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Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study

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Abstract

Background—Perfluoroalkyl substances (PFASs) are widespread and persistent environmental pollutants. Previous studies, primarily among non-pregnant individuals, suggest positive associations between PFAS levels and certain blood lipids. If there is a causal link between PFAS concentrations and elevated lipids during pregnancy, this may suggest a mechanism by which PFAS exposure leads to certain adverse pregnancy outcomes, including preeclampsia.

Methods—This cross-sectional analysis included 891 pregnant women enrolled in the Norwegian Mother and Child (MoBa) Cohort Study in 2003–2004. Non-fasting plasma samples were obtained at mid-pregnancy and analyzed for nineteen PFASs. Total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, and triglycerides were measured in plasma. Linear regression was used to quantify associations between each PFAS exposure and each lipid outcome. A multiple PFAS model was also fitted.

Results—Seven PFASs were quantifiable in >50% of samples. Perfluorooctane sulfonate (PFOS) concentration was associated with total cholesterol, which increased 4.2 mg/dL per interquartile shift (95% CI=0.8, 7.7) in adjusted models. Five of the seven PFASs studied were positively associated with HDL cholesterol, and all seven had elevated HDL associated with the

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highest quartile of exposure. Perfluoroundecanoic acid showed the strongest association with HDL: HDL increased 3.7 mg/dL per interquartile shift (95% CI=2.5, 4.9).

Conclusion—Plasma concentrations of PFASs were positively associated with HDL cholesterol, and PFOS was positively associated with total cholesterol in this sample of pregnant Norwegian women. While elevated HDL is not an adverse outcome per se, elevated total cholesterol associated with PFASs during pregnancy could be of concern if causal.

Keywords

The Norwegian Mother and Child Cohort Study; MoBa; perfluoroalkyl substances; perfluorooctanoic acid; perfluorooctane sulfonate

1. Introduction

Perfluoroalkyl substances (PFASs[1]) are persistent environmental contaminants detectable in the blood of human populations worldwide (Fromme et al., 2009; Kannan et al., 2004). PFASs are used in the manufacturing process of numerous industrial and consumer products, including surface treatments for fabrics and food packaging, fire-fighting foam, surfactants and pesticide additives (Giesy and Kannan, 2002). They may enter the environment through release from industrial sources or through consumer products (Prevedouros et al., 2006).

Sources of exposure to PFASs in the general population may include food, drinking water, house dust, air, and breast milk for infants (Fromme et al., 2010; Haug et al., 2011). PFASs are highly resistant to degradation in the environment or metabolism in the body (Fromme et al., 2009; Lau et al., 2007). The elimination half-lives of perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorooctanoate (PFOA) from human serum have been estimated to be 4.8 years, 7.3 years, and 2.3 years, respectively (Bartell et al., 2010; Olsen et al., 2007). The geometric mean elimination half-lives of PFOS and PFHxS were estimated using serial serum measurements collected over three to five years in 26 former fluorochemical workers who had retired within four years of the beginning of the study (Olsen et al., 2007). The median PFOA elimination half-life was estimated using serial serum measurements from 200 individuals previously exposed to PFOA-contaminated drinking water (Bartell et al., 2010). In that study, up to six serum samples were collected during the 15 months following the installation of a granular activated carbon water filtration system (Bartell et al., 2010). Both studies suggested that subject-specific elimination rates may vary considerably (Bartell et al., 2010; Olsen et al., 2007). PFASs are not lipophilic, and while the tissue distribution in humans is unknown, animal studies suggest that PFASs likely reside primarily in the liver, kidneys, and blood (Kennedy et al., 2004).

Animal studies have demonstrated a number of adverse health effects associated with high-dose oral exposure to PFOS and PFOA, including hepatotoxicity, tumorigenesis, immunotoxicity, and developmental toxicity (DeWitt et al., 2009; Lau et al., 2007). Some adverse effects in animals are believed to be mediated through the binding of PFASs to the peroxisome proliferator-activated receptor alpha (PPARα), which plays a role in the regulation of lipid and glucose metabolism in humans and rodents (Abbott et al., 2007).

[1]*Abbreviations*: BMI, body mass index; CI, confidence interval; DAG, directed acyclic graph; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; LOQ, limit of quantification; MBRN, Medical Birth Registry of Norway; MoBa, Norwegian Mother and Child Cohort Study; PFASs, perfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid.

In epidemiologic studies of highly exposed populations as well as populations with background levels of exposure, PFAS concentrations have been associated with altered lipid profiles that are consistent with increased risk of cardiovascular disease. These lipid alterations have included elevated plasma cholesterol and triglycerides (Nelson et al., 2010; Olsen et al., 2003; Steenland et al., 2009). The two PFASs typically present in human blood at the highest concentrations, PFOS and PFOA, have been studied most extensively, but recently researchers have begun to examine the lipid correlates of PFASs present at lower concentrations, including PFHxS and perfluorononanoic acid (PFNA) (Fisher et al., 2013; Nelson et al., 2010). The observed associations between PFASs and lipid concentrations have not been consistent across studies or across populations, possibly owing to the different age and sex distributions of the groups studied, the different sizes of the study populations, or the different settings (occupational versus general populations).

To our knowledge, no previous studies of the associations between PFAS levels and plasma lipid concentrations have been conducted among pregnant women. The relation between PFASs and lipids during pregnancy is particularly important to quantify for the following reasons: 1) environmental contaminants present in maternal blood may have adverse effects on both the mother and developing fetus; 2) pregnant women differ in their relative and absolute lipid concentrations (Piechota and Staszewski, 1992), and may show different associations between PFASs and lipids as compared with non-pregnant women; and 3) altered plasma lipids during pregnancy, particularly elevated plasma triglycerides, are associated with a number of adverse outcomes, including preeclampsia (Sattar et al., 1997a) and pregnancy-induced hypertension (Vrijkotte et al., 2012).

A retrospective study of pregnancy outcomes among women in Ohio and West Virginia exposed to PFOA-contaminated drinking water found elevated odds of preeclampsia associated with higher levels of PFOA (Savitz et al., 2012). If pregnant women with higher PFAS levels demonstrate the same altered lipid patterns previously described in non-pregnant populations, then this may suggest a mechanism by which plasma PFAS concentrations could be linked to an increased risk of preeclampsia. In particular, elevations in triglycerides and certain types of low-density lipoprotein (LDL) particles are considered “pro-atherogenic” during pregnancy and may promote oxidative stress and endothelial damage, leading to preeclampsia (Llurba et al., 2005).

