

# Prenatal Exposures to Perfluoroalkyl Acids and Associations with Markers of Adiposity and Plasma Lipids in Infancy: An Odense Child Cohort Study

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**BACKGROUND:** Perfluoroalkyl acids (PFAA) are repellants that cross the placental barrier, enabling interference with fetal programming. Maternal PFAA concentrations have been associated with offspring obesity and dyslipidemia in childhood and adulthood, but this association has not been studied in infancy.

**OBJECTIVES:** We investigated associations between maternal PFAA concentrations and repeated markers of adiposity and lipid metabolism in infancy.

**METHODS:** In the prospective Odense Child Cohort, maternal pregnancy serum concentrations of five PFAA: Perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) were measured in 649 women. Offspring were examined at birth ( $n=613$ ) and at 3 months ( $n=602$ ) and 18 months ( $n=503$ ) of age. Total cholesterol, LDL, HDL, and triglyceride were evaluated at 3 months ( $n=262$ ) and 18 months ( $n=198$ ) of age. Mixed effects linear regression models estimated associations between PFAA and standardized (SDS) body mass index (BMI), ponderal index, and waist circumference. Associations between PFAA and body fat% (BF%) and plasma lipids SDS at 3 months and 18 months of age were investigated with linear regression models.

**RESULTS:** PFNA and PFDA were associated with higher BMI SDS [adjusted  $\beta=0.26$ ; 95% confidence interval (CI): 0.03, 0.49 and  $\beta=0.58$ ; 95% CI:  $-0.03$ , 1.19, respectively, for 1-ng/mL increases] and ponderal index SDS ( $\beta=0.36$ ; 95% CI: 0.13, 0.59 and  $\beta=1.02$ ; 95% CI: 0.40, 1.64, respectively) at 3 and 18 months of age (pooled) in girls. Corresponding estimates for boys were closer to the null but not significantly different from estimates for girls. In boys and girls (combined), PFNA and PFDA were associated with BF% at age 3 months (for 1-ng/mL PFDA,  $\beta=0.40$ ; 95% CI: 0.04, 0.75), and PFDA was associated with total cholesterol SDS at 18 months ( $\beta=1.06$ ; 95% CI: 0.08, 2.03) ( $n=83$ ).

**DISCUSSION:** Prenatal PFAA were positively associated with longitudinal markers of adiposity and higher total cholesterol in infancy. These findings deserve attention in light of rising rates of childhood overweight conditions and dyslipidemia. <https://doi.org/10.1289/EHP5184>

## Introduction

Perfluoroalkyl acids (PFAA) are persistent chemicals used as surface repellants in fabrics and food packaging due to water-, stain-, and grease-resistant properties (ATSDR 2018). Prevalent routes of PFAA exposure are mainly dietary and, in some populations, also through drinking water. PFAA can be measured in the majority of humans with elimination half-lives ranging from 4 to 8 y (ATSDR 2018). Two of the previously most used and studied PFAA, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been phased out by some of the major manufacturers (ATSDR 2018; Glynn et al. 2012). Nonetheless, other PFAA, such as perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid

(PFDA), are still in production, and these PFAA are less studied.

PFAA cross the placental barrier (Monroy et al. 2008), and PFAA have been detected in human amniotic fluid (Stein et al. 2012) and umbilical cord blood (Monroy et al. 2008). Some PFAA have endocrine-disrupting abilities and may influence the fetal endocrine programming related to growth patterns and lipid metabolism (Buhrke et al. 2013; Wolf et al. 2012; Ye et al. 2012; Yu et al. 2009; Zhao et al. 2011). Data from previous infancy studies investigating associations between PFAA concentrations and anthropometry differed in sample size, and anthropometric outcomes were obtained at different stages of infancy and from various sources (Table S1) (Alkhalawi et al. 2016; Andersen et al. 2010; Gyllenhammar et al. 2018; Karlsen et al. 2017; Maisonet et al. 2012; Manzano-Salgado et al. 2017; Shoaff et al. 2018; Starling et al. 2019). Three prospective studies suggested that increased maternal PFAA concentrations was linked to higher weight at 3 months of age (Gyllenhammar et al. 2018), significantly increased percent fat mass at 5 months of age in boys (Starling et al. 2019), and significantly higher weight gain z-scores [ $\beta=0.13$ ; (95% CI: 0.01, 0.26); with a doubling of PFOA] at 6 months of age in boys (Manzano-Salgado et al. 2017). However, a Danish study found that a one-unit increase (1-ng/mL) in maternal pregnancy concentrations of PFOS was associated with small reductions in 12-month-old offspring in body mass index (BMI) z-score [ $\beta=0.007$ ; (95% CI:  $-0.011$ ,  $-0.002$ )] (Andersen et al. 2010). Two studies demonstrated that higher maternal concentration of PFOS was associated in late infancy with higher BMI z-scores and overweight risk in Faroese children 18 months old (Karlsen et al. 2017) and with increased weight in British girls

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20 months old (Maisonet et al. 2012). No significant association was demonstrated between PFAA and repeated pooled measurements of ponderal index (Alkhalawi et al. 2016) during the first year of infancy, whereas there were indications of negative associations between PFOA and pooled BMI z-scores from 4 wk to 2 years of age (Shoaff et al. 2018); however, during this period, the rate of change in anthropometry was not modified by PFAA (Shoaff et al. 2018). In relation to lipid metabolism, PFOS and PFOA concentrations have been associated with hyperlipidemia in adults from the general population (Nelson et al. 2010; Steenland et al. 2009). Moreover, prenatal PFAA concentrations could affect metabolic function in offspring girls and boys differently, and five studies analyzing sex-dimorphic anthropometry found significant associations for females only in three studies (Halldorsson et al. 2012; Høyer et al. 2015; Mora et al. 2017) and for males only in two studies (Andersen et al. 2010; Manzano-Salgado et al. 2017). Five of the eight infancy studies investigated associations between PFAA exposure and repeated anthropometric measurements throughout infancy (Alkhalawi et al. 2016; Andersen et al. 2010; Gyllenhammar et al. 2018; Maisonet et al. 2012; Shoaff et al. 2018), whereas others measured anthropometry only at a single time point during infancy (Karlsen et al. 2017; Manzano-Salgado et al. 2017; Starling et al. 2019). Moreover, some studies were restricted to outcomes that were either self-reported (Andersen et al. 2010) or obtained from medical records (Alkhalawi et al. 2016; Gyllenhammar et al. 2018; Manzano-Salgado et al. 2017), whereas others collected data from regular clinical examinations (Karlsen et al. 2017; Maisonet et al. 2012; Shoaff et al. 2018; Starling et al. 2019). In general, the PFAA studies during infancy were of a sample size <447 children (Alkhalawi et al. 2016; Gyllenhammar et al. 2018; Karlsen et al. 2017; Maisonet et al. 2012; Shoaff et al. 2018; Starling et al. 2019). To the best of our knowledge, no study has investigated associations between maternal pregnancy PFAA concentrations and lipid metabolism in infancy.

We hypothesized that prenatal PFAA exposure may induce metabolic dysfunction, resulting in increased markers of adiposity and lipid metabolism observed in infancy. To our knowledge, this is the first study to prospectively explore associations of maternal PFHxS, PFOS, PFOA, PFNA, and PFDA concentrations in early pregnancy with repeated markers of adiposity and lipid metabolism in infancy.

## Materials and Methods

### Study Population

The present exploratory study is part of Odense Child Cohort (OCC) ( $n=2,874$ ), a longitudinal birth cohort conducted in Denmark (Kyhl et al. 2015). Eligible women were those residing in the Municipality of Odense, Region of Southern Denmark, and recruited in early pregnancy [gestational age (GA) <16] between 2010 and 2012. Following enrollment, the pregnant women were asked to donate a blood sample for PFAA assessment ( $n=649$ ) and to respond to a questionnaire on current general health. Infant anthropometric measurements were conducted at birth, and children were invited to a clinical examination, including anthropometry, at 3 months and 18 months of age (Figure 1). All parents of children participating at the clinical examination at 3 and 18 months were approached to consent for blood sample collection from the child for lipid assessment (Figure 1).

