BMI) and waist circumference (WC) in a later follow-up of >800 children from the same cohort at age 7 (Andersen et al. 2013). Prenatal PFOS and/or PFOA exposure also was associated with higher weight at 20 mo in a study of 320 girls from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort (Maisonet et al. 2012), higher risk of waist-to-height ratio (WHtR) >0.5 at 5–9 y in 1,022 children from Greenland and Ukraine (Høyer et al. 2015), higher adiposity at 8 y in 204 American children (Braun et al. 2016), and higher BMI among 345 Danish women, but not among 320 Danish men, at age 20 (Halldorsson et al. 2012). Prenatal PFAS exposure was associated with higher BMI, skinfold thickness, and total fat mass, measured using dual-energy X-

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ray absorptiometry (DXA) in 7-y-old girls ($n = 466$), but not boys ($n = 522$) from a U.S. birth cohort (Mora et al. 2016). However, estimated early-life PFOA exposure was not associated with self-reported BMI at 20–40 y among 8,764 adults who resided near a PFOA manufacturing facility in the United States (Barry et al. 2014).

In addition to overweight and adiposity, cardiometabolic risk factors include elevated blood pressure (BP), lipid abnormalities, and abnormal glucose homeostasis, all of which are considered components of metabolic syndrome (Kassi et al. 2011). A cross-sectional study of adolescent participants (12–19 y of age) in the U.S. National Health and Nutrition Examination Survey (NHANES) study reported inverse associations between perfluorononanoic acid (PFNA) and metabolic syndrome, but positive associations of PFOS with markers of abnormal glucose homeostasis (Lin et al. 2009), while a second NHANES study reported that PFOA and PFOS were inversely associated with hypertension in adolescents (Geiger et al. 2014b). Two additional cross-sectional studies of PFOA and PFOS include a study of Danish children at 8–10 y of age ($n = 342$–499) that reported no associations with cardiometabolic risk factors in the population as a whole (Timmermann et al. 2014), and a cross-sectional study of >12,000 U.S. children and adolescents (1–18 y) with potential exposure via contaminated drinking water, which reported positive associations with serum lipid levels (Frisbee et al. 2010). Two longitudinal studies of PFAS and cardiometabolic risk factors, other than overweight and adiposity, include a study of girls in the ALSPAC cohort, which reported that increasing prenatal PFOA concentrations within the lowest tertile of the distribution (but not in the second or third tertiles) was positively associated with total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) at 7 and 15 y (111 and 88 girls, respectively) (Maisonet et al. 2015). A U.S. study reported no association between prenatal PFAS and insulin resistance at approximately 8 y of age ($n = 441$), though concurrent serum PFOA and PFOS were inversely associated with insulin resistance in a cross-sectional analysis ($n = 541$) (Fleisch et al. 2016).

In the present study, we evaluated prenatal exposures to four PFAS in association with individual cardiometabolic risk factors (anthropometric measurements, blood pressure, and serum lipids) in early and midchildhood among participants in a Spanish birth cohort. In addition, we estimated longitudinal associations with a combined CM-risk score as an alternative predictor of overall cardiometabolic risk (Eisenmann 2008; Pandit et al. 2011).

Methods

Study Population

We used data from the INMA (INfancia y Medio Ambiente, Environment and Childhood) birth cohort study including three Spanish regions: Gipuzkoa, Sabadell, and Valencia. A total of 2,150 pregnant women were recruited in the first trimester of pregnancy [gestational age: median = 12.71 wk; interquartile range (IQR) = 5.85] during the years 2003–2008. The inclusion criteria were: 16 y old or older, intention of giving birth at the reference hospital, singleton pregnancy, no language barrier, and no assisted pregnancy (Guxens et al. 2012). Child anthropometric data at 6 mo was abstracted from medical records. Mother–child pairs were then assessed when children were 4 and 7 y old. Maternal PFAS concentrations were measured in serum samples from 1,243 women (58% of the initial cohort) who participated in follow-up when their children were 4 y of age (Manzano-Salgado et al. 2016). The present analysis was limited to mother–child pairs with prenatal PFAS concentrations and weight gain data for children at 6 mo ($n = 1,154$), and with BMI at 4 y ($n = 1,230$) and 7 y ($n = 1,086$) (Figure 1). In addition, WC and BP measurements were available at 4 y of age for children from the Valencia and Sabadell subcohorts ($n = 839$, 68%); blood lipid levels were available for a subset of children from all three of the subcohorts ($n = 627$, 51%); and our derived CM-risk score, which was based on WC, BP, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) (as described in detail below), was available for 386 children (31%) (Figure 1). All participating women signed written informed consent. This study was approved by the regional ethical committees of each cohort (Guxens et al. 2012).

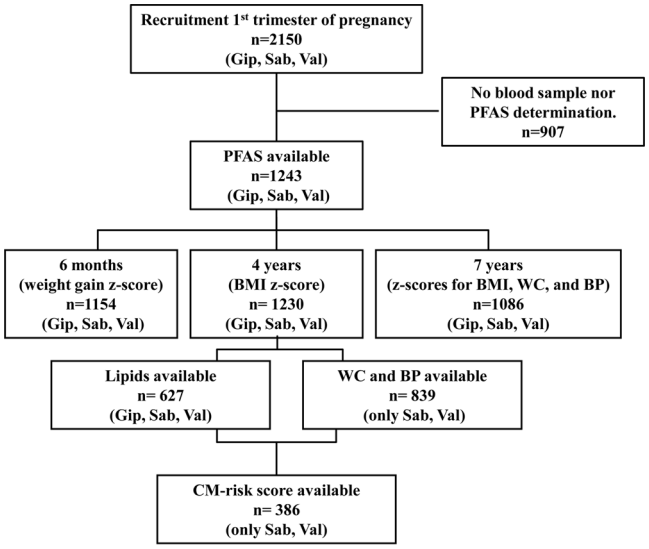

Figure 1. Flow chart of sample populations in our study. Note: BP, blood pressure; CM, cardiometabolic; Gip, Gipuzkoa; PFAS, perfluoroalkyl substances; Sab, Sabadell; Val, Valencia; WC, waist circumference.