We therefore measured cross-sectional associations between PFAS concentrations at midpregnancy and the levels of the following lipid parameters: total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. The purpose of this study was to increase understanding of the physiologic correlates of plasma PFAS concentration during pregnancy and to evaluate a possible mechanism of any associations that may exist between plasma PFAS concentrations and adverse pregnancy outcomes, including preeclampsia.

2. Materials and Methods

2.1. Cohort description and eligibility criteria

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2006; Nilsen et al., 2009; Ronningen et al., 2006). The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. Participants were recruited from all over Norway in 1999–2008. In total, 39% of invited women participated. Informed consent was obtained from each MoBa participant upon recruitment. At the time of enrollment, women provided information via questionnaire regarding a number of demographic and lifestyle characteristics, as well as events in their

reproductive and medical history. Blood samples were obtained from the mother during pregnancy (median = 18 weeks of gestation). Data obtained in MoBa were linked to the Medical Birth Registry of Norway (Irgens, 2000). Further details may be found at www.fhi.no/morogbarn. The current study is based on version 4.301 of the quality-assured data files released for research.

The current analysis uses data that were previously collected for a case-control study of PFASs and subfecundity (Whitworth et al., 2012). For the previous study, 400 pregnancies were randomly selected from all eligible MoBa participants who met the criteria for subfecundity (defined as self-reported time to pregnancy of greater than 12 months), and 550 pregnancies were randomly selected from all eligible MoBa participants who reported a time to pregnancy of any duration. To be eligible for selection into the previous study, women must have enrolled in MoBa in 2003–2004, delivered a live-born child, provided a mid-pregnancy plasma sample, and provided complete information about time to pregnancy on the enrollment questionnaire.

From the 950 participants in the previous study, 891 women (94%) with complete information on covariates and outcomes of interest were included in the present analysis. Each of the individual covariates had missing data for <3% of participants. Participants ranged in age from 19 to 44 (Table 1). Half of the women had no previous live births or stillbirths (50%), while 3% had three or more previous pregnancies. A total of 8% reported smoking during pregnancy. The range of gestational age at the time of blood draw was from 12 weeks to 37 weeks; however, the majority of women (99%) provided plasma samples during their second trimester of pregnancy (14–26 weeks' gestation), and 73% of participants provided plasma samples between 17 and 20 weeks of gestation.

2.2. Collection and storage of plasma samples

At the time of study enrollment, maternal non-fasting blood samples were collected in EDTA tubes at hospitals and maternity units across Norway and shipped at ambient temperature to the MoBa biorepository in Oslo. Most samples were received and processed the day after collection (Ronningen et al., 2006). At the biorepository, plasma was separated, aliquoted, and stored at −80 degrees Celsius. Changes in PFAS concentrations in transit are believed to be negligible, as PFASs are chemically stable (Fromel and Knepper, 2010), and a recent study showed no evidence of change over time in concentrations of four PFASs in serum maintained at room temperature for 10 days (Kato et al., 2013). Lipid concentrations are also expected to be relatively unchanged during shipping; a previous study demonstrated that lipid measurements changed by only a small percentage (<7% for LDL, <4% for HDL, total cholesterol, and triglycerides) when whole blood was kept at room temperature (21 degrees Celsius) in EDTA tubes for up to 7 days (Clark et al., 2003).

2.3. Exposure measurement

Concentrations (ng/mL) of nineteen PFASs were measured in maternal plasma using high-performance liquid chromatography/tandem mass spectrometry at the Norwegian Institute of Public Health. Calibration solutions were prepared in serum from newborn calves using PFAS standards purchased from Wellington Laboratories (Guelph, Ontario, Canada). The calibration samples contained concentrations of PFASs ranging from 0.050 to 75 ng/mL serum for all analytes. In brief, 150 μL of plasma from each participant or matrix-matched calibration solution was transferred to a centrifugation tube and internal standards and methanol were added and mixed. The samples were then centrifuged and the supernatant was transferred to a glass autosampler vial, and 0.1 M formic acid was added and mixed. The extracts were analyzed by injection of 400 μL on a column switching liquid chromatography system coupled to a triple quadrupole mass spectrometer. A Betasil C8

column (10 mm × 4 mm × 5 μm particles) from Thermo Scientific was used as the on-line SPE column and a Betasil C8 (50 mm × 2.1 mm × 3 μm particles) from Thermo Scientific as the analytical column. The limit of quantification (LOQ) was 0.05 ng/mL for all PFASs, except for perfluorobutanoic acid (0.1 ng/mL), perfluorotetradecanoate (0.2 ng/mL) and perfluorodecane sulfonate (0.2 ng/mL). Further details on the analytical method are described elsewhere (Haug et al., 2009a).

Statistical analyses were restricted to the seven PFASs quantifiable in at least 50% of samples: perfluorooctane sulfonate (PFOS), perfluoroheptane sulfonate (PFHpS), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA). The method blanks analyzed with each batch of samples did not contain any of the PFASs above the LOQ. For quantification of PFOS, the total area of linear and branched isomers was integrated (peaks not chromatographically separated). A total of 50 blinded, pooled specimens were analyzed in the same batches as the sample specimens for quality assurance/quality control (QA/QC) purposes. Inter-assay coefficients of variation were calculated for each PFAS and are shown in Table 2.

2.4. Outcome measurement

Plasma lipid parameters (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) were measured in the same mid-pregnancy, non-fasting plasma sample that was used to evaluate PFAS concentrations. All analytes were measured with an Olympus AU400e Clinical Chemistry Analyzer at the National Institute of Environmental Health Sciences, using reagents from Beckman Coulter. The method used to measure LDL was direct enzymatic (N-geneous® LDL-ST cholesterol reagent). Inter-assay coefficients of variation were calculated for all lipid parameters based on the 50 QA/QC samples.