In this study, we excluded multiple pregnancy ( $n=56$ ), miscarriage ( $n=103$ ), stillbirth ( $n=10$ ), and mothers with no serum PFAA ( $n=2,056$ ) (Figure 1). Of the eligible 649 mother-child pairs with PFAA concentrations, 4 women were pregnant more than once within the inclusion period (only first pregnancy

included in data set), 27 children born preterm (GA <37) were excluded, and 5 children had missing anthropometric data from birth (Figure 1). Anthropometric measurements of children were performed at birth ( $n=613$ ), at a median age of 3.2 months [interquartile range (IQR): 2.8, 3.7] ( $n=602$ ) and 19.2 months (IQR: 18.6, 19.6) ( $n=530$ ). Among these, 84 children (51% boys, 49% girls) had a blood sample drawn from both clinical visits at 3 and 18 months of age (Figure 1).

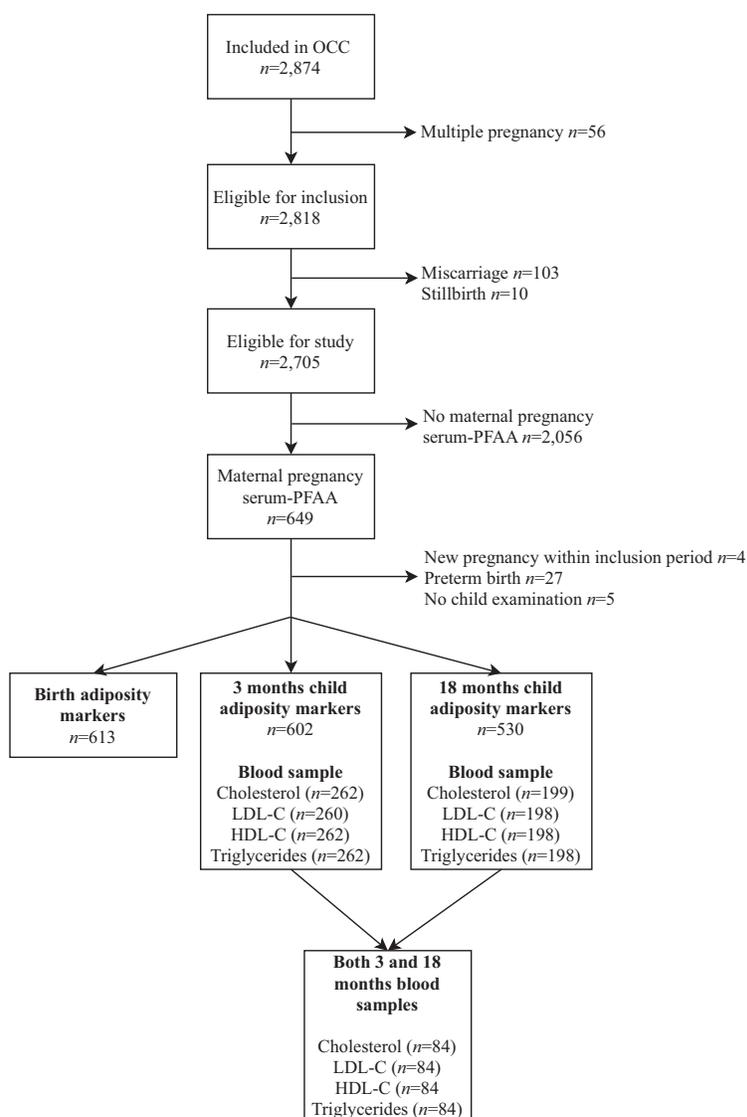
### PFAA Assessment

Assessment of maternal serum PFAA concentrations included the following compounds: PFHxS, PFOS, PFOA, PFNA, and PFDA. Serum PFAA concentrations were assessed based on blood samples obtained at inclusion [median GA (IQR): 11.3 (9.9, 14.3) weeks] in a subsample of 649 pregnant women. Of the 649 women with serum PFAA concentrations, 200 blood samples were randomly selected, and the remaining 449 blood samples were chosen based on obtainability of data from birth records, questionnaires and a clinical examination of the children at 3 months of age. PFAA concentrations were estimated using online solid phase extraction followed by liquid chromatography and triple quadrupole mass spectrometry (LC-MS/MS) at the Department of Environmental Medicine, University of Southern Denmark (Jensen et al. 2015). The analyses were performed between September 2011 and September 2013. The within-batch coefficients of variation (CVs) were <3% and the between-batch CVs were <5.2%. The Limit of Quantification (LOQ) was 0.03 ng/mL for all compounds. PFOS, PFOA, PFNA, and PFDA were detectable in all samples in this study (LOQ >0.03 ng/mL), but 7 (1.1%) of the participants had a PFHxS concentration below the LOQ that was reported as LOQ/2.

### Markers of Adiposity

Birth weight, length, and waist circumference (WC) were registered by midwives after parturition, and data were obtained from birth records. Weight, length, WC, and skinfold thickness were assessed at clinical examinations at ages 3 months and 18 months. Three health care professionals performed the clinical examination blinded to the prenatal PFAA concentrations. Weight was measured without clothing using an electronic scale (Seca 717; Seca), and recumbent length was determined to the nearest millimeter (Seca 416; Seca). WC was assessed as an indirect measure of central adiposity (Cornier et al. 2011) using a plastic measuring tape (Seca 212, Seca) to the nearest millimeter around a horizontal plane midway between the lower lateral rib border and the upper lateral hip crest border. BMI and ponderal index were proxies for total body adiposity (Hetherington-Rauth et al. 2017). BMI (in kg/m<sup>2</sup>) was calculated as weight (kg) divided by the length squared (m<sup>2</sup>), and ponderal index (in kg/m<sup>3</sup>) was calculated as weight (kg) divided by the length cubed (m<sup>3</sup>). Triceps and subscapular skinfold thicknesses were each measured three times with a Harpenden skinfold caliper (C.M.S. Weighing Equipment Ltd.) to the nearest 0.1 mm, and measures were averaged. Body fat percentage (BF%) was calculated according to Slaughter et al.'s formula (Slaughter et al. 1988) applying triceps and subscapular skinfolds according to sex.

Age- (month-by-month) and sex-specific standard deviation score (SDS) for BMI were calculated according to 2014 Danish reference data (Tinggaard et al. 2014), and age- (month-by-month) and sex-specific SDS for waist circumference and BF% SDS were calculated according to a Danish mother-child reference cohort (Wohlfahrt-Veje et al. 2014). Age- (month-by-month) and sex-specific SDS for ponderal index was calculated on basis of cohort specific internal reference values from the OCC.



**Figure 1.** Flowchart of mother–child pairs.

SDS expresses the number of standard deviations a given measurement of interest lies below or above the sample mean from an age- and sex-specific reference population.

### Lipid Assessment

Lipid assays were completed on nonfasting blood samples collected at 3 months and 18 months of age. Plasma total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglyceride (TG) concentrations were assayed using ARCHITECT (Abbott). When TG concentrations were less than 4 mmol/L, LDL results were calculated using the Friedewald equation (Friedewald et al. 1972). The quality of the lipid analyses was assured by participation in external quality control programs from The Reference Institute for Bioanalytics (RfB), Bonn, Germany, using “Clinical chemical analytes in serum” (8/y) and “Lipoproteins” (4/y).

Age- (month-by-month) and sex-specific SDS for biomarkers of lipid status were calculated on basis of cohort specific internal reference values from the OCC.

### Covariates

Maternal age at delivery and parity were derived from hospital records using maternal civil registration numbers. Prepregnancy BMI, smoking status (yes; no) during pregnancy, breastfeeding duration (months) for the current pregnancy, and educational level (high school or less; high school + 1–4 y; high school + > 4 y) were extracted from questionnaires. If educational information was missing in questionnaires, they were retrieved from hospital records. Maternal ethnicity (European; non-European) was based on information on her mother’s country of birth obtained from Odense Municipality. Characteristics of the children, including gestational age at birth and sex, were obtained from hospital records.

### Ethical Approval

The study was performed in accordance with the Helsinki Declaration II and approved by the Regional Scientific Ethical Review Committee for Southern Denmark (Project ID S-20090130) and the Danish Data Protection Agency (J. No. 18/33119). All participants received written and oral information and provided their written consent for participation in the study.

## Statistical Analyses

Maternal pregnancy serum PFAA concentrations were reported as median (5th–95th percentiles) in subgroups according to maternal and child characteristics. Kruskal-Wallis and Wilcoxon rank sum tests were used to compare PFAA concentrations between subgroups, and pairwise Spearman correlations were calculated for each pair of PFAA. Characteristics of included mother–child pairs in the study were compared with those of the rest of the women in the OCC using Wilcoxon rank sum tests for BMI and gestational age, and *t*-test for age, and a chi square test for smoking, ethnicity, parity, child sex, and educational level.