Perfluoroalkyl Substances Determination

We collected maternal blood samples during the first trimester of pregnancy (median = 12.71 wk; IQR = 5.85). Plasma was aliquoted in 1.8 mL cryotube vials (Sigma-V7884, Nunc® CryoTubes®) and stored at −80°C until their analysis at the Institute for Occupational Medicine, RWTH Aachen University (Aachen, Germany), as previously described (Manzano-Salgado et al. 2015). Briefly, plasma concentrations of perfluorohexanesulfonic acid (PFHxS), PFOS, PFOA, and PFNA were determined by column-switching liquid chromatography (Agilent 1,100 Series high-performance liquid chromatography apparatus; Agilent Technologies) coupled with tandem mass spectrometry (Sciex API 3,000 liquid chromatography coupled with tandem mass spectrometry system in electrospray ionization-negative mode; Applied Biosystems), according to a modified protocol described by Kato et al. (2011). The limits of quantification (LOQ) and detection (determined as a signal-to-noise ratio of 6 in the vicinity of the analytes) were 0.20 ng/mL and 0.10 ng/mL, respectively, for PFHxS, PFOS, and PFOA, and 0.10 ng/mL and 0.05 ng/mL, respectively, for PFNA (Manzano-Salgado et al. 2015).

Anthropometric Measurements

Assessment of anthropometric outcomes in INMA has been previously described (Valvi et al. 2013, 2015). Briefly, we abstracted height and weight measurements at birth and 6 mo from medical registries, and calculated age- and sex-specific *z*-scores for weight gain from birth to 6 mo using World Health Organization (WHO) reference values (de Onis et al. 2009, 2007). Early rapid

growth during the first months of life has been associated with later obesity (Monteiro and Victora 2005); therefore, we also evaluated early rapid growth, defined as a z-score >0.67 standard deviation (SD) for weight gain from birth until 6 mo.

At 4 and 7 y, we measured weight (nearest 0.10 g) and height (nearest 0.10 cm) using a standard protocol (with no shoes and in light clothing) (Valvi et al. 2013). We calculated BMI (weight in kg/height in cm^2) and age- and sex-specific BMI z-scores using the WHO reference (de Onis et al. 2009, 2007), and defined overweight as BMI z-score ≥85th percentile (de Onis et al. 2009, 2007). WC was measured using an inelastic tape (SECA model 201; SECA) at the midpoint between the right lower rib and the iliac crest, and after a slight breath out. We derived age-, sex-, and region-specific WC z-scores as the standardized residuals from a regression model of WC as the dependent variable, and age, sex, and region as the predictors, following the method of Eisenmann (2008), with standardization by region used to account for differences among the three regional subcohorts (Table S1) (Manzano-Salgado et al. 2016). We used WC (cm) and height (cm) to calculate the WHtR, and defined high abdominal adiposity as WHtR > 0.5 based on previous studies (Graves et al. 2014; Martin-Calvo et al. 2016; Mokha et al. 2010).

Blood Pressure

We used a digital automatic monitor (OMRON CPII) to measure systolic and diastolic BP (SBP and DBP). At age 4 y, we did a single measurement after five min of rest only, while at age 7 y, we did two measurements (with an additional 5-min rest period in between) and averaged the paired values for SBP and DBP, respectively. We derived average BP at each age as the mean of the SBP and DBP values. Similar to WC, we used regression models to derive BP z-scores standardized by age, sex, height, and region (Sabadell and Valencia at 4 y; Gipuzkoa, Sabadell, and Valencia at 7 y).

Lipids

In INMA, lipids were measured in all the children that agreed to provide blood samples in the follow-up at 4 y ($n = 740$). For the purpose of the present study, we selected 627 children because they also had matched maternal PFAS concentrations. Lipids were measured using nonfasting blood samples (samples were fasting for Valencia, but not for Sabadell or Gipuzkoa) collected by venipuncture. We measured total TC, HDL-C, and TG levels using standard analytical techniques (ABX-Pentra 400; Horiba Medical). LDL-C was calculated based on TC, HDL-C, and TG concentrations using the Friedewald formula (Fukuyama et al. 2008). As for WC, we derived age-, sex-, and region-specific z-scores for TC and each individual lipid.

Cardiometabolic Risk Score

We derived a continuous CM-risk score at 4 y of age that is similar to the pediatric metabolic syndrome (MetS) score derived for the IDEFICS study by Ahrens et al. (2014) for >16,000 children at 2–9 y of age from eight European countries. Specifically, our cardiometabolic (CM)-risk score was derived as the sum of the standardized z-scores for WC, BP, and the mean of the HDL-C and TG z-scores, with HDL-C multiplied by −1 because it is inversely associated with cardiometabolic risk:

$$\text{CM-risk score} = (\text{WC } z\text{-score}) + (\text{BP } z\text{-score}) + \left[\frac{(-\text{HDL-C } z\text{-score} + \text{TGs } z\text{-score})}{2}\right]$$

Our CM-risk score differs from the Identification and prevention of dietary- and lifestyle-induced health effects in children and infants (IDEFICS) MetS score in that it does not include a measure of glucose homeostasis; blood lipid levels were not always measured in fasting blood samples; z-scores for individual components were standardized by INMA study region (and by height for BP), as well as by age and sex; and we used the average of SBP and DBP, rather than mean arterial pressure, to represent the BP component. A higher CM-risk score suggests higher cardiometabolic risk.