2.5. Covariates

Information on maternal characteristics was derived from the MoBa baseline questionnaire and from the Medical Birth Registry of Norway (MBRN). Variables considered to be potential confounders based on the prior literature were: maternal age (Chateau-Degat et al., 2010; Fisher et al., 2013; Nelson et al., 2010; Steenland et al., 2009), pre-pregnancy body mass index (BMI) (Fei et al., 2007), nulliparous or most recent interpregnancy interval (Fei et al., 2007; Whitworth et al., 2012), duration of breastfeeding most recent child (Whitworth et al., 2012), maternal years of education (Fisher et al., 2013; Steenland et al., 2009), current smoking at mid-pregnancy (Chateau-Degat et al., 2010; Fisher et al., 2013; Nelson et al., 2010; Steenland et al., 2009), gestational weeks at blood draw (Fei et al., 2007), and amount of oily fish consumed daily at the time of the mid-pregnancy questionnaire (Brantsaeter et al., 2013; Chateau-Degat et al., 2010). Weight gain (kg) from pre-pregnancy to mid-pregnancy was calculated by subtracting the self-reported pre-pregnancy weight from the self-reported current weight on the MoBa baseline questionnaire.

Additionally, for models with HDL cholesterol as the outcome, adjustment for plasma albumin concentration (g/dL; quartiles) was considered due to the positive correlation observed between HDL and plasma albumin. Albumin was measured in the mid-pregnancy maternal plasma sample with an Olympus AU400e Clinical Chemistry Analyzer at the National Institute of Environmental Health Sciences, using reagents from Beckman Coulter. The method of measurement was direct and utilized a neutral buffered solution of bromocresol green as a dye binding indicator.

2.6. Statistical analysis

Weighted multiple linear regression was used to estimate the association between each PFAS concentration and each lipid outcome. Weights were based on the inverse probability of selection into the original case-control study (Whitworth et al., 2012), as described in Richardson et al. (2007). A sensitivity analysis was performed to examine whether restricting to the women who were selected without regard to subfecundity (the 'base sample') would produce results that closely resembled the results of the weighted analysis.

Concentrations of PFASs were treated in two ways for the purposes of analysis: (1) as quartiles, with the lowest quartile serving as the referent category (with the exception of PFDA, which was categorized only at or above versus below the median due to >25% of values below the limit of quantification) and (2) as natural-log transformed continuous variables to assess linear trends. In models using quartiles of exposure, beta-coefficients represent the change in lipid parameter (mg/dL or ln-mg/dL) associated with each of the upper quartiles, relative to the lowest quartile of PFAS concentration. In models using natural log-transformed continuous exposure variables, the beta-coefficients represent the change in lipid concentration (mg/dL or ln-mg/dL) associated with each natural log-unit increase in each PFAS. Additionally, the change in lipid concentration (mg/dL or ln-mg/dL) associated with an interquartile-range (IQR) shift in each continuous ln-PFAS (from the 25^{th} percentile to the 75^{th} percentile of the observed exposure distribution) is reported. For PFDA, because more than 25% of values were below the LOQ, the IQR was estimated from the observed portion of the log-normal distribution. For all PFASs, values below the LOQ were replaced by the expected value of the log-normal distribution, given that the value was below the limit of quantification; this was calculated as the mean of values below the limit of quantification randomly drawn from the estimated log-normal distribution (Richardson and Ciampi, 2003).

Each lipid parameter was treated as a continuous outcome variable in a separate model with a single PFAS exposure variable. Plasma triglycerides were natural-log transformed in order to meet the modeling assumption of normally distributed residuals. For simplicity and comparability, models for all four lipid parameters were adjusted for the same covariate set. Covariates were selected through the construction of a directed acyclic graph (DAG) representing the existing literature, and the identification of a minimally sufficient set of variables to control confounding. The DAG was primarily based on expected confounders of the PFAS-HDL association but was also generalizable to the other lipid outcomes. The minimally sufficient adjustment set was identified using DAGitty v1.0 (www.dagitty.net).

All covariates were modeled as categorical variables to allow for non-linear associations. The categorization of covariates was as follows: maternal age (<24, 25–29, 30–34, >=35 years); pre-pregnancy BMI (<25, 25–29.99, >=30 kg/m^2); maternal education (less than high school, completed high school, some college, 4 or more years of college); smoking at mid-pregnancy (yes/no), oily fish consumed (0–3.0, 3.1–7.5, 7.6–14.9, 15–100 g/day); gestational age at blood draw (12–16, 17–18, 19–20, 21–37 weeks); nulliparous or most recent interpregnancy interval (nulliparous, 4–23, 24–47, 48–245 months); and breastfeeding duration in previous pregnancy (nulliparous or <1, 1–5, 6–11, 12–17, 18–36 months). Subjects were excluded from the analysis if they had missing values for any modeled covariates. Additionally, a sensitivity analysis was performed with the inclusion of weight gain (kg) from pre-pregnancy to mid-pregnancy as a continuous covariate, although this variable was not part of the original DAG.

Spearman rank-order correlations were calculated between each pair of PFASs. In order to explore the possible influence of confounding by other, correlated PFASs in single-pollutant models, a multiple-pollutant model was estimated for HDL cholesterol. In the multiple-

pollutant model, all seven PFASs quantifiable in >50% of samples were included in the same model as natural-log transformed continuous variables. The variance inflation factors for each of the exposures in this model were examined to assess multicollinearity. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

3. Results and Discussion

3.1. Measured PFAS concentrations compared to previous studies

Of the nineteen PFASs measured, only seven were quantifiable in greater than 50% of samples: PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS, and PFOS. Further analyses were restricted to these seven compounds. PFDA was quantifiable in 70% of samples, while the six other PFASs were quantifiable in at least 88% of samples (Table 2). PFOS and PFOA were quantifiable in 100% of samples. The highest median concentration was observed for PFOS (13.03 ng/mL), followed by PFOA (2.25 ng/mL), then PFHxS (0.60 ng/mL), PFNA (0.39 ng/mL), PFUnDA (0.22 ng/mL), PFHpS (0.13 ng/mL), and finally PFDA (0.09 ng/mL). The median plasma concentrations of the seven PFASs in this study were comparable to reported serum levels from 2006 in Norway (Haug et al., 2009b) and somewhat lower than reported serum levels from the United States in the same year (Kato et al., 2011). Plasma and serum measurements of PFASs have been shown to be approximately equal for a particular subject at a given time (Ehresman et al., 2007). Therefore the PFAS concentrations measured in the current study are comparable to the magnitude of exposure observed in previous studies of background-exposed populations.