Random mixed effects linear regression models were used to investigate associations between maternal pregnancy PFAA concentrations and longitudinal offspring markers of adiposity, including BMI SDS, ponderal index SDS, and WC SDS, from 3 months and 18 months of age and adjusting for outcome at birth (baseline) to take baseline imbalance into account. Mother–child pairs were included as random effects to account for the repeated measurements of the children. For the outcomes BMI SDS, ponderal index SDS, and WC SDS, we modeled interaction terms between maternal PFAA concentrations and an indicator for study visit (3 months or 18 months) in the random mixed effects regression models to investigate differences in associations with the outcomes according to age and report pooled estimates for associations with outcomes at 3 months and 18 months of age (combined) when the difference in slopes was not significant ( $p > 0.05$ ), in addition to estimates for visit-specific outcomes from the same models. The random mixed effects linear regression models estimated the difference ( $\beta$ -estimates) in offspring markers of adiposity (pooled and visit-specific) per 1-ng/mL increase in the maternal PFAA concentration.

Multiple linear regression models were used to examine associations between maternal pregnancy PFAA concentrations and markers of adiposity (BMI SDS, ponderal index SDS, and WC SDS) at birth. Because BF% SDS and lipid data SDS were available only at 3 months and 18 months of age, random mixed effects regression modeling could not be performed, when also adjusting for baseline (3 months) to take baseline imbalance into account, as explained below. Thus, multiple linear regression models were conducted to exploratively investigate associations between maternal PFAA concentrations and BF% SDS and lipid concentration SDS at ages 3 months and 18 months, because data for these outcomes were not available at birth. The multiple linear regression models estimated the difference ( $\beta$ -estimates) in markers of adiposity and lipid metabolism in children per 1-ng/mL increase in the maternal PFAA concentration. Thus, it was not possible to evaluate differences by visit for BF% and lipid concentrations, and visit-specific estimates by default were reported for these outcomes.

To address potential baseline imbalance of prenatal growth measures, the random mixed effects linear regression models at 3 months and 18 months of age were adjusted for the birth measurement of the respective outcome (BMI SDS, ponderal index SDS, and WC SDS) (Vickers and Altman 2001). Additionally, this adjustment will correct for any effect of exposures on growth until birth. The multiple linear regression models with BF% and markers of lipid metabolism as outcome of interest at 18 months of age were adjusted for the baseline measurement at 3 months of age (Vickers and Altman 2001). Confounders and intermediate factors were identified based on *a priori* review of published evidence and using directed acyclic graphs (DAGs) (Figure S1). PFAA concentrations have been reported to change across categories of parity (Brantsæter et al. 2013), age (Bjerregaard-Olesen et al. 2016), education (Bjerregaard-Olesen et al. 2016), offspring sex (Frisbee et al. 2010), BMI (Brantsæter et al. 2013), and

smoking (Bjerregaard-Olesen et al. 2016). These covariates have also been associated with childhood adiposity and lipid metabolism (Woo Baidal et al. 2016). Thus, in mixed effects linear regression models we *a priori* included the following potential confounders: maternal age at delivery (continuous), parity (nulliparous; parous), prepregnancy BMI (continuous), maternal educational level (high school or less; high school + 1–4 y; high school + > 4 y), maternal smoking (yes; no), offspring sex (girl; boy), visit (three months; 18 months), and adiposity measurements at birth (continuous). The adjusted linear regression models at birth and 3 months of age included the potential confounders: maternal age at delivery, parity, prepregnancy BMI, maternal educational level, maternal smoking, and offspring sex. The linear regression models at 18 months of age were additionally adjusted for the outcome at 3 months. As a sensitivity analysis, lipid outcomes at ages 3 months and 18 months were additionally adjusted for offspring BMI SDS at respective ages.

In all adjusted statistical models, maternal prepregnancy BMI was additionally included as a second-degree polynomial to allow for nonlinear associations. Moreover, effect modification by child sex on the associations between PFAA concentrations and markers of adiposity and lipid metabolism was evaluated by including an interaction term (between PFAA concentration and sex) in all the adjusted models. These models were used to obtain sex-specific results.

Model assumptions of all models were validated through thorough residual analyses, and multicollinearity was assessed using variance inflation factors. Missing data were assumed to occur at random, and all statistical analyses were based on complete cases. A two-sided significance level of 5% was used in all analyses, including evaluation of effect modifiers. Data were analyzed using STATA/IC version 15.1 (StataCorp).

## Results

Of the 613 mother–child pairs, mothers were predominantly nulliparous (57.9%) with a mean age of 30.2 y ( $\pm 4.5$  y) at the time of parturition; 97.7% were of European origin, 18.4% were in the highest educational group (completed high school + > 4 y), and 3.4% smoked during pregnancy (Table 1). Included mother–child pairs did not differ statistically from the rest of the OCC with regard to major characteristics, but were more often nulliparous, of European origin, smoked less, and had a higher prepregnancy BMI (Table S2). The included children were evenly distributed by sex (47% girls and 53% boys) with a mean BMI SDS of 0.02 ( $\pm 1.01$ ), 0.07 ( $\pm 0.97$ ), and  $-0.20$  ( $\pm 1.01$ ) at birth, 3 months, and 18 months of age, respectively (Table S3). The subgroup of children with lipid outcomes at both 3 months and 18 months of age ( $n = 84$ ) did not differ in relation to major maternal or child characteristics when compared with the subset of mother–child pairs with anthropometric measurements but were more often nulliparous, of European origin, smoked less, and had a higher prepregnancy BMI when compared with the rest of the OCC (Table S2).

All pregnant women had detectable serum concentrations of at least four PFAA, but 1.1% of the women had a PFHxS concentration below the LOQ (Table S4). The five maternal PFAA concentrations were weakly to strongly intercorrelated with Spearman's correlation coefficients ranging from 0.33 (PFHxS and PFDA) to 0.74 (PFNA and PFDA) (Table S5). Concentrations of all five PFAA were higher among nulliparous vs. parous women and among women giving birth at 37–40 wk vs.  $\geq 40$  wk of gestational age (Table 1). PFOS and PFOA decreased with increasing maternal age and higher educational level, PFNA and PFDA decreased with higher prepregnancy BMI; and PFHxS, PFOA, and PFNA