Covariates

At enrollment (during the first trimester of pregnancy), mothers provided blood samples and completed self-reported questionnaires on sociodemographic and dietary factors, including the maternal country of birth (Spain or other), region of residence (Gipuzkoa, Sabadell, and Valencia), parity (0, 1, and ≥2), age (in years), and weekly intakes of fish and seafood consumption during the previous 3 mo of pregnancy (based on a food frequency questionnaire) (Manzano-Salgado et al. 2016). In INMA, mothers self-reported the duration of previous breastfeeding as the total number of weeks for any previous pregnancy. Then we combined all the durations into a single variable and classified their previous breastfeeding as none, <4 mo, 4–6 mo, or >6 mo. However, in the present study, we used the continuous variable in the models, that is, the total number of weeks of any previous breastfeeding. Regarding prepregnancy BMI, in INMA, height was measured, and prepregnancy weight was self-reported. Self-reported prepregnancy weight and measured weight at 12 wk of pregnancy were highly correlated; $r = 0.96$; $p < 0.0001$, (Casas et al. 2013). We then used the reported prepregnancy weight and measured height to calculate BMI (kg/m^2), and classified mothers as underweight, normal weight, overweight, and obese. Further, the association between PFAS and fetal growth may be confounded by maternal glomerular filtration rate (GFR) during pregnancy (Verner et al. 2015). Therefore, we also measured maternal plasma creatinine ($n = 800$) and calculated GFR using the Cockcroft-Gault formula [GFR = (140-maternal age) × weight (kg) × 1.04/serum creatinine (μmol/L)]. Because plasma albumin is the binding site of PFAS (D'eon et al. 2010), we measured maternal albumin levels using the same maternal plasma sample that was used for measuring PFAS concentrations ($n = 800$). Finally, in the follow-ups at the ages of 14 mo, 4 y, and 7 y, mothers completed questionnaires with information on postnatal characteristics of the index child, such as the duration of breastfeeding (total number of weeks) and the level of physical activity (Guxens et al. 2012).

Statistical Analysis

Since maternal PFAS concentrations were skewed to the right, we transformed PFAS concentrations to a 2-base logarithm. For PFAS with values under the LOQ (<4% for all compounds), we used multiple imputations to assign a value between 0 and the LOQ (0.20 ng/mL, and 0.10 ng/mL for PFNA) using the Stata 14.0 command ice. A detailed description of this procedure is provided in Table S2. We used generalized additive models (GAMs) of each $\log_2$–transformed PFAS and each outcome (adjusted for maternal region of residence, country of birth, parity, prepregnancy BMI, previous breastfeeding, and by the age at follow-up and sex of the child) to assess potential departures from linearity (p-value < 0.05). Based on this criterion, we found evidence of nonlinearity for exposure–outcome relations at age 7 (data not shown), and therefore modeled associations with PFAS concentrations categorized into quartiles, as well as associations with $\log_2$ PFAS as continuous variables; otherwise, we modeled continuous $\log_2$ PFAS concentrations only. We estimated

associations between individual PFAS and each outcome using multivariable linear regression models for continuous outcomes (CM-risk score and z-scores for weight gain, BMI, WC, BP, TC, HDL-C, LDL-C, and TG) and Poisson regression models for binary outcomes (rapid growth, overweight, and WHtR > 0.5). Linear regression coefficients represent the unit difference in each outcome (where 1 unit is equivalent to a 1-SD difference in z-scores, or a 1-unit difference in the CM-risk score) with a doubling of prenatal PFAS concentration. Covariates included in the model were selected based on our previous study of determinants of PFAS concentrations in our cohort (Manzano-Salgado et al. 2016) and whether these determinants were associated with at least one of the outcomes (p-value < 0.10). We further adjusted all of our models by the age and sex of the child. Final models were adjusted for maternal country of birth (Spain or other), parity (number of pregnancies), previous breastfeeding (number of weeks), age (years), prepregnancy BMI (kg/m^2), and by the age and sex of the child. We used multiple imputations to impute missing covariate data (<5%) (Donders et al. 2006; Sterne et al. 2009), under the assumption that covariate data were missing at random. We used Stata 14.0 command ice to create 20 different datasets for each age point (i.e., 6 mo, 4 y, and 7 y) and region of residence (i.e. Gipuzkoa, Sabadell, and Valencia), and imputed missing values as maternal and child characteristics (age, country of birth, fish intake during pregnancy, education, etc.). A detailed description of the imputation procedure is provided in Table S2. Distributions of covariates in all the imputed datasets were similar to those observed (see Table S3 for 4-y-old children from Sabadell; otherwise, data not shown).

We performed various sensitivity analyses. Because PFAS effect may differ by sex (Andersen et al. 2010; Halldorsson et al. 2012), we evaluated if the sex of the child modified our results by including the interaction term in the models (i.e., considered significant if p-value for PFAS * sex-interaction < 0.05) and by stratifying our analysis. Moreover, children with low birth weight (LBW) (i.e., <2,500 g) or preterm (i.e., before 37 wk of gestation) might have a different growth pattern than the rest of children, so we repeated our analysis excluding children with LBW ($n = 56$) and born preterm ($n = 48$). Further, we excluded children born by cesarean section ($n = 210$) because a higher risk of obesity later in life has been suggested (Blustein et al. 2013). We also introduced maternal GFR as a confounder to evaluate if it changed our associations. Because albumin levels decrease during pregnancy (Glynn et al. 2012; van den Akker et al. 2008), we introduced albumin levels during pregnancy as a potential confounder in our models. Further, we stratified our models by breastfeeding duration of the index child as a proxy of postnatal PFAS exposure [never, short-term (<4 mo), long-term (4–6 mo), and very long-term (>6 mo)]. Finally, as PFAS are correlated in our cohort (Manzano-Salgado et al. 2015, 2016), we assessed whether introducing all PFAS into a single model changed the estimates for associations with CM-risk scores only.

We used the STATA 14.1 statistical software (Stata Corporation) for our analysis. We considered a p-value < 0.05 to be statistically significant.