A number of the PFASs demonstrated moderate to high pairwise correlations (Table 3). The most strongly correlated PFASs were PFNA and PFDA (ρ=0.71). The least correlated PFASs among the seven were PFUnDA and PFHpS (ρ=0.17). In general, the sulfonates (PFHxS, PFHpS, PFOS) were relatively highly correlated with one another (ρ=0.53–0.60) while the pairwise correlations among the carboxylates (PFOA, PFNA, PFDA, PFUnDA) were more variable (ρ=0.26–0.71).

3.2. Distribution of lipid outcome variables

The median plasma concentration for total cholesterol was 211 mg/dL, for HDL was 67 mg/dL, for LDL was 125 mg/dL, and for triglycerides was 124 mg/dL (Table 4). The distribution of plasma total cholesterol, HDL cholesterol, and LDL cholesterol was approximately normal while the distribution of plasma triglycerides demonstrated positive skew. Triglycerides were therefore natural-log transformed in subsequent analyses. Two implausibly low values for HDL cholesterol (2.5 mg/dL) and triglycerides (2.5 mg/dL) were treated as missing. Lipid concentrations were moderately to highly correlated with one another. The highest correlation was observed between total cholesterol and LDL (ρ=0.88), while total cholesterol was only moderately correlated with HDL (ρ=0.36) and ln-triglycerides (ρ=0.35). HDL and LDL were very weakly correlated (ρ=0.06, $p>0.05$), while HDL and ln-triglycerides were inversely correlated (ρ= −0.29, $p<0.05$).

3.3. PFASs and total cholesterol

Total cholesterol was positively associated with ln-PFOS as a continuous variable (Table 5). Each ln-unit increase in PFOS was associated with an increase of 8.96 mg/dL (95% CI=1.70, 16.22) in total cholesterol. For each IQR-unit increase in ln-PFOS, total cholesterol increased by 4.25 mg/dL (95% CI=0.81, 7.69). The latter change represents an increase of 2.0% over the median concentration of total cholesterol in this population. The Pearson correlation between ln-PFOS and total cholesterol was 0.08 ($p<0.05$). The third and fourth quartiles of PFOS had elevated total cholesterol as compared to the first quartile, but the confidence intervals were imprecise. None of the other PFASs had notable linear

associations with total cholesterol; in addition, the quartile estimates generally did not provide support for monotonic dose-response relationships between PFASs and total cholesterol.

Several previous studies have described positive associations between PFASs and total cholesterol. Both PFOA and PFOS have been associated with total cholesterol in a general population sample (Nelson et al., 2010) and among highly PFOA-exposed adults (Steenland et al., 2009) and children (Frisbee et al., 2010). Among highly exposed, predominantly male, adult workers PFOA was associated with total cholesterol (Costa et al., 2009; Olsen et al., 2003; Sakr et al., 2007). There is prior evidence that associations with total cholesterol may vary among PFASs. One study of non-pregnant adults in the general U.S. population described positive associations between PFOS, PFOA, PFNA and total cholesterol, but an inverse association between PFHxS and total cholesterol (Nelson et al., 2010); while a recent study of non-pregnant adult Canadians reported a positive association between PFHxS and total cholesterol, but no significant associations between PFOA or PFOS and total cholesterol in weighted analyses (Fisher et al., 2013).

3.4. PFASs and HDL cholesterol

In adjusted quartile analyses, all seven PFASs had higher HDL cholesterol associated with the highest quartile of exposure, relative to the lowest quartile of exposure (or, in the case of PFDA, associated with concentration at or above the median versus below the median) (Table 5). Additionally, PFOS, PFNA, PFDA, PFUnDA, and PFHxS showed positive linear associations with HDL cholesterol in adjusted models. The strongest evidence supporting a monotonic dose-response relationship with HDL was observed for PFUnDA, which had the highest quartile-specific associations as well as the largest associated change in HDL. For each natural log-unit increase in PFUnDA, HDL increased by 4.05 mg/dL (95% CI=2.75, 5.35). For each IQR-unit increase in ln-PFUnDA, HDL increased by 3.71 mg/dL (95% CI=2.52, 4.89). This change represents an increase of 5.5% over the median concentration of HDL cholesterol in this sample.

The other six PFASs also demonstrated some evidence of an exposure-response relationship with HDL in adjusted quartile analyses, although in general the association was driven by the highest quartile of exposure. Each IQR-unit increase in ln-PFNA was associated with an increase of 1.66 mg/dL HDL (95% CI=0.57, 2.76); each IQR-unit increase in ln-PFDA was associated with an increase of 2.55 mg/dL HDL (95% CI=1.22, 3.88); and each IQR-unit increase in ln-PFOS was associated with an increase of 2.08 mg/dL HDL (95% CI=1.12, 3.04).

The observed associations between PFASs and HDL cholesterol are consistent with the findings of some previous cross-sectional studies, but inconsistent with others. Serum PFOS has been positively associated with HDL cholesterol among non-pregnant Inuit adults (Chateau-Degat et al., 2010) as well as among children and adolescents from a highly PFOA-exposed community in Ohio and West Virginia (Frisbee et al., 2010). However, other studies have reported no association between PFASs and HDL cholesterol (Nelson et al., 2010; Steenland et al., 2009), and one recent study of highly PFOA-exposed male workers in China reported an inverse association between PFOA and HDL (Wang et al., 2012).

Adjustment for plasma albumin concentration (quartiles) tended to reduce the magnitude of the associations between each PFAS quartile and HDL cholesterol (Supplemental Table). On average, the difference in HDL cholesterol between the lowest and highest quartile of PFAS was reduced by 15% with the inclusion of plasma albumin in the model. The coefficients for the linear associations between continuous natural-log transformed PFAS variables and continuous HDL were attenuated by 16% on average, but remained elevated.