**Table 1.** Maternal pregnancy serum PFAA concentration according to maternal and offspring characteristics.

Variables	n (%)	Median (5th, 95th percentile)				
		PFHxS (ng/mL)	PFOS (ng/mL)	PFOA (ng/mL)	PFNA (ng/mL)	PFDA (ng/mL)
Total	613 (100)	0.30 (0.08, 0.66)	8.04 (3.82, 15.46)	1.62 (0.67, 4.03)	0.66 (0.33, 1.52)	0.26 (0.15, 0.53)
<b>Maternal characteristics</b>						
Maternal age (y)						
<25	61 (10.0)	0.27 (0.13, 0.56)	8.68 (4.98, 14.80)	1.87 (0.73, 4.50)	0.72 (0.29, 1.75)	0.26 (0.15, 0.44)
25–29	220 (35.9)	0.30 (0.06, 0.66)	8.36 (3.65, 17.25)	1.84 (0.73, 4.34)	0.72 (0.32, 1.63)	0.28 (0.13, 0.59)
30–34	222 (36.2)	0.29 (0.08, 0.59)	7.82 (4.22, 15.17)	1.56 (0.67, 3.85)	0.63 (0.37, 1.32)	0.26 (0.16, 0.46)
>35	110 (17.9)	0.30 (0.10, 0.72)	7.16 (3.61, 13.24)	1.28 (0.54, 3.98)	0.62 (0.27, 1.33)	0.24 (0.14, 0.64)
<i>p</i> -Value		0.92	0.01	<0.01	0.08	0.21
Prepregnancy BMI (kg/m <sup>2</sup> )						
<18.5	15 (2.4)	0.34 (0.03, 0.78)	8.36 (3.41, 16.72)	1.87 (0.31, 3.28)	0.84 (0.27, 1.52)	0.29 (0.12, 0.58)
18.5–25.0	357 (58.2)	0.30 (0.07, 0.66)	8.14 (3.79, 15.46)	1.67 (0.67, 4.49)	0.69 (0.34, 1.50)	0.28 (0.15, 0.56)
≥25.0	241 (39.3)	0.29 (0.09, 0.59)	7.73 (3.83, 15.30)	1.57 (0.73, 3.81)	0.62 (0.31, 1.52)	0.25 (0.15, 0.43)
<i>p</i> -Value		0.26	0.49	0.87	0.08	0.02
Parity						
Nulliparous	355 (57.9)	0.36 (0.12, 0.70)	8.85 (4.23, 16.81)	2.01 (0.86, 4.61)	0.73 (0.37, 1.64)	0.28 (0.16, 0.59)
Parous	258 (42.1)	0.24 (0.05, 0.52)	6.80 (3.60, 14.23)	1.16 (0.54, 3.13)	0.57 (0.29, 1.16)	0.24 (0.14, 0.48)
<i>p</i> -Value		<0.01	<0.01	<0.01	<0.01	<0.01
Smoking						
Yes	21 (3.4)	0.30 (0.09, 0.59)	8.71 (5.50, 12.30)	1.94 (1.20, 2.82)	0.62 (0.41, 1.10)	0.23 (0.16, 0.45)
No	592 (96.6)	0.30 (0.08, 0.66)	7.85 (3.79, 15.67)	1.60 (0.67, 4.14)	0.66 (0.33, 1.52)	0.26 (0.15, 0.53)
<i>p</i> -Value		0.93	0.23	0.09	0.90	0.26
Educational level						
High school or less	184 (30.0)	0.28 (0.09, 0.59)	8.51 (3.91, 16.46)	1.83 (0.74, 4.78)	0.67 (0.32, 1.74)	0.26 (0.14, 0.58)
High school+1–4 y	308 (50.2)	0.29 (0.07, 0.63)	8.13 (3.82, 15.31)	1.53 (0.63, 3.85)	0.65 (0.34, 1.47)	0.26 (0.14, 0.47)
High school+ > 4 y	113 (18.4)	0.32 (0.10, 0.70)	7.23 (3.65, 15.67)	1.58 (0.65, 3.78)	0.71 (0.35, 1.47)	0.28 (0.15, 0.53)
Missing	8 (1.4)					
<i>p</i> -Value		0.18	0.02	0.01	0.20	0.36
Ethnicity						
European	599 (97.7)	0.30 (0.08, 0.63)	8.07 (3.83, 15.67)	1.63 (0.69, 4.01)	0.66 (0.33, 1.51)	0.26 (0.15, 0.52)
Non-European	14 (2.3)	0.21 (0.03, 0.85)	5.07 (1.25, 13.44)	1.12 (0.31, 9.71)	0.71 (0.27, 4.40)	0.33 (0.12, 1.75)
<i>p</i> -Value		0.38	0.01	0.22	0.77	0.23
<b>Child characteristics</b>						
Child sex						
Boys	325 (53.0)	0.31 (0.12, 0.63)	8.47 (4.07, 15.46)	1.70 (0.74, 4.61)	0.70 (0.35, 1.55)	0.27 (0.14, 0.59)
Girls	288 (47.0)	0.28 (0.07, 0.66)	7.51 (3.61, 15.17)	1.57 (0.58, 3.70)	0.63 (0.30, 1.45)	0.26 (0.15, 0.47)
<i>p</i> -Value		0.04	0.07	<0.01	0.03	0.27
Gestational age (weeks)						
37–40	244 (39.8)	0.31 (0.12, 0.63)	8.51 (3.94, 17.18)	1.80 (0.71, 4.14)	0.70 (0.35, 1.58)	0.27 (0.15, 0.53)
>40	369 (60.2)	0.28 (0.07, 0.66)	7.78 (3.65, 14.33)	1.52 (0.67, 3.98)	0.64 (0.31, 1.43)	0.26 (0.14, 0.52)
<i>p</i> -Value		0.01	<0.01	<0.01	0.03	0.04
Exclusive breastfeeding (months)						
0	100 (16.3)	0.33 (0.09, 0.71)	8.30 (4.14, 16.24)	1.71 (0.69, 3.81)	0.67 (0.38, 1.57)	0.25 (0.15, 0.60)
>0–3	222 (36.2)	0.28 (0.07, 0.61)	8.53 (3.94, 16.50)	1.82 (0.78, 4.90)	0.70 (0.30, 1.57)	0.27 (0.15, 0.57)
3–6	182 (29.7)	0.30 (0.09, 0.62)	7.73 (3.82, 15.67)	1.47 (0.66, 3.58)	0.63 (0.34, 1.31)	0.26 (0.15, 0.47)
>6	31 (5.1)	0.29 (0.13, 0.60)	8.11 (3.58, 13.24)	1.44 (0.54, 3.70)	0.70 (0.31, 1.84)	0.28 (0.15, 0.43)
Missing	78 (12.7)					
<i>p</i> -Value		0.44	0.17	0.02	0.27	0.36
Birth weight						
<2,500 g	2 (0.3)	0.53 (0.33, 0.72)	8.95 (7.80, 10.11)	2.48 (2.33, 2.63)	0.71 (0.58, 0.84)	0.26 (0.22, 0.30)
2,500–4,499 g	587 (95.8)	0.30 (0.08, 0.66)	8.06 (3.82, 15.67)	1.65 (0.67, 4.03)	0.67 (0.33, 1.52)	0.26 (0.15, 0.53)
>4,499 g	24 (3.9)	0.28 (0.07, 0.54)	7.38 (4.21, 14.23)	1.28 (0.70, 2.39)	0.64 (0.40, 0.99)	0.27 (0.17, 0.36)
<i>p</i> -Value		0.34	0.68	0.02	0.84	0.99

Note: BMI, body mass index; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid. *p*-Value, when comparing PFAA concentrations between subgroups according to maternal and child characteristics using Kruskal-Wallis or Wilcoxon rank sum tests.

were higher in women with male offspring in comparison with women with female offspring.

At birth, 1-ng/mL increases in PFOS and PFHxS were associated with increases in the ponderal index SDS of 0.03 (95% CI: 0.01, 0.05) and 0.24 (95% CI: 0.01, 0.47), respectively, after confounder adjustment (Table 2). In the adjusted random mixed effects linear regression models including the whole study group, interaction terms ( $p_{\text{int}}$ ) between PFAA and visit indicated no significant differences in the slopes at 3 months and 18 months of age for offspring BMI SDS (all  $p_{\text{int}} \geq 0.42$ ), ponderal index SDS ( $p_{\text{int}} \geq 0.43$ ), and WC SDS ( $p_{\text{int}} \geq 0.06$ ). In adjusted analyses at 3 months and 18 months of age (pooled), 1-ng/mL increases in

PFOA, PFNA, and PFDA were associated with average increases in the ponderal index SDS of 0.07 (95% CI: 0.01, 0.13), 0.24 (95% CI: 0.08, 0.41), and 0.60 (95% CI: 0.18, 1.02), respectively. Likewise, 1-ng/mL increases in PFNA, PFDA, and PFOA at 3 months and 18 months of age were associated with average increases in the BMI SDS of 0.18 (95% CI: 0.02, 0.34), 0.42 (95% CI: 0.01, 0.84), and 0.04 (95% CI: -0.01, 0.10), respectively. When stratified according to child sex, in girls at 3 months and 18 months of age, PFNA and PFDA concentrations were associated with increased BMI SDS [PFNA: 0.26 (95% CI: 0.03, 0.49), PFDA: 0.58 (95% CI: -0.03, 1.19)] and ponderal index SDS [PFNA: 0.36 (95% CI: 0.13, 0.59), PFDA: 1.02 (95% CI:

**Table 2.** Average difference ( $\beta$ ) in marker of adiposity in offspring at 3 and 18 months of age pooled, and at respective visits for 1-unit (ng/mL) increase in maternal pregnancy serum PFAA concentration.