Results

Mothers included in our study were more likely to be older, nulliparous, and with university studies compared to mothers without PFAS measured (this comparison does not include children who were excluded from analyses of lipids, BP, or CM-risk score) (Table S4). Geometric mean concentrations of PFAS ranged between 0.61 ng/mL for PFHxS and 5.80 ng/mL for PFOS (Table 1). PFAS were moderately correlated, with PFOA and PFNA (Spearman rho: 0.68 p-value < 0.001) being the most correlated, and PFHxS and PFNA being the least correlated (Spearman rho: 0.43 p-value < 0.001). Twenty-four percent of our children were classified as rapid growers at 6 mo, and 27% and 34% were classified as overweight at 4 and 7 y, respectively (Table 2). At 4 and 7 y, 26% and 19% of children had high abdominal adiposity (i.e., WHtR > 0.5), respectively (Table 2). Overall, the associations between PFAS and rapid growth, overweight, and WHtR > 0.5 were close to the null and not significant at any age (see Table S5). In this study, we describe the results from the adjusted models because we consider that they are closer to the true association of PFAS on cardiometabolic risk during childhood (for unadjusted models, see Table S6). Because no major differences in the magnitude or direction of the effect estimates were observed between the analyses using imputed data vs. complete cases only ($n \leq 1{,}100$) (Table S7), we only present the results based on imputed datasets. Differences in the CM-risk scores were more pronounced, but we do not consider this will affect interpretation (Table S7).

Table 1. Perfluoroalkyl substance (PFAS) concentrations (ng/mL) in maternal plasma during pregnancy.

Compound	n(%) > LOD	p5	p25	GM	p75	p95
PFHxS	1,185 (96.3)	0.28	0.43	0.61	0.84	1.39
PFOS	1,230 (100)	2.54	4.52	5.80	7.84	11.40
PFOA	1,230 (100)	0.97	1.63	2.32	3.31	5.24
PFNA	1,222 (99.3)	0.30	0.50	0.66	0.90	1.49

Note: Concentrations are from the study population at 4 y ($n = 1{,}230$). GM, geometric mean; LOD, limit of detection; p5, 25, 75, 95, percentiles 5, 25, 75, and 95; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.

We observed few associations between prenatal PFAS concentrations, anthropometric measurements, and BP at any age (Table 3). Maternal PFOA concentrations were positively associated with weight gain z-scores at 6 mo in boys [for a doubling of PFOA, $\beta = 0.13$; 95% confidence interval (CI): 0.01, 0.26] but not girls ($\beta = -0.03$; 95% CI: −0.14, 0.08) (p-value for sex-interaction = 0.28) (Table 3). PFHxS was negatively associated with weight gain from birth to 6 mo in the overall population ($\beta = -0.06$; 95% CI: −0.15, 0.03) and in boys and girls, though the association was not statistically significant (Table 3). There were no other significant associations for the other PFAS at this age (Table 3). At 4 and 7 y, we did not observe any statistically significant association between PFAS and anthropometric measurements and BP (Table 3). However, we observed a pattern of inverse associations between PFHxS, BMI, and WC z-scores in the overall population and in boys (Table 3), whereas for girls, positive associations were observed (p-value for sex-interaction at 4 y ≥ 0.12 and at 7 y ≥ 0.16). At these ages, PFOS, PFOA, and PFNA showed patterns of positives associations with BMI z-scores in the overall population and in boys (p-value for sex-interaction > 0.18). Further, at 7 y, we observed nonsignificant associations between PFOA and BP z-scores that were positive in boys but negative in girls (p-value for sex-interaction = 0.11). Finally, at age 7, we repeated the analysis using quartiles of PFAS exposure (instead of the continued variable) because GAM models showed nonlinearity for exposure–outcome relations at this age. Using quartiles of PFAS exposure yielded patterns of positive associations, although not statically significant, between PFOS, WC, and BP z-scores, and PFNA, BMI, and WC z-scores at 7 y (Figure S1). On the contrary, a pattern of inverse associations was observed between PFOA and BP z-scores at 7 y (Figure S1).

As for the lipids measured at 4 y, we did not observe any statistically significant association between PFAS and z-scores of TC, HDL-C, or LDL-C at 4 y. In general, associations between

Table 2. Summary of child characteristics in our study.

Child characteristic	6 mo $n = 1{,}154$	4 y $n = 1{,}230$	7 y $n = 1{,}086$
Age (months or years) (mean ± SD)	6.1 ± 0.1	4.4 ± 0.2	7.4 ± 0.5
Anthropometric measurements			
Weight (kg) (mean ± SD)	7.7 ± 0.9	18.1 ± 2.6	27.3 ± 5.7
Height (cm) (mean ± SD)	67.0 ± 2.4	105.7 ± 4.5	125.2 ± 6.0
Weight gain *z*-score (mean ± SD)	0.09 ± 1.0	—	—
Rapid growth (yes) *n* (%)[a]	270 (24)	—	—
BMI (kg/m^2) (mean ± SD)	17.1 ± 1.4	16.2 ± 1.6	17.2 ± 2.6
BMI *z*-score (mean ± SD)	−0.9 ± 0.9	0.6 ± 1.0	0.8 ± 1.2
Overweight (yes) *n* (%)	—	336 (27)	367 (34)
Waist circumference (cm) (mean ± SD) ($n = 839$ at 4 y)	—	51.2 ± 4.2[b]	58.2 ± 6.6
Waist circumference *z*-score ($n = 839$ at 4 y) (mean ± SD)	—	−0.01 ± 0.95[b]	−0.01 ± 1.0
Waist-to-height ratio >0.5 (yes) *n* (%) ($n = 839$ at 4 y)	—	219 (26)[b]	204 (19)[b]
BP, mmHg (mean ± SD) ($n = 839$ at 4 y)			
Systolic BP	—	102.5 ± 15.6[b]	107.3 ± 9.8
Diastolic BP	—	66.0 ± 15.4[b]	63.5 ± 8.5
BP *z*-score	—	0.01 ± 1.0[b]	0.00 ± 1.0
Lipids, mg/dL (mean ± SD) ($n = 627$)			
Total cholesterol	—	167.7 ± 26.6	—
HDL-C	—	51.5 ± 12.1	—
LDL-C	—	100.8 ± 22.0	—
Triglycerides	—	76.6 ± 36.9	—
CM-risk score (mean ± SD) ($n = 386$)[b,c]	—	−0.84 ± 4.0	—