As multiple PFASs tend to be correlated with one another, it is possible that the results observed in single-exposure models are partially due to the confounding influence of one or more correlated PFAS species. All seven PFASs were included as continuous natural-log transformed exposure variables in the same model to determine their mutually-adjusted associations with HDL. The coefficients for all PFASs were attenuated, with the exception of PFUnDA, which remained strongly associated with HDL (Figure 1). The change in HDL for each IQR-unit of PFNA was reduced from 1.66 mg/dL (95% CI=0.57, 2.76) to −1.19 mg/dL (95% CI= −2.97, 0.59) in the adjusted multiple pollutant model. The change in HDL for each IQR-unit of PFUnDA decreased only slightly from 3.71 mg/dL (95% CI=2.52, 4.89) to 3.63 mg/dL (95% CI=1.88, 5.39). The influence of including the other six PFASs in each model was generally much stronger than the influence of adjustment for other covariates. The multiple pollutant model did not show evidence of multicollinearity; all variance inflation factors for the PFAS variables were <4. It is notable that PFUnDA, with an alkyl chain length of 10, was the longest-chain PFAS examined here, but we are not aware of research suggesting that PFUnDA has higher potency or biological activity in humans relative to the shorter-chain PFASs.

3.5. PFASs and LDL cholesterol

In adjusted models for LDL cholesterol, the beta-coefficient for an ln-unit change in PFOS was elevated, but the confidence interval was wide and included the null (Table 5); each IQR-unit shift in ln-PFOS was associated with a change of 3.07 mg/dL LDL (95% CI= −0.03, 6.18). The second quartile of PFOS concentration was associated with a decrease in LDL concentration relative to the first quartile, while the third and fourth quartiles of PFOS concentration were associated with elevated LDL. None of the other six PFASs were associated with LDL.

The existing literature on this topic is inconclusive, with previous studies reporting both positive (Frisbee et al., 2010; Sakr et al., 2007; Steenland et al., 2009) and no associations between PFASs and LDL cholesterol (Chateau-Degat et al., 2010). In the present study, PFOS was positively associated with both total cholesterol and HDL cholesterol, and less strongly associated with LDL cholesterol. This finding of the same direction of association with both HDL and LDL cholesterol is not necessarily paradoxical. While certain medications and foods can increase HDL cholesterol while decreasing LDL cholesterol (Mensink et al., 2003; Soudijn et al., 2007), other foods such as saturated fats can increase both HDL and LDL cholesterol (Hayek et al., 1993). Positive associations between PFOS and both HDL and LDL cholesterol were also observed in a previous study among children and adolescents (Frisbee et al., 2010).

3.6. PFASs and triglycerides

Linear associations between PFASs and triglycerides were not observed (Table 5). The fourth quartile of PFUnDA concentration was associated with small decrease in ln-triglycerides (−0.08 ln-mg/dL, 95% CI= −0.16, −0.01) relative to the first quartile. Additionally, the second quartile of PFHpS concentration was associated with slightly lower ln-triglycerides (−0.08 ln-mg/dL, 95% CI= −0.15, −0.01) relative to the first quartile. None of the other PFASs were associated with triglycerides in quartile-based or continuous models.

Previous studies have reported positive associations between PFOA and PFOS and triglycerides, among highly exposed workers (Olsen et al., 2003) and among adults in the highly PFOA-exposed Ohio and West Virginia population (Steenland et al., 2009). One occupational study reported no association between PFOA and triglycerides (Sakr et al., 2007).

3.7. Sensitivity analyses

Restricting the analysis to the 525 women with complete covariate and outcome data who were selected without regard to subfecundity produced quartile effect estimates that were similar to those produced by the weighted models, although the confidence limits were wider due to the smaller sample size (results not shown). Additional inclusion of weight gain (kg) from pre-pregnancy to mid-pregnancy as a continuous covariate did not materially change the interpretation of any effect estimates (results not shown), and therefore was not included in the adjusted models for which results are shown.

3.8. Interpretation of findings

This study is the first to specifically examine associations between PFASs and lipids in pregnant women. Some previous studies (Chateau-Degat et al., 2010; Nelson et al., 2010) excluded pregnant women, who are known to have altered lipid metabolism relative to non-pregnant women. Notably, in normal pregnancy total cholesterol rises 25–50% over non-pregnant levels (Piechota and Staszewski, 1992) and triglycerides are typically elevated 200–400% times over pre-pregnant levels by late pregnancy (Fahraeus et al., 1985; Sattar et al., 1997b). HDL cholesterol also increases by approximately 40% over non-pregnant levels (Fahraeus et al., 1985). The composition of HDL particles also changes during pregnancy, such that each particle contains a greater proportion of cholesterol and acylglycerols, but without an increase in phospholipid content (Aurell and Cramer, 1966).

PFOA and PFOS concentrations in pregnant women have been reported to be somewhat lower than in non-pregnant women (Woodruff et al., 2011); in one study this difference persisted after adjustment for hemoglobin/hematocrit ratio as well as demographic and lifestyle factors (Javins et al., 2013). The observed differences may be due to the residual confounding effects of normal plasma volume expansion during pregnancy, but may also suggest transfer of PFASs to the fetus, or increased excretion of PFASs during pregnancy (Loccisano et al., 2013). Given these differences in both lipids and PFASs during pregnancy, it is plausible that whatever causal or non-causal mechanism leads to observed associations between PFASs and lipids, the associations may differ in pregnant women relative to non-pregnant women.

The biological mechanism that may lead to positive associations between plasma PFAS concentrations and lipids in humans is largely unknown. PFAS are known to activate PPARα, a receptor involved in regulating gene expression related to lipid and glucose metabolism, but the activation of this receptor in humans tends to produce lower lipid concentrations (Hertz et al., 1995). While some of the toxic effects of PFOA in animals are believed to operate through activation of PPARα, evidence suggests that the toxicity of PFOS may occur through alternate mechanisms, independent of PPARα (Abbott et al., 2009; Abbott et al., 2007). Recently, toxicologists have begun to study PFASs other than PFOA and PFOS, and have demonstrated varying strength of PPARα activation associated with PFASs of different chain lengths (Wolf et al., 2012). The relative proportion of biological effects due to PPARα-dependent mechanisms and PPARα-independent mechanisms may vary across PFASs.