Compound (ng/mL)	BMI SDS			Ponderal index SDS			Waist circumference SDS		
	$\beta$ (95% CI)	<i>p</i>	<i>p</i> <sub>int</sub> <sup>a</sup>	$\beta$ (95% CI)	<i>p</i>	<i>p</i> <sub>int</sub> <sup>a</sup>	$\beta$ (95% CI)	<i>p</i>	<i>p</i> <sub>int</sub> <sup>a</sup>
PFHxS pooled 3 and 18 months									
Crude <sup>b</sup>	0.04 (−0.16, 0.25)	0.68		0.07 (−0.13, 0.28)	0.48		−0.01 (−0.20, 0.18)	0.94	
Adjusted <sup>c</sup>	0.04 (−0.16, 0.24)	0.67	0.90	0.08 (−0.12, 0.29)	0.43	0.69	0.01 (−0.18, 0.19)	0.95	0.62
Respective visits									
Birth <sup>d</sup>	0.19 (−0.04, 0.43)	0.11		0.24 (0.01, 0.47)	0.05		0.13 (−0.11, 0.36)	0.28	
3 months <sup>e</sup>	0.05 (−0.18, 0.28)	0.66		0.10 (−0.14, 0.33)	0.42		0.03 (−0.18, 0.25)	0.76	
18 months <sup>e</sup>	0.04 (−0.19, 0.26)	0.76		0.05 (−0.18, 0.29)	0.67		−0.02 (−0.24, 0.20)	0.84	
PFOS pooled 3 and 18 months									
Crude <sup>b</sup>	−0.01 (−0.03, 0.01)	0.46		−0.01 (−0.03, 0.02)	0.62		−0.01 (−0.03, 0.004)	0.12	
Adjusted <sup>c</sup>	−0.01 (−0.03, 0.01)	0.53	0.84	−0.004 (−0.03, 0.02)	0.77	0.85	−0.01 (−0.03, 0.01)	0.31	0.18
Respective visits									
Birth <sup>d</sup>	0.02 (−0.01, 0.04)	0.15		0.03 (0.01, 0.05)	0.02		0.001 (−0.02, 0.02)	0.95	
3 months <sup>e</sup>	−0.01 (−0.03, 0.02)	0.63		−0.005 (−0.03, 0.016)	0.63		−0.02 (−0.04, 0.004)	0.13	
18 months <sup>e</sup>	−0.01 (−0.03, 0.02)	0.52		−0.003 (−0.03, 0.020)	0.78		−0.001 (−0.02, 0.02)	0.93	
PFOA pooled 3 and 18 months									
Crude <sup>b</sup>	0.03 (−0.03, 0.08)	0.35		0.05 (−0.003, 0.11)	0.06		−0.01 (−0.06, 0.04)	0.73	
Adjusted <sup>c</sup>	0.04 (−0.01, 0.10)	0.14	0.42	0.07 (0.01, 0.13)	0.02	0.43	0.01 (−0.04, 0.07)	0.62	0.06
Respective visits									
Birth <sup>d</sup>	0.02 (−0.04, 0.09)	0.50		0.05 (−0.01, 0.12)	0.10		−0.02 (−0.08, 0.05)	0.59	
3 months <sup>e</sup>	0.03 (−0.03, 0.10)	0.29		0.06 (−0.004, 0.12)	0.07		−0.01 (−0.07, 0.05)	0.72	
18 months <sup>e</sup>	0.06 (−0.01, 0.13)	0.09		0.09 (0.02, 0.16)	0.02		0.05 (−0.02, 0.12)	0.13	
PFNA pooled 3 and 18 months									
Crude <sup>b</sup>	0.13 (−0.04, 0.30)	0.13		0.20 (0.03, 0.36)	0.02		0.03 (−0.12, 0.18)	0.70	
Adjusted <sup>c</sup>	0.18 (0.02, 0.34)	0.03	0.74	0.24 (0.08, 0.41)	<0.01	0.82	0.09 (−0.06, 0.24)	0.25	0.62
Respective visits									
Birth <sup>d</sup>	−0.04 (−0.23, 0.15)	0.65		0.03 (−0.16, 0.21)	0.79		−0.10 (−0.30, 0.09)	0.28	
3 months <sup>e</sup>	0.17 (−0.01, 0.35)	0.07		0.23 (0.05, 0.42)	0.01		0.07 (−0.11, 0.24)	0.45	
18 months <sup>e</sup>	0.20 (0.01, 0.39)	0.04		0.25 (0.06, 0.45)	0.01		0.12 (−0.07, 0.30)	0.22	
PFDA pooled 3 and 18 months									
Crude <sup>b</sup>	0.33 (−0.10, 0.76)	0.13		0.53 (0.11, 0.96)	0.02		0.03 (−0.37, 0.42)	0.90	
Adjusted <sup>c</sup>	0.42 (0.01, 0.84)	0.05	0.89	0.60 (0.18, 1.02)	<0.01	0.92	0.10 (−0.28, 0.49)	0.60	0.97
Respective visits									
Birth <sup>d</sup>	0.08 (−0.40, 0.56)	0.74		0.20 (−0.27, 0.67)	0.41		−0.07 (−0.55, 0.41)	0.78	
3 months <sup>e</sup>	0.41 (−0.04, 0.87)	0.08		0.61 (0.14, 1.07)	0.01		0.10 (−0.34, 0.54)	0.66	
18 months <sup>e</sup>	0.38 (−0.12, 0.88)	0.12		0.59 (0.07, 1.10)	0.03		0.11 (−0.36, 0.57)	0.65	

Note: BMI, body mass index; CI, confidence interval; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, standard deviation scores.

<sup>a</sup>Interaction between the compound and visit (3 months or 18 months), tested with the likelihood-ratio test.

<sup>b</sup>Mixed modeling (*n* = 600 for BMI SDS and ponderal index SDS, *n* = 596 for waist circumference SDS).

<sup>c</sup>Mixed modeling: Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, sex, visit, and adiposity marker at birth. (*n* = 593 for BMI SDS and ponderal index SDS, *n* = 589 for waist circumference SDS).

<sup>d</sup>Linear regression: Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, and sex. (*n* = 593 for BMI SDS and ponderal index SDS, *n* = 589 for waist circumference SDS).

<sup>e</sup>Mixed modeling: Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, sex, and adiposity marker at birth. (*n* = 593 for BMI SDS and ponderal index SDS; *n* = 589 for waist circumference SDS).

0.40, 1.64] (Table 3). Corresponding estimates for boys were closer to the null (e.g., for PFNA and BMI SDS, 0.10; 95% CI: −0.12, 0.33), but were not significantly different from estimates for girls (*p*<sub>int</sub> 0.07–0.42).

As BF% SDS and lipid concentration SDS were available only at 3 months and 18 months of age, it was not feasible to evaluate differences by visit and also adjust for baseline (3 months of age). By default, we report separate effect estimates for age-specific BF% SDS and lipid concentration SDS only.

PFNA and PFDA were positively associated with BF% SDS at 3 months of age (adjusted  $\beta$  = 0.20; 95% CI: 0.06, 0.34 and  $\beta$  = 0.40; 95% CI: 0.04, 0.75 for 1-ng/mL increases, respectively), but not at 18 months of age (Table 4). Associations between maternal PFAA and BF% were similar in girls and boys (all *p*<sub>int</sub>  $\geq$  0.50) (Table 4).

Maternal PFDA and PFNA were associated with higher total cholesterol SDS in offspring at 18 months (adjusted  $\beta$  = 1.06;

95% CI: 0.08, 2.03 and  $\beta$  = 0.37; 95% CI: −0.05, 0.79 for 1-ng/mL increases, respectively) but not at 3 months of age (Table 5). In addition, there was a positive association between maternal PFDA and TG SDS at 18 months of age (adjusted  $\beta$  = 0.92; 95% CI: −0.11, 1.95 for 1-ng/mL increase). Gender-specific associations between maternal PFAA and lipid outcomes were based on data for 125–126 girls and 133–134 boys at 3 months of age, and 40 girls and 43 boys at 18 months of age; therefore, estimates are imprecise and should be interpreted with caution, especially for outcomes measured at 18 months of age (Table 6). There were no clear differences between girls and boys for associations with lipid outcomes at 3 months of age, with the exception of HDL SDS, which showed inverse associations among girls and positive associations among boys for PFHxS, PFOS, PFNA, and PFDA (*p*<sub>int</sub> = 0.02–0.08). For LDL SDS in offspring at 18 months, associations with PFOA, PFNA, and PFDA were positive for girls and inverse for boys (*p*<sub>int</sub> of 0.01, 0.07,

**Table 3.** Adjusted average difference ( $\beta$ ) in adiposity marker in offspring at 3 and 18 months of age (pooled data) for 1-unit (ng/mL) increase in maternal pregnancy serum PFAA concentration stratified according to sex obtained from random mixed effects linear regression models.