Note: —, no data; BMI, body mass index; BP, blood pressure; CM, cardiometabolic; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.
[a]Rapid growth was defined as a weight gain *z*-score from birth until 6 mo >0.67 SD.
[b]Only Sabadell and Valencia subcohorts have available data for this outcome.
[c]CM-risk score is the *z*-scores for WC, BP, and the mean of the HDL-C and TG *z*-scores, with HDL-C multiplied by −1.

prenatal PFAS and blood lipids at 4 y were close to the null (overall and when stratified by sex) (Table 3). One exception was the association between PFHxS and TGs at age 4 y, which was positive overall (β = 0.11; 95% CI: 0.01, 0.21) and in boys (β = 0.16; 95% CI: 0.03, 0.30) and girls (β = 0.07; 95% CI: −0.08, 0.22) (*p*-value for sex-interaction = 0.85). In addition, while the association between PFOA and HDL-C was close to the null in the combined population (β = −0.04; 95% CI: −0.15, 0.08), it was positive for girls (β = 0.12; 95% CI: −0.02, 0.27) and negative for boys (β = −0.20; 95% CI: −0.37, −0.03), with *p*-value for sex-interaction = 0.10 (Table 3).

Children with available CM-risk scores had lower BMI (mean BMI: 16.05 vs. 16.26; *p*-value = 0.03) and TGs (mean TGs: 71.08 vs. 85.34 mg/dL; *p*-value <0.001), but higher HDL-C levels (mean HDL-C: 54.96 vs. 46.05 mg/dL; *p*-value < 0.001) than the full sample at 4 y (data not shown). Prenatal exposures to PFOS, PFOA, and PFNA were positively associated with CM-risk scores at 4 y in the overall population (Figure 2, Table 3), with a significant association for PFNA (β = 0.60; 95% CI: 0.04, 1.16) that was similar in magnitude for boys and girls (*p*-value for sex-interaction = 0.86). There were no significant differences in associations with CM-risk scores between boys and girls, though associations with PFOA were on opposite sides of the null (*p*-value for sex-interaction = 0.45).

Regarding the sensitivity analyses, the associations between PFOA and BMI *z*-scores at 4 and 7 y increased with breastfeeding duration of the index child, and these associations were statistically significant in very long-term breastfeeding duration, i.e., at 4 y: β = 0.14 (0.03, 0.26; *p*-value for breastfeeding-interaction = 0.09) and at 7 y: β = 0.17 (0.02, 0.32; *p*-value for breastfeeding-interaction = 0.01) (Figure S2). Associations with the CM-risk score differed when estimated using a model that included all four PFAS (compared with estimates from single PFAS models), with coefficients suggesting a stronger positive association with PFNA (β = 0.85; 95% CI: 0.01, 1.69), a weaker positive association with PFOS (β = 0.11; 95% CI: −0.73, 0.95), and a stronger negative association with PFHxS (β = −0.36; 95% CI: −1.05, 0.33), while the association with PFOA changed from positive to negative (β = −0.26; 95% CI: −1.20, 0.68). However, the mutually adjusted estimates were imprecise, and, with the exception of the association with PFNA, none were clearly different from the null. After including GFR in our models, all estimated associations remained close to null, but the association between PFHxS and BMI *z*-scores at 4 y changed from negative to positive, and the CIs were reduced for the associations between PFOS and PFOA and BMI *z*-scores at 4 y (Table S8). Similar results were observed after including maternal albumin levels in our models (Table S9). Finally, estimates remained similar after excluding cesarean section (Table S10), LBW, or preterm infants (data not shown because few observations were excluded).

Discussion

In this study with low prenatal PFAS concentrations, we observed little or no association with cardiometabolic risk components from birth until 7 y. None of the PFAS were significantly associated with anthropometric measurements or BP at 4 or 7 y. A doubling of prenatal PFHxS was associated with significantly higher TGs *z*-scores at 4 y of age, and prenatal PFNA was associated with a significantly higher CM-risk score. We did not observe any other statistically significant associations between PFAS concentrations and lipid *z*-scores or the CM-risk score in the overall population, or when stratified by sex.

In our study, low exposure levels of PFAS were not significantly associated with weight gain, BMI, overweight, or WC at any age. Other studies with similar exposure levels found that prenatal PFOS and PFOA exposure was associated with WHtR > 0.5 at 7 y ($n = 1{,}022$) (Høyer et al. 2015), and PFAS were also associated with higher BMI, WC, skinfold thickness, and DXA total fat mass in girls aged 7 ($n = 1{,}006$) (Mora et al. 2016). In the Health Outcomes and Measures of the Environment Study ($n = 204$) with PFOA exposure levels above the U.S. average (median: 5.3 ng/mL vs. 2.3 ng/mL), prenatal PFOA exposure was associated with higher BMI, WC, and adiposity at 8 y

Table 3. Adjusted associations between maternal perfluoroalkyl substance (PFAS) concentrations (log_2-transformed, in ng/mL) and cardiometabolic components during childhood.