The investigation of potential health effects due to relatively low-concentration plasma contaminants such as PFUnDA, PFNA, and PFHxS may be particularly important given that the effects of human exposure to these PFASs have not received as much attention in the literature as the effects of exposure to PFOA and PFOS. While the measured plasma concentrations of these PFASs were substantially lower in our study than the concentrations of PFOA and PFOS, serum concentrations of PFNA may still be increasing in some populations (Haug et al., 2009b; Kato et al., 2011). Moreover, certain PFASs that previously

appeared in human serum at low concentrations, such as PFHxS, have been used to replace PFOS in household stain-proofing and other applications following the phase-out of PFOS in 2000–2002, and therefore we may expect higher human exposures in the future (Beesoon et al., 2012; Glynn et al., 2012).

A limitation of this study is the cross-sectional design, which does not allow causal interpretation of the findings. Non-causal explanations for the findings may include unmeasured confounding or pharmacokinetics. A recent laboratory study explored whether previously observed cross-sectional associations between PFASs and plasma lipid parameters could be explained by the distribution of PFASs into lipoprotein fractions, and concluded that this partitioning was not a sufficient explanation for the dose-response associations observed (Butenhoff et al., 2012). However, the same study found that of the small proportion of each PFAS (for example, <25% of PFOS) that resided in the non-lipoprotein-depleted portion of the plasma, a somewhat larger percentage was recovered from the HDL subfraction relative to the LDL and VLDL subfractions (Butenhoff et al., 2012), raising the possibility of a special affinity between HDL and some PFASs. Researchers may wish to further examine whether this affinity could account for the association observed here between HDL and PFASs in plasma.

Another potential limitation of this study is the use of non-fasting plasma lipid measurements. Fasting for 8–12 hours is typically recommended prior to lipid screening. However, researchers have observed minimal changes in lipid profiles following typical food intake (Langsted et al., 2008). Additionally, non-fasting triglycerides may be more strongly associated with cardiovascular disease risk in women than fasting triglycerides (Bansal et al., 2007). Our study also employed a single measurement of plasma lipid concentrations. As these measurements may vary over time, multiple measures are preferred to establish lipid profiles for cardiovascular risk (Davis et al., 1990).

PFASs have been previously correlated with other environmental pollutants such as PCBs and PBDEs (Haug et al., 2010), possibly due to common exposure sources such as fish and shellfish. We adjusted for daily intake of oily fish in our models; however we cannot exclude the possibility of residual confounding due to measurement error in fish intake, or to unmeasured environmental pollutants. Our study did not consider dietary components other than oily fish that may influence lipid concentrations.

In general, the findings lend only modest support to a possible mechanism by which PFAS concentrations during pregnancy may be related to adverse pregnancy outcomes. Lipid disturbances in early and late pregnancy associated with preeclampsia include elevated triglycerides (Baker et al., 2009; Sattar et al., 1997a), as well as higher total cholesterol and LDL, and typically lower HDL cholesterol (Bayhan et al., 2005; Enquobahrie et al., 2004), although one study noted slightly higher HDL at mid-pregnancy among women who subsequently developed severe preeclampsia as compared to controls (Baker et al., 2009). We found no evidence of elevated triglycerides associated with PFAS concentrations in pregnant women. The positive associations we observed between multiple PFASs and HDL cholesterol are not consistent with elevated risk of preeclampsia. However, we did observe a positive linear association between PFOS and total cholesterol, and higher total cholesterol is associated with elevated risk of preeclampsia. We plan to directly examine the associations between mid-pregnancy PFASs and preeclampsia in another study within the MoBa cohort.

4. Conclusions

This study provides evidence of PFAS-specific associations with lipids in pregnant women. Specifically, plasma PFOS concentration during pregnancy was positively associated with total cholesterol, and multiple PFASs were positively associated with HDL cholesterol. While the latter finding is not consistent with lipid profiles that have been associated with adverse pregnancy outcomes, the association between PFOS concentrations and cholesterol during pregnancy merits further investigation in a longitudinal study to determine whether or not it is likely to be causal. Elevated total cholesterol during pregnancy is associated with adverse pregnancy outcomes, including preeclampsia, which can result in harm to the mother and developing fetus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We examined associations between perfluoroalkyl substances and lipids in pregnancy.
- Plasma PFOS was positively associated with total cholesterol among pregnant women.
- Seven perfluoroalkyl substances were positively associated with HDL cholesterol.
- Multiple pollutant models reduced all associations with HDL except PFUnDA.
- Cholesterol findings may suggest a possible mechanism linking PFOS to preeclampsia.