Compound (ng/mL)	BMI SDS			Ponderal index SDS			Waist circumference SDS		
	Girls (n = 275) $\beta$ (95% CI)	Boys (n = 318) $\beta$ (95% CI)	$p_{int}^a$	Girls (n = 275) $\beta$ (95% CI)	Boys (n = 318) $\beta$ (95% CI)	$p_{int}^a$	Girls (n = 274) $\beta$ (95% CI)	Boys (n = 315) $\beta$ (95% CI)	$p_{int}^a$
PFHxS									
Adjusted <sup>b</sup>	0.03 (-0.19, 0.24)	0.13 (-0.34, 0.60)	0.70	0.02 (-0.14, 0.82)	0.34 (-0.14, 0.82)	0.24	0.02 (-0.19, 0.22)	-0.05 (-0.49, 0.39)	0.80
PFOS									
Adjusted <sup>b</sup>	-0.01 (-0.04, 0.02)	-0.004 (-0.03, 0.02)	0.84	-0.01 (-0.04, 0.02)	-0.001 (-0.03, 0.03)	0.64	-0.01 (-0.04, 0.01)	-0.01 (-0.03, 0.02)	0.80
PFOA									
Adjusted <sup>b</sup>	0.06 (-0.02, 0.14)	0.03 (-0.04, 0.11)	0.67	0.09 (0.004, 0.17)	0.06 (-0.02, 0.13)	0.58	-0.01 (-0.09, 0.07)	0.03 (-0.04, 0.11)	0.40
PFNA									
Adjusted <sup>b</sup>	0.26 (0.03, 0.49)	0.10 (-0.12, 0.33)	0.33	0.36 (0.13, 0.59)	0.13 (-0.10, 0.36)	0.16	0.14 (-0.08, 0.35)	0.04 (-0.17, 0.25)	0.52
PFDA									
Adjusted <sup>b</sup>	0.58 (-0.03, 1.19)	0.24 (-0.31, 0.80)	0.42	1.02 (0.40, 1.64)	0.25 (-0.32, 0.82)	0.07	0.07 (-0.50, 0.65)	0.13 (-0.38, 0.64)	0.89

Note: BMI, body mass index; CI, confidence interval; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, standard deviation scores.

<sup>a</sup>Interaction between the compound and sex, tested with the likelihood-ratio test.

<sup>b</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, visit, and adiposity marker at birth.

and <0.01, respectively), whereas associations between the same PFAA and triglyceride SDS at 18 months of age were positive for boys and close to the null for girls (all  $p_{int} < 0.01$ ). PFNA and PFDA were positively associated with total cholesterol SDS at 18 months of age in boys and girls, without significant differences by gender ( $p_{int}$  0.59 and 0.41, respectively). Associations between maternal PFAA and lipid outcomes at 3 months and 18 months of age did not markedly change when additionally adjusted for BMI SDS at the same ages (Tables S6–S7).

## Discussion

Maternal PFNA and PFDA concentrations during pregnancy were associated with offspring having higher BMI SDS and ponderal index SDS at 3 months and 18 months of age, and higher BF% SDS at 3 months of age. PFNA and PFDA concentrations were positively associated with BMI SDS and ponderal index SDS in girls at 3 months and 18 months of age. In boys, corresponding associations were also positive, but estimates were closer to the null, and they were not significantly different from estimates for girls.

In the present study, maternal pregnancy PFNA concentrations were comparable with findings reported in a Spanish study (Manzano-Salgado et al. 2017), whereas prenatal maternal PFOS and PFOA concentrations were generally lower than those reported in previous studies (Andersen et al. 2010; Braun et al. 2016; Maisonet et al. 2012; Mora et al. 2017). These differences may reflect temporal and/or spatial differences in PFAA exposures.

Our results support the study hypothesis that prenatal PFAA exposure may induce metabolic dysfunction resulting in increased markers of adiposity in infancy, and the findings expand and are in line with observations from other infancy studies. Also, for study participants at 3 months of age, a recent Swedish study demonstrated nonsignificant positive associations between maternal concentrations of PFHxS, PFOS, PFNA, and PFOA and weight SDS in infants (Gyllenhammar et al. 2018). Moreover, maternal plasma PFOA concentration was associated with greater weight gain until 6 months of age in Spanish boys, but not in girls (Manzano-Salgado et al. 2017). Maternal serum PFOS concentration was associated with increased BMI SDS and overweight risk in 18-month-old Faroese children (Karlsen et al. 2017) and with increased weight in British girls 20 months of age (Maisonet et al. 2012). It is interesting to note that we found that higher maternal pregnancy PFNA and PFDA concentrations were associated with increased BF% SDS at 3 months of age, but not at 18 months of age. Likewise, a recent American study reported that higher maternal pregnancy concentrations of PFNA and PFOA were associated with greater fat mass in boys at 5 months of age, but they had only one fat mass measurement during infancy (Starling et al. 2019). We still demonstrated positive associations between PFNA and PFDA concentrations and BMI SDS and ponderal index SDS at 18 months of age. This finding may reflect that our markers of adiposity are sensitive to different compartments of body composition, because BMI and ponderal index are proxies for total adiposity, with restricted ability to detect specific changes in fat percentage throughout infancy in comparison with the skinfold thickness BF% marker (Rodríguez et al. 2005). Moreover, early infancy represents a period of rapid changes and unequal growth rates. The postnatal weight gain is primarily due to the increase in the infant's fat mass, which peaks around 6 months of age (Demerath and Fields 2014; Wohlfahrt-Veje et al. 2014). This increase is followed by a slowed growth rate of fat mass in comparison with the increasing growth rate of fat-free mass, resulting in a declined BF% (Demerath and Fields 2014; Wohlfahrt-Veje et al. 2014). This decline in percentage body fat after 6 months in infants could

**Table 4.** Adjusted difference ( $\beta$ ) in body fat % (BF%) in offspring at 3 months and 18 months of age for 1-unit (ng/mL) increase in maternal pregnancy serum PFAA concentration obtained from linear regression models.

Compound (ng/mL)	BF% SDS			$p_{int}^a$
	All $\beta$ (95% CI)	Girls $\beta$ (95% CI)	Boys $\beta$ (95% CI)	
PFHxS				
3 months <sup>b</sup>	0.04 (−0.14, 0.22)	0.04 (−0.15, 0.23)	0.03 (−0.39, 0.44)	0.96
18 months <sup>c</sup>	−0.08 (−0.27, 0.11)	−0.08 (−0.29, 0.12)	−0.07 (−0.53, 0.39)	0.95
PFOS				
3 months <sup>b</sup>	−0.0004 (−0.02, 0.02)	−0.001 (−0.02, 0.02)	−0.003 (−0.02, 0.02)	0.99
18 months <sup>c</sup>	0.01 (−0.01, 0.03)	0.01 (−0.01, 0.04)	0.01 (−0.02, 0.02)	0.69
PFOA				
3 months <sup>b</sup>	0.05 (−0.002, 0.10)	0.06 (−0.03, 0.14)	0.07 (−0.002, 0.13)	0.50
18 months <sup>c</sup>	0.02 (−0.04, 0.07)	0.03 (−0.05, 0.11)	0.002 (−0.08, 0.08)	0.61
PFNA				
3 months <sup>b</sup>	0.20 (0.06, 0.34)	0.20 (0.002, 0.40)	0.19 (−0.004, 0.39)	0.94
18 months <sup>c</sup>	0.05 (−0.12, 0.21)	0.03 (−0.19, 0.26)	0.06 (−0.17, 0.29)	0.89
PFDA				
3 months <sup>b</sup>	0.40 (0.04, 0.75)	0.41 (−0.13, 0.94)	0.39 (−0.09, 0.86)	0.96
18 months <sup>c</sup>	0.10 (−0.33, 0.54)	−0.04 (−0.65, 0.57)	0.24 (−0.37, 0.85)	0.52

Note: BF%, body fat %; CI, confidence interval; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, standard deviation scores.

<sup>a</sup>Interaction between the compound and sex, adjusted as described under <sup>b</sup> or <sup>c</sup>, tested with the Wald test.

<sup>b</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, and sex (not in sex-specific analyses) (All:  $n = 585$ , girls:  $n = 272$ , boys:  $n = 313$ ).