Cardiometabolic components	n	PFHxS β (95% CI)	PFHxS p-Value sex interaction	PFOS β (95% CI)	PFOS p-Value sex interaction	PFOA β (95% CI)	PFOA p-Value sex interaction	PFNA β (95% CI)	PFNA p-Value sex interaction
From birth until 6 mo									
Weight gain z-score, overall	1,154	−0.06 (−0.15, 0.02)		−0.02 (−0.11, 0.07)		0.04 (−0.04, 0.12)		0.01 (−0.07, 0.09)	
Girls	568	−0.09 (−0.20, 0.02)	0.93	−0.09 (−0.21, 0.04)	0.54	−0.03 (−0.14, 0.08)	0.28	0.00 (−0.11, 0.11)	0.86
Boys	586	−0.03 (−0.15, 0.10)		0.05 (−0.08, 0.19)		0.13 (0.01, 0.26)		0.04 (−0.09, 0.17)	
At 4 y									
BMI z-score, overall	1,230	−0.02 (−0.10, 0.07)		0.04 (−0.05, 0.13)		0.04 (−0.04, 0.13)		0.05 (−0.03, 0.13)	
Girls	600	−0.02 (−0.13, 0.08)	0.70	0.02 (−0.10, 0.14)	0.99	0.00 (−0.11, 0.10)	0.31	0.02 (−0.08, 0.12)	0.26
Boys	630	−0.02 (−0.15, 0.11)		0.05 (−0.08, 0.18)		0.09 (−0.03, 0.22)		0.08 (−0.04, 0.19)	
WC z-score[b], overall	839	−0.04 (−0.14, 0.05)		−0.03 (−0.13, 0.07)		0.00 (−0.09, 0.10)		0.02 (−0.07, 0.10)	
Girls	412	0.03 (−0.10, 0.16)	0.12	−0.04 (−0.18, 0.10)	0.73	−0.03 (−0.16, 0.10)	0.78	0.02 (−0.10, 0.14)	0.97
Boys	427	−0.11 (−0.25, 0.03)		−0.02 (−0.17, 0.13)		0.04 (−0.10, 0.18)		0.02 (−0.11, 0.14)	
BP z-score[b], overall	839	−0.01 (−0.10, 0.09)		−0.05 (−0.15, 0.06)		−0.06 (−0.16, 0.04)		−0.01 (−0.10, 0.08)	
Girls	412	0.10 (−0.09, 0.18)	0.60	−0.06 (−0.22, 0.09)	0.74	−0.04 (−0.18, 0.10)	0.99	0.05 (−0.08, 0.18)	0.39
Boys	427	−0.06 (−0.20, 0.08)		−0.02 (−0.18, 0.14)		−0.08 (−0.23, 0.07)		−0.07 (−0.20, 0.06)	
Lipids									
TC z-score, overall	627	0.02 (−0.09, 0.12)		0.02 (−0.10, 0.15)		0.02 (−0.10, 0.15)		0.00 (−0.11, 0.12)	
Girls	318	0.04 (−0.12, 0.20)	0.96	0.05 (−0.13, 0.23)	0.74	0.09 (−0.08, 0.26)	0.53	0.05 (−0.11, 0.21)	0.85
Boys	309	−0.02 (−0.17, 0.13)		0.00 (−0.18, 0.17)		−0.05 (−0.22, 0.13)		−0.05 (−0.22, 0.12)	
HDL-C z-score, overall	627	−0.01 (−0.11, 0.10)		−0.03 (−0.14, 0.09)		−0.04 (−0.15, 0.08)		−0.03 (−0.14, 0.08)	
Girls	318	0.04 (−0.10, 0.19)	0.97	−0.03 (−0.18, 0.13)	0.71	0.12 (−0.02, 0.27)	0.10	0.04 (−0.10, 0.18)	0.34
Boys	309	−0.06 (−0.22, 0.10)		−0.02 (−0.20, 0.16)		−0.20 (−0.01, −0.03)		−0.10 (−0.27, 0.07)	
LDL-C z-score, overall	627	−0.01 (−0.12, 0.09)		0.02 (−0.10, 015)		0.03 (−0.08, 0.15)		0.01 (−0.10, 0.12)	
Girls	318	0.00 (−0.15, 0.15)	0.94	0.07 (−0.11, 0.25)	0.51	0.04 (−0.12, 0.21)	1.00	0.02 (−0.13, 0.18)	0.71
Boys	309	−0.04 (−0.18, 0.10)		−0.02 (−0.20, 0.15)		0.02 (−0.15, 0.19)		−0.01 (−0.17, 0.16)	
Triglycerides z-score, overall	627	0.11 (0.01, 0.21)		0.05 (−0.06, 0.17)		0.04 (−0.07, 0.15)		0.03 (−0.07, 0.14)	
Girls	318	0.07 (−0.08, 0.22)	0.85	0.01 (−0.17, 0.19)	0.80	−0.01 (−0.17, 0.16)	0.79	0.05 (−0.11, 0.20)	0.68
Boys	309	0.16 (0.03, 0.30)		0.09 (−0.06, 0.24)		0.08 (−0.06, 0.22)		0.02 (−0.12, 0.16)	
CM-risk score[b,c] overall	386	−0.09 (−0.64, 0.45)		0.28 (−0.33, 0.89)		0.27 (−0.35, 0.89)		0.60 (0.04, 1.16)	
Girls	197	−0.14 (−0.93, 0.64)	0.77	0.10 (−0.73, 0.93)	0.73	−0.22 (−1.10, 0.66)	0.45	0.50 (−0.27, 1.27)	0.86
Boys	189	−0.09 (−0.86, 0.68)		0.47 (−0.44, 1.37)		0.72 (−0.17, 1.62)		0.70 (−0.13, 1.54)	
At 7 y									
BMI z-score, overall	1,086	−0.04 (−0.14, 0.06)		0.03 (−0.08, 0.14)		0.03 (−0.08, 0.13)		0.06 (−0.04, 0.16)	
Girls	535	0.02 (−0.10, 0.14)	0.44	0.05 (−0.09, 0.20)	0.60	−0.01 (−0.13, 0.12)	0.48	0.00 (−0.12, 0.13)	0.18
Boys	551	−0.10 (−0.25, 0.05)		0.02 (−0.15, 0.19)		0.07 (−0.10, 0.23)		0.12 (−0.04, 0.28)	
WC z-score, overall	1,086	−0.04 (−0.12, 0.04)		0.00 (−0.09, 0.09)		−0.02 (−0.11, 0.06)		0.02 (−0.07, 0.10)	
Girls	535	0.04 (−0.07, 0.15)	0.16	0.04 (−0.09, 0.17)	0.42	−0.05 (−0.16, 0.07)	0.49	−0.03 (−0.14, 0.08)	0.26
Boys	551	−0.11 (−0.23, 0.01)		−0.02 (−0.16, 0.11)		0.01 (−0.12, 0.14)		0.07 (−0.05, 0.19)	
BP z-score, overall	1,086	0.04 (−0.04, 0.13)		0.06 (−0.04, 0.15)		−0.02 (−0.11, 0.07)		0.00 (−0.08, 0.09)	
Girls	535	0.07 (−0.06, 0.19)	0.99	0.06 (−0.09, 0.20)	0.92	−0.08 (−0.21, 0.04)	0.11	0.00 (−0.12, 0.13)	0.70
Boys	551	0.00 (−0.11, 0.11)		0.04 (−0.08, 0.17)		0.04 (−0.08, 0.16)		−0.01 (−0.12, 0.11)	

Note: Coefficients represent the average difference in the outcome with a doubling of the exposure. BMI, body mass index; BP, blood pressure; CI, confidence interval; CM, cardiometabolic; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; TC, total cholesterol; WC, waist circumference.