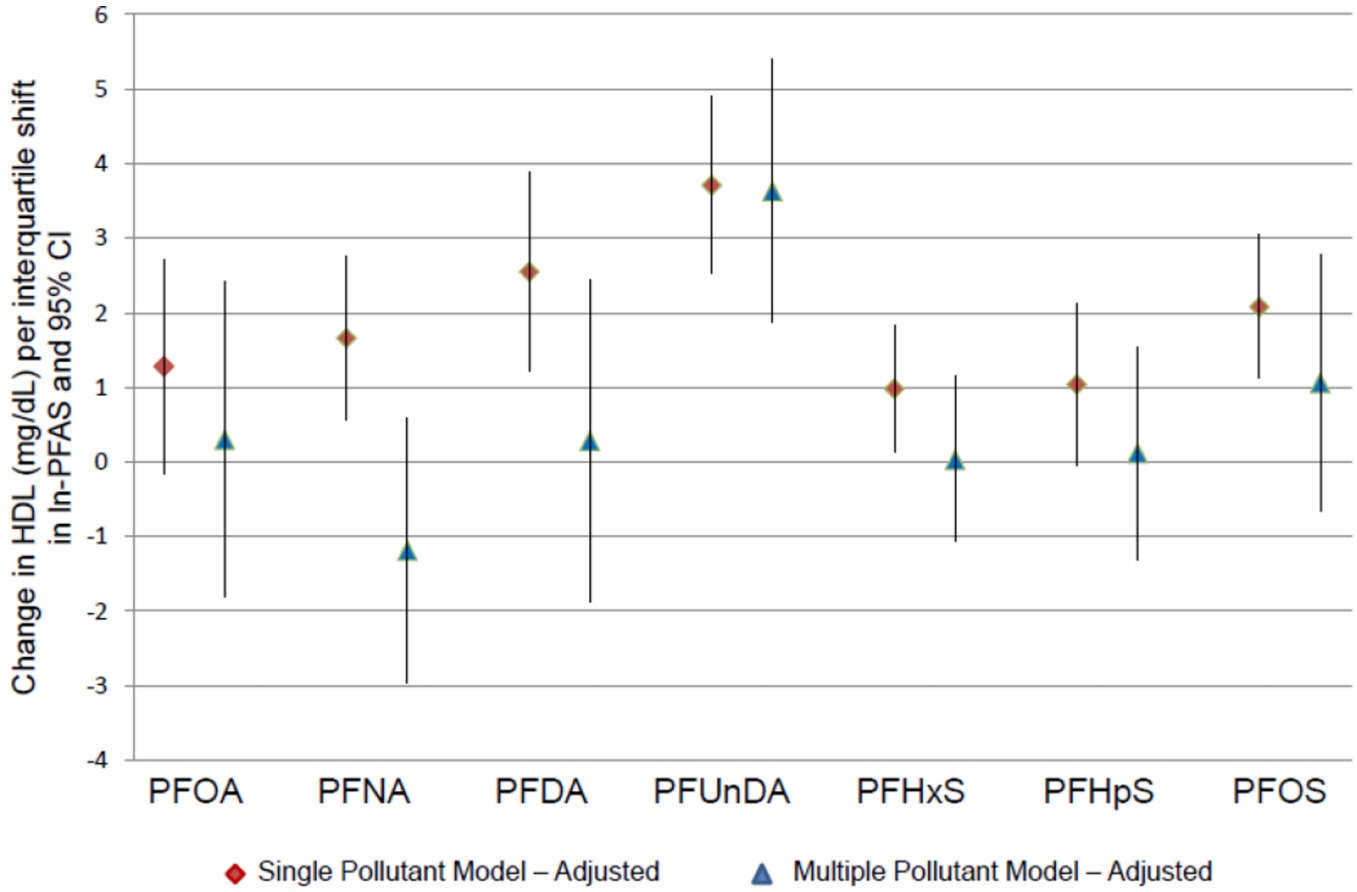

Figure 1.
Weighted linear regression coefficients for single and multiple pollutant models of the association between natural log-transformed PFASs (ln-ng/mL) and HDL cholesterol (mg/dL).

Table 1

Characteristics of 891 pregnant women enrolled in the Norwegian Mother and Child Cohort Study (2003–2004) and previously selected for a case-control study of subfecundity

	N	%
Age (years)		
19–24	54	6
25–29	262	29
30–34	399	45
35–44	176	20
Pre-pregnant body mass index (kg/m^2)		
14.9–<25.0	529	59
25.0–<30.0	241	27
30.0–45.4	121	14
Education completed		
Less than high school	74	8
Completed high school	287	32
Some college	373	42
4 or more years college	157	18
Smoking at mid-pregnancy		
Yes	72	8
No	819	92
Oily fish consumed (g/day)		
0–3.0	230	26
3.1–7.5	224	25
7.6–14.9	216	24
15–100	221	25
Gestational age at blood draw (weeks)		
12–16	151	17
17–20	653	73
21–37	87	10
Trimester of pregnancy at blood draw		
First (12–13 weeks)	6	0.7
Second (14–26 weeks)	881	99
Third (27–37 weeks)	4	0.4
Time to pregnancy (months)		
<3	292	33
3–6	146	16
7–12	54	6
>12	397	45
Previous live births or stillbirths		
0	447	50
1	308	35

	N	%
2	111	12
3 +	25	3
Interpregnancy interval (months)		
No previous births/stillbirths	447	50
4–23	106	12
24–47	178	20
48–245	160	18
Breastfeeding duration in previous pregnancy (months)		
No previous births/stillbirths	447	50
<1	114	13
1–5	39	4
6–11	146	16
12–17	120	13
18–36	25	3

Table 2

Plasma concentrations (ng/mL) of seven perfluoroalkyl substances quantitated in >50% of samples from 891 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003–2004

	Abbreviation	Percent Quantifiable	Carbon Chain Length[a]	Percentile 5th	25th	50th	75th	95th	CV[b]
Perfluorocarboxylic Acids									
Perfluorooctanoic acid	PFOA	100.0	7	1.05	1.66	2.25	3.03	4.43	6.7
Perfluorononanoic acid	PFNA	99.9	8	0.17	0.29	0.39	0.51	0.81	15.6
Perfluorodecanoic acid	PFDA	70.1	9	<LOQ[c]	<LOQ	0.09	0.15	0.27	18.1
Perfluoroundecanoic acid	PFUnDA	94.1	10	<LOQ	0.13	0.22	0.33	0.57	32.8
Perfluorosulfonic Acids									
Perfluorohexane sulfonate	PFHxS	99.8	6	0.27	0.44	0.60	0.87	1.87	13.2
Perfluoroheptane sulfonate	PFHpS	88.0	7	<LOQ	0.09	0.13	0.19	0.32	44.5
Perfluorooctane sulfonate	PFOS	100.0	8	6.90	10.31	13.03	16.60	24.34	11.3

[a] Refers to the number of carbons in the fully-fluorinated alkyl chain.

[b] Coefficient of variation, inter-assay, was calculated as (weighted standard deviation of batch means / weighted mean of batch means)*100 for the 50 pooled control samples, weighted by the number of pooled control samples in each analytic batch.

[c] Limit of quantification.

Table 3

Spearman correlation coefficients among plasma concentrations (ng/mL) of seven perfluoroalkyl substances quantitated in >50% of samples from 891[a] pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003–2004

	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	-						
PFNA	0.62	-					
PFDA	0.42	0.71	-				
PFUnDA	0.26	0.56	0.60	-			
PFHxS	0.57	0.51	0.32	0.32	-		
PFHpS	0.48	0.43	0.24	0.17	0.53	-	
PFOS	0.64	0.66	0.44	0.44	0.59	0.60	-

[a]Values below the limit of quantification are excluded; sample size differs for each pairwise correlation.