<sup>c</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, sex (not in sex-specific analyses), and BF% at age 3 months (All:  $n = 473$ , girls:  $n = 218$ , boys:  $n = 255$ ).

potentially have attenuated our associations between PFNA and PFDA concentrations and BF% SDS at 18 months of age. In addition, as the infant grows, the impact of prenatal PFAA exposure on the measured markers of adiposity at 18 months of age may decline, and the child's own exposure to environmental factors, such as PFAA, may start to have a greater influence.

We estimated that higher maternal serum PFNA and PFDA concentrations were associated with increased total cholesterol SDS at 18 months of age in girls and boys, but estimates were based on a subset of observations [ $n = 83$  (40 girls and 43 boys)]. Previous reports on associations between PFAA concentrations and lipid metabolism in childhood and adolescence (Frisbee et al. 2010; Geiger et al. 2014; Manzano-Salgado et al. 2017; Zeng et al. 2015) included only a single time point measurement of

lipid metabolism (Frisbee et al. 2010; Geiger et al. 2014; Manzano-Salgado et al. 2017; Zeng et al. 2015) or were of a cross-sectional design (Frisbee et al. 2010; Geiger et al. 2014; Zeng et al. 2015), thus limiting an extensive assessment of dyslipidemia over time. However, a recent prospective Spanish study (Manzano-Salgado et al. 2017) reported that maternal PFHxS concentrations were associated with increased TG SDS in children 4 years of age ( $n = 627$ ). In comparison with our study population, the mean maternal pregnancy PFHxS concentration was two-fold higher in the Spanish study (Manzano-Salgado et al. 2017).

Evidence from experimental studies suggests that PFAA may affect complex metabolic pathways relevant to childhood adiposity and lipid metabolism by interfering with peroxisome proliferator-

**Table 5.** Adjusted difference ( $\beta$ ) in lipid outcome in offspring at 3 months and 18 months of age for 1-unit (ng/mL) increase in maternal pregnancy serum PFAA concentration obtained from linear regression models.

Compound (ng/mL)	Total cholesterol SDS		LDL SDS		HDL SDS		Triglyceride SDS	
	$\beta$ (95% CI)	$p$	$\beta$ (95% CI)	$p$	$\beta$ (95% CI)	$p$	$\beta$ (95% CI)	$p$
PFHxS								
3 months <sup>a</sup>	−0.08 (−0.33, 0.17)	0.54	0.01 (−0.24, 0.26)	0.93	−0.08 (−0.34, 0.18)	0.55	0.18 (−0.07, 0.44)	0.16
18 months <sup>b</sup>	−0.06 (−0.32, 0.21)	0.68	−0.06 (−0.35, 0.22)	0.65	0.02 (−0.23, 0.27)	0.89	−0.24 (−0.51, 0.04)	0.09
PFOS								
3 months <sup>a</sup>	0.01 (−0.03, 0.04)	0.66	0.02 (−0.02, 0.05)	0.41	0.003 (−0.04, 0.04)	0.89	−0.01 (−0.05, 0.02)	0.50
18 months <sup>b</sup>	0.02 (−0.05, 0.08)	0.60	0.04 (−0.03, 0.10)	0.28	−0.03 (−0.09, 0.02)	0.26	0.001 (−0.06, 0.07)	0.99
PFOA								
3 months <sup>a</sup>	−0.08 (−0.18, 0.03)	0.14	−0.02 (−0.13, 0.08)	0.65	−0.07 (−0.17, 0.04)	0.22	−0.03 (−0.13, 0.08)	0.61
18 months <sup>b</sup>	0.07 (−0.07, 0.22)	0.32	0.04 (−0.12, 0.20)	0.63	−0.05 (−0.19, 0.09)	0.48	0.10 (−0.05, 0.26)	0.19
PFNA								
3 months <sup>a</sup>	−0.15 (−0.44, 0.13)	0.30	−0.06 (−0.35, 0.23)	0.67	−0.04 (−0.34, 0.26)	0.78	−0.10 (−0.39, 0.20)	0.52
18 months <sup>b</sup>	0.37 (−0.05, 0.79)	0.08	0.24 (−0.21, 0.70)	0.29	−0.001 (−0.41, 0.40)	0.98	0.18 (−0.26, 0.63)	0.41
PFDA								
3 months <sup>a</sup>	−0.23 (−0.90, 0.43)	0.49	−0.05 (−0.73, 0.62)	0.87	0.004 (−0.68, 0.69)	0.99	−0.21 (−0.88, 0.47)	0.55
18 months <sup>b</sup>	1.06 (0.08, 2.03)	0.03	0.64 (−0.43, 1.71)	0.24	−0.13 (−1.08, 0.83)	0.79	0.92 (−0.11, 1.95)	0.08

Note: CI, confidence interval; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, standard deviation scores.

<sup>a</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, and sex ( $n = 260$  for total cholesterol SDS, HDL SDS, and triglyceride SDS; and  $n = 258$  for LDL SDS).

<sup>b</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, sex, and lipid outcome at age 3 months ( $n = 83$  for all markers of lipid metabolism).

**Table 6.** Adjusted difference ( $\beta$ ) in lipid concentration in offspring at 3 months and 18 months of age for 1-unit (ng/mL) increase in maternal pregnancy serum PFAA concentration stratified according to sex, obtained from linear regression models.

Compound (ng/mL)	Total cholesterol SDS				LDL SDS				HDL SDS				Triglyceride SDS				
	Girls		Boys		Girls		Boys		Girls		Boys		Girls		Boys		
	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	$\beta$ (95% CI)	$p_{int}^a$	
PFHxS																	
3 months <sup>b</sup>	-0.11 (-0.37, 0.16)	0.53	0.13 (-0.58, 0.85)	0.53	0.05 (-0.22, 0.32)	0.39	-1.19 (-0.46, 0.08)	0.73 (0.001, 1.47)	0.02	0.21 (-0.06, 0.48)	0.02	-0.02 (-0.21, 0.16)	0.48	-0.02 (-0.21, 0.16)	0.48	-0.02 (-0.21, 0.16)	0.48
18 months <sup>c</sup>	-0.05 (-0.32, 0.21)	0.95	-0.10 (-1.41, 1.21)	0.95	-0.08 (-0.37, 0.21)	0.53	0.01 (-0.25, 0.27)	0.15 (-1.10, 1.41)	0.82	-0.22 (-0.50, 0.06)	0.82	-0.62 (-1.95, 0.70)	0.56	-0.62 (-1.95, 0.70)	0.56	-0.62 (-1.95, 0.70)	0.56
PFOS																	
3 months <sup>b</sup>	0.01 (-0.04, 0.06)	0.99	0.01 (-0.04, 0.06)	0.99	0.04 (-0.01, 0.09)	0.22	-0.03 (-0.09, 0.02)	0.04 (-0.02, 0.09)	0.07	-0.01 (-0.06, 0.04)	0.07	-0.02 (-0.07, 0.04)	0.86	-0.02 (-0.07, 0.04)	0.86	-0.02 (-0.07, 0.04)	0.86
18 months <sup>c</sup>	0.01 (-0.07, 0.09)	0.67	0.03 (-0.06, 0.12)	0.67	0.04 (-0.04, 0.13)	0.76	-0.07 (-0.14, 0.01)	0.01 (-0.08, 0.09)	0.18	-0.04 (-0.12, 0.05)	0.18	0.05 (-0.05, 0.14)	0.18	0.05 (-0.05, 0.14)	0.18	0.05 (-0.05, 0.14)	0.18
PFOA																	
3 months <sup>b</sup>	-0.07 (-0.22, 0.08)	0.88	-0.08 (-0.23, 0.06)	0.88	-0.02 (-0.17, 0.13)	0.99	-0.11 (-0.26, 0.05)	-0.03 (-0.18, 0.12)	0.48	0.01 (-0.14, 0.16)	0.48	-0.06 (-0.21, 0.08)	0.52	-0.06 (-0.21, 0.08)	0.52	-0.06 (-0.21, 0.08)	0.52
18 months <sup>c</sup>	0.14 (-0.03, 0.31)	0.10	-0.12 (-0.39, 0.16)	0.10	0.16 (-0.02, 0.34)	0.01	-0.05 (-0.22, 0.12)	-0.05 (-0.32, 0.21)	0.98	-0.02 (-0.19, 0.15)	0.98	0.43 (0.16, 0.70)	<0.01	0.43 (0.16, 0.70)	<0.01	0.43 (0.16, 0.70)	<0.01
PFNA																	
3 months <sup>b</sup>	-0.08 (-0.49, 0.33)	0.64	-0.22 (-0.62, 0.18)	0.64	0.08 (-0.34, 0.49)	0.35	-0.35 (-0.77, 0.08)	0.25 (-0.16, 0.67)	0.05	0.09 (-0.33, 0.51)	0.05	-0.28 (-0.69, 0.13)	0.22	-0.28 (-0.69, 0.13)	0.22	-0.28 (-0.69, 0.13)	0.22
18 months <sup>c</sup>	0.40 (-0.07, 0.87)	0.59	0.25 (-0.68, 1.19)	0.59	0.45 (-0.05, 0.95)	0.07	-0.11 (-0.58, 0.36)	0.36 (-0.56, 1.29)	0.38	-0.11 (-0.59, 0.36)	0.38	1.39 (0.44, 2.35)	<0.01	1.39 (0.44, 2.35)	<0.01	1.39 (0.44, 2.35)	<0.01
PFDA																	
3 months <sup>b</sup>	-0.39 (-1.43, 0.66)	0.71	-0.12 (-1.00, 0.75)	0.71	-0.01 (-1.07, 1.04)	0.92	-0.83 (-1.96, 0.29)	0.64 (-0.56, 1.85)	0.08	0.002 (-0.97, 0.97)	0.08	-1.32 (-3.18, 0.49)	0.21	-1.32 (-3.18, 0.49)	0.21	-1.32 (-3.18, 0.49)	0.21
18 months <sup>c</sup>	1.29 (0.17, 2.42)	0.41	0.27 (-1.86, 2.40)	0.41	1.51 (0.34, 2.68)	<0.01	-1.36 (-1.49, 0.77)	0.61 (-1.49, 2.71)	0.43	-0.10 (-1.18, 0.99)	0.43	4.44 (2.37, 6.53)	<0.01	4.44 (2.37, 6.53)	<0.01	4.44 (2.37, 6.53)	<0.01