[a]Model adjusted by: maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, and prepregnancy BMI), and the age and sex of the child. Note that sex of the child was not included in the models stratified by sex.

[b]Only Sabadell and Valencia subcohorts have available data for this outcome.

[c]CM-risk score is the z-scores for WC, BP, and the mean of the HDL-C and TG z-scores, with HDL-C multiplied by −1.

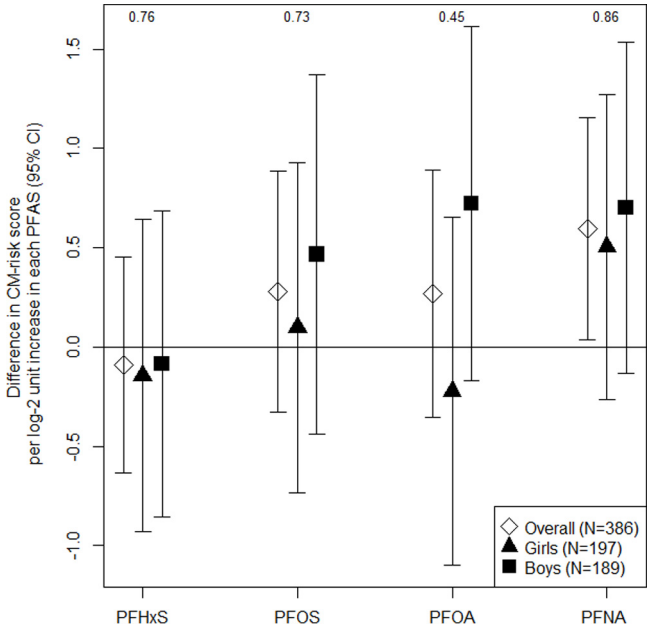

Figure 2. Adjusted associations between maternal PFAS concentrations (log_2–transformed, in ng/mL) and cardiometabolic risk score at 4 y. Abbreviations: CI, confidence interval; CM, cardiometabolic; PFHxS, perfluorohexanesulfonic acid; PFOS, perfluorooctanesulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid. Model adjusted by: maternal characteristics (i.e., region of residence, country of birth, previous breastfeeding, age, and prepregnancy BMI), and the age and sex of the child. Only Sabadell and Valencia subcohorts have available data for this outcome. CM-risk score is the *z*-scores for waist circumference (WC), blood pressure (BP), and the mean of the high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) *z*-scores, with HDL-C multiplied by -1. Note: Coefficients represent the average difference in the outcome with a doubling of the exposure. Upper values represent the *p*-values of the sex interaction term.

(Braun et al. 2016). On the contrary, the DNBC, where PFOS exposure levels (median: 33.18 ng/mL) are considerably higher than ours, prenatal exposure to PFOS and PFOA was nonsignificantly associated with self-reported lower BMI ($n = 811$), overweight, and WC ($n = 804$) at age 7 (Andersen et al. 2013). Differences in the outcome measure might explain the conflicting results in prospective studies, though there are many other causal and noncausal factors that also might contribute to variation among studies.

We did not find clear or consistent evidence of differences in associations between boys and girls. Prenatal PFOA exposure was associated with higher weight gain from birth until 6 mo in boys, but not girls, though the difference was not significant (*p*-value for sex-interaction = 0.28). The opposite pattern was seen in DNBC, where prenatal PFOA exposure (median: 5.25 ng/mL) was associated with lower weight and BMI at 5 and 12 mo in boys, but not in girls (Andersen et al. 2010). Effects might differ between the present study and the DNBC because exposures were higher in the DNBC, though other causal and noncausal explanations are also possible.

Early-life BP is predictive of cardiovascular health in adult life (Bao et al. 1995). In our study, we did not observe any statistically significant association between PFAS concentrations and BP at any age. In line with our results, cross-sectional data from the United States ($n = 1{,}655$) showed no association between PFAS and hypertension in children 12–18 y old (Geiger et al. 2014b). In the present study, BP was measured twice at 7 y of age and averaged as recommended (Pickering et al. 2005), but was measured only once at 4 y of age, and only in children from two of the three study regions. We used the average of SBP and DBP as our BP outcome, consistent with the study of Ahrens et al. (2014), though other studies have evaluated SBP and DBP as separate outcomes, or have used mean arterial pressure (Eisenmann 2008; Geiger et al. 2014b; Sardinha et al. 2016; Shafiee et al. 2013).

Elevated TG levels during childhood have been associated with metabolic syndrome later in life (Berenson et al. 1998; Miller et al. 2011). TGs tend to accumulate in the liver, and PFAS are known to activate peroxisome proliferator–activated alpha receptor, which is a nuclear receptor that regulates lipid homeostasis in the liver (Lau et al. 2007; Rosen et al. 2008). In our study, higher prenatal PFHxS concentrations were associated with higher TG levels at 4 y, with higher point estimate for boys, but given the low precision of the estimates and *p*-value for sex-interaction = 0.85, there is not clear evidence of a stronger association in boys than in girls. Few studies have assessed PFAS effect on lipids during childhood and adolescence, and even though they are of cross-sectional design, they suggest that PFAS, especially PFOS and PFOA, alter the lipid profile in children (Frisbee et al. 2010; Geiger et al. 2014a; Lin et al. 2009; Zeng et al. 2015). The study of Frisbee et al. (2010) from the C8 project ($n > 12{,}000$ children aged 1–18 y old) only measured PFOS and PFOA, and observed positive associations of both with TC and LDL-C, with PFOS also positively associated with HDL-C. The study of Geiger et al. (2014a) from the NHANES ($n = 814$ children aged 12–18 y old) only evaluated PFOA and PFOS, and reported that PFOA was positively associated with LDL-C > 110 mg/dL and with HDL-C < 40 mg/dL (these were used as parameters of dyslipidemia), and PFOS was positively associated with LDL-C > 110 mg/dL only. The study from Lin et al. (2009), also from NHANES ($n = 474$ children aged 12–18 y old), assessed PFHxS, PFOS, PFOA, and PFNA, and observed that PFNA negatively associated with HDL-C. The study of Zeng et al. (2015) in 225 Taiwanese children (aged 12–15 y), measured eight PFAS, and observed that PFOS and PFNA associated with TC, LDL-C, and TGs. Also, in a prospective study, prenatal PFOA in the lowest tertile was positively associated with LDL-C, but not with TC, HDL-C, or TG in girls at 7 and 15 y old (Maisonet et al. 2015).