Note: CI, confidence interval; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, standard deviation scores.

<sup>a</sup>Interaction between the compound and sex, adjusted as described under <sup>b</sup> or <sup>c</sup>, tested with the Wald test.

<sup>b</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, and smoking (For total cholesterol SDS, HDL SDS, and triglyceride SDS  $n = 126$  girls and  $n = 134$  boys; and for LDL SDS  $n = 125$  girls and  $n = 133$  boys).

<sup>c</sup>Adjusted for maternal age, parity, prepregnancy BMI, prepregnancy BMI<sup>2</sup>, educational level, smoking, and lipid outcome at age 3 months (For all lipid outcomes  $n = 40$  girls, and  $n = 43$  boys).

activated receptors (PPAR), cortisol and thyroid metabolism, and sex hormone receptors (Buhrke et al. 2013; Wolf et al. 2012; Ye et al. 2012; Yu et al. 2009; Zhao et al. 2011). Rodent and human *in vitro* studies demonstrated that PFAA are able to activate the PPAR $\alpha$  and PPAR $\gamma$ , which are involved in lipid and glycogen metabolism (Buhrke et al. 2013; Wolf et al. 2008; Wolf et al. 2012). Non-PPAR-mechanistic pathways have been suggested, because *in vitro* studies in both rat and human cells have shown PFAA as potent inhibitors of both 11 $\beta$ -hydroxysteroid dehydrogenase 1 (Ye et al. 2012) and 2 (Zhao et al. 2011), thus potentially influencing available cortisol to the fetus. In a recent prospective cohort study, we demonstrated that PFOS concentration in early pregnancy was significantly inversely associated with diurnal urinary concentrations of cortisone in late pregnancy, indicating reduced activity of 11 $\beta$ -hydroxysteroid dehydrogenase 2 (Dreyer et al. 2020). A rodent study (Yu et al. 2009) indicated that both prenatal exposure and postnatal PFOS exposure were associated with decreased thyroxine levels (T4) in offspring, which is linked to decreased basal metabolic rate and increased BMI in humans (Knudsen et al. 2005). Finally, findings from this study and others (Halldorsson et al. 2012; Høyser et al. 2015; Manzano-Salgado et al. 2017; Mora et al. 2017) provide some evidence of differences in associations by sex in regard to the association between *in utero* PFAA exposure and anthropometry measurements, where females appear to be more susceptible (Halldorsson et al. 2012; Høyser et al. 2015; Mora et al. 2017). However, we also found evidence of positive associations in girls between PFAA exposure and LDL SDS at 18 months of age that were accompanied by even stronger inverse associations in boys; however, lipid data should be interpreted with caution due to the small sample size by gender at 18 months of age. An *in vitro* study (Kjeldsen and Bonefeld-Jørgensen 2013) on human cells demonstrated that PFAA induced estrogenic and antiandrogenic activities, but the sensitivity to the effects of PFAA between the two sexes may hypothetically differ due to the natural androgen-estrogen homeostasis (Jensen et al. 2020; Kuijper et al. 2013).

The major strength of the study is the prospective follow-up design, with a sizable number of mother-child pairs for most of the outcomes. Maternal PFAA concentrations were determined in early pregnancy, and we presume that the results reflect the PFAA concentration during much of the pregnancy due to the long half-lives of PFAA (ATSDR 2018). Three trained health care professionals blinded to the PFAA concentrations carried out the consecutive clinical examinations, hence minimizing misclassification bias with respect to the outcomes. Repeated objective standardized markers of adiposity and lipid metabolism were obtained in offspring.

The study also has some limitations. Included mothers were more often nulliparous, of European origin, smoked less, and had a higher prepregnancy BMI in comparison with the rest of the women in the OCC. Potential confounding by parity, smoking, and prepregnancy BMI was addressed by adjustment in the analyses. However, residual confounding by other covariates related to prenatal PFAA concentrations and markers of adiposity and lipid metabolism in infancy cannot be dismissed; these covariates include family cholesterol levels and exposure to other unmeasured correlated endocrine disrupting chemicals during pregnancy and in infancy. Moreover, the possibility of unmeasured or residual confounding by diet cannot be ruled out, because diet may be a common cause of both PFAA exposure (Vestergren et al. 2012) and markers of adiposity and lipids in infancy (Emmett and Jones 2015). Unfortunately, data on maternal and child diet were not available in this study. The observed trends of reduced maternal pregnancy PFAA concentrations with increasing maternal prepregnancy BMI could suggest that fat biopsies may be a matrix

of interest, even though PFAA do not tend to accumulate in fatty compartments (Pérez et al. 2013). Fat biopsies were not collected in the present study; however, that information may be a focus for future studies. Because the study hypothesis was tested across several markers of adiposity and lipid metabolism, multiple comparison bias should be considered. We acknowledge the variability in the magnitude of the associations between PFAA and markers of lipid metabolism. The variability may depend on age at blood sampling, infant sex, and other intrinsic factors, such as hormones. As concentrations of androgens and gonadotropins differ between girls and boys during minipuberty in early childhood (Jensen et al. 2020; Kuiri-Hänninen et al. 2014), it cannot be excluded that the investigated associations between PFAA and markers of lipid metabolism may be influenced by sex differences in hormones. In addition, the results on maternal PFAA concentrations and markers of offspring lipid metabolism at 18 months of age, especially the gender-specific estimates, warrants cautious interpretation due to the small sample size limiting the statistical precision. However, the study had an exploratory approach in the analysis of associations between maternal pregnancy serum PFAA concentrations and infancy metabolism investigated from multiple angles, including adiposity measurements and lipid status.

Collectively, our longitudinal data suggest that fetal life is a sensitive period for exposure to maternal PFAA concentrations with future implications on markers of adiposity and lipid metabolism already in infancy. Childhood obesity is increasing dramatically (Kumar and Kelly 2017); in addition, childhood overweight and abnormal lipid metabolism are predictive of obesity (Singh et al. 2008) and cardiovascular risk factors (Juonala et al. 2011; Nicklas et al. 2002) in adulthood, including dyslipidemia, hypertension, and type 2 diabetes, conditions considered to be major challenges to human health (Unnikrishnan et al. 2017).

## Conclusions

Maternal PFOA, PFNA, and PFDA concentrations in pregnancy serum were associated with increased BMI SDS and ponderal index SDS in the offspring at 3 months and 18 months of age, and with higher BF% SDS at 3 months. The PFDA concentration was also associated with increased total cholesterol SDS at age 18 months of age.

The possible impact of developmental PFAA exposure on metabolic dysfunction in childhood and in later life deserves attention.

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