Our CM-risk score included three of four individual components (e.g., anthropometric measurements, BP, and lipids) that are typically used to define metabolic syndrome (Eisenmann 2008). In our study, we observed higher CM-risk scores with higher PFOS, PFOA, and PFNA, but the association was only significant for PFNA. In contrast, Lin et al. (2009) reported that serum PFHxS, PFOA, PFOS, and PFNA concentrations in NHANES participants 12–19 y of age were inversely associated with the prevalence of metabolic syndrome (based on ≥three of the following conditions: high WC, high serum TG, low serum HDL, elevated SBP or DBP, medication for hypertension, or elevated fasting blood glucose or medication to reduce blood glucose), with a significant negative association for PFNA. However, direct comparisons between our study and Lin et al. (2009) are not possible given differences in the study design, population age, and outcome. In a Danish birth cohort study, prenatal exposure to PFOA was associated with overweight at 20 y in women, but not men (Halldorsson et al. 2012). Future research should include a prospective assessment of prenatal PFAS exposure and follow-up beyond early and midchildhood, with evaluation of sex-specific associations in larger populations.

Maternal excretion rates during pregnancy may influence the associations between prenatal exposure to PFAS and weight of the child (Verner et al. 2015). In our study, we adjusted our models by maternal GFR, showing that excretion rates are unlikely to

have confounded the association between maternal PFAS concentration in plasma and childhood cardiometabolic outcomes. In the Project Viva cohort (United States), Fleisch et al. (2016) reported that after adjusment for GFR, their exposure–outcome estimates did not change by more than 10%. In the same cohort, Mora et al. (2016) reported that adjusting for GFR marginally strenghtened the associations between prenatal PFAS exposure and adiposity in midchildhood, suggesting some confounding by GFR. Both in the Project Viva and in our cohort, maternal PFAS concentrations were measured early in pregnancy when changes in GFR might not have a big impact on PFAS concentrations (Verner et al. 2015).

The main strengths of our study are its prospective design and large sample size for analyses of BMI and weight gain, and the ability to estimate associations between prenatal PFAS exposures and outcomes that may contribute to future cardiometabolic risk, including weight gain from birth to 6 mo; BMI, WC, and BP at 4 and 7 y of age; and blood lipids and a composite CM-risk score (based on WC, BP, and lipids) at age 4. Nevertheless, some methodological limitations should be considered. First, lipid levels were measured using fasting samples in children from the Valencia region, but nonfasting samples for children from Sabadell and Gipuzkoa, which may influence lipid levels, especially TGs. In addition, lipid concentrations were measured in only a subset of children ($n = 627$) at 4 y of age. Second, our CM-risk score does not include a marker of glucose homeostasis, which is one of the components that is normally used to define metabolic syndrome (Ahrens et al. 2014; Eisenmann 2008). Therefore, our CM-risk score might not fully characterize the potential impact of PFAS on the prevalence of metabolic syndrome at age 4 or the future risk of cardiometabolic disease. Future follow-ups with available information on glucose homeostasis or insulin resistance at later ages are recommended. Third, we could only calculate the CM-risk score in 386 children that were generally healthier than the rest, thus limiting the extrapolation of our result to the full sample at 4 y ($n = 1{,}230$). Fourth, we observed a pattern of positive associations between PFOA and BMI z-scores at ages 4 and 7 y with longer duration of breastfeeding for the index child. This finding suggests that postnatal PFAS exposure may play a role on childhood cardiometabolic risk, as similarly seen in other studies (e.g., Domazet et al. 2016; Zeng et al. 2015); however, we lack a direct measurement of postnatal PFAS exposure in our cohort. Fifth, women included in this study were more likely to be older, nulliparous, and with higher education than those excluded from the analysis. Given that older and nulliparous women tend to have higher PFAS levels (Manzano-Salgado et al. 2016), we probably included women and children with exposures that were higher than exposures in the cohort as a whole. Sixth, we cannot rule out the possibility of chance findings due to multiple comparisons in our study, or the possibility of uncontrolled confounding or bias due to measurement errors or missing data. Finally, in this study, small sample sizes for some of our analyses resulted in unstable or imprecise estimates of association, particularly for the CM-risk score, lipids, WC, and BP at 4 y, and for estimates stratified by sex.

Conclusions

In this study population exposed to low levels of PFAS, we found little evidence of an association between prenatal PFAS exposure and cardiometabolic risk during childhood. Although concerns have been raised about the potential for bias due to changes in maternal GFR during pregnancy, we measured PFAS early in pregnancy (before such changes are likely to be pronounced), and adjusting for GFR in a sensitivity analysis had little effect on our findings. Moreover, we evaluated multiple outcomes that may contribute to cardiometabolic risk, including anthropometric measurements, BP, lipid levels, as well as a combined risk score. Our findings are consistent with two previous prospective studies that reported little or no evidence of associations between prenatal PFAS exposure and obesity during early and midchildhood (Andersen et al. 2013; Mora et al. 2016). Future studies with follow-ups beyond midchildhood and with measures of glucose homeostasis are needed to further elucidate the effect of prenatal PFAS exposure on cardiometabolic risk.

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