Table 4

Distribution of plasma lipid concentrations (mg/dL) among 891 pregnant women enrolled in the Norwegian Mother and Child Cohort Study, 2003–2004

	Percentile					
	5th	25th	50th	75th	95th	CV[a]
Total cholesterol	158	189	211	233	276	2.5
High-density lipoprotein cholesterol	47	58	67	75	88	4.0
Low-density lipoprotein cholesterol	81	107	125	147	178	4.3
Triglycerides	74	98	124	158	218	8.7

[a]Coefficient of variation, inter-assay, was calculated as (weighted standard deviation of batch means / weighted mean of batch means)*100 for the 50 pooled control samples, weighted by the number of pooled control samples in each analytic batch.

Table 5

Weighted[a], adjusted[b] linear regression coefficients between perfluoroalkyl substances (ng/mL) and lipid parameters (mg/dL).

		Total Cholesterol			HDL Cholesterol			LDL Cholesterol			Ln-Triglycerides		
		LSM[c]/β	95% CI[d]		LSM/β	95% CI		LSM/β	95% CI		LSM/β	95% CI	
PFOA	Q1	206.80			61.14			125.86			4.92		
	Q2	1.49	−6.49,	9.48	0.22	−2.38,	2.83	0.94	−6.08,	7.96	0.03	−0.04,	0.11
	Q3	3.54	−4.51,	11.59	2.31	−0.59,	5.20	4.16	−3.19,	11.50	0.01	−0.08,	0.09
	Q4	3.90	−5.00,	12.80	3.42	0.56,	6.28	3.35	−4.35,	11.06	−0.04	−0.12,	0.04
	Per ln-ng/mL[e]	2.58	−4.32,	9.47	2.13	−0.26,	4.51	2.25	−3.97,	8.48	0.00	−0.07,	0.06
	Per IQR[f]	1.55	−2.60,	5.69	1.28	−0.15,	2.71	1.36	−2.38,	5.10	0.00	−0.04,	0.04
PFNA	Q1	210.05			61.61			129.42			4.94		
	Q2	−5.28	−12.75,	2.19	−0.06	−2.60,	2.47	−5.04	−11.78,	1.70	−0.03	−0.10,	0.04
	Q3	−3.84	−11.55,	3.86	0.48	−2.09,	3.06	−3.82	−10.71,	3.07	−0.02	−0.09,	0.05
	Q4	2.22	−6.47,	10.90	3.26	0.47,	6.05	−0.81	−8.30,	6.69	−0.02	−0.09,	0.06
	Per ln-ng/mL	0.01	−5.98,	6.00	2.84	0.97,	4.71	−2.15	−7.31,	3.02	−0.02	−0.07,	0.03
	Per IQR	0.01	−3.51,	3.52	1.66	0.57,	2.76	−1.26	−4.29,	1.77	−0.01	−0.04,	0.02
PFDA	<median	207.85			61.28			127.25			4.95		
	>=median	2.07	−3.33,	7.46	2.72	0.89,	4.55	0.88	−3.86,	5.63	−0.06	−0.11,	0.00
	Per ln-ng/mL	1.84	−2.12,	5.79	2.54	1.22,	3.87	0.19	−3.30,	3.69	−0.03	−0.07,	0.01
	Per IQR[g]	1.84	−2.13,	5.81	2.55	1.22,	3.88	0.20	−3.31,	3.70	−0.03	−0.07,	0.01
PFUnDA	Q1	207.63			59.31			129.05			4.97		
	Q2	0.55	−6.50,	7.60	3.06	0.58,	5.54	−1.90	−8.14,	4.34	−0.07	−0.13,	0.00
	Q3	0.22	−7.23,	7.67	4.41	1.86,	6.95	−3.50	−10.29,	3.30	−0.07	−0.14,	0.00
	Q4	4.41	−3.46,	12.27	7.61	4.98,	10.25	−2.40	−9.45,	4.64	−0.08	−0.16,	−0.01
	Per ln-ng/mL	0.89	−3.28,	5.06	4.05	2.75,	5.35	−2.36	−5.97,	1.25	−0.04	−0.08,	0.00
	Per IQR	0.81	−3.00,	4.63	3.71	2.52,	4.89	−2.16	−5.46,	1.15	−0.04	−0.07,	0.00
PFHxS	Q1	207.09			60.40			127.00			4.95		
	Q2	0.65	−6.87,	8.17	1.57	−0.95,	4.09	0.44	−6.19,	7.08	−0.04	−0.11,	0.02
	Q3	1.62	−6.08,	9.32	2.69	0.06,	5.31	0.50	−6.15,	7.16	−0.02	−0.10,	0.05
	Q4	4.25	−3.88,	12.39	3.21	0.77,	5.65	1.48	−5.89,	8.85	−0.02	−0.09,	0.05

		Total Cholesterol			HDL Cholesterol			LDL Cholesterol			Ln-Triglycerides		
		LSM[c]/β	95% CI[d]		LSM/β	95% CI		LSM/β	95% CI		LSM/β	95% CI	
	Per ln-ng/mL	3.00	−1.75,	7.76	1.46	0.19,	2.73	1.92	−2.50,	6.33	−0.01	−0.05,	0.03
	Per IQR	2.01	−1.17,	5.19	0.98	0.13,	1.82	1.28	−1.67,	4.23	0.00	−0.03,	0.02
PFHpS	Q1	209.29			61.60			127.05			4.96		
	Q2	−1.25	−8.75,	6.25	−0.27	−2.58,	2.05	2.54	−3.99,	9.08	−0.08	−0.15,	−0.01
	Q3	−2.36	−10.02,	5.30	0.32	−2.21,	2.86	−1.06	−7.82,	5.70	−0.01	−0.08,	0.06
	Q4	0.91	−7.23,	9.05	3.00	0.53,	5.47	2.05	−4.99,	9.10	−0.06	−0.14,	0.01
	Per ln-ng/mL	−0.60	−5.11,	3.91	1.30	−0.06,	2.66	−0.03	−3.99,	3.94	−0.03	−0.07,	0.01
	Per IQR	−0.48	−4.08,	3.13	1.04	−0.05,	2.13	−0.02	−3.19,	3.14	−0.02	−0.06,	0.01
PFOS	Q1	207.59			60.34			127.04			4.93		
	Q2	−3.35	−10.34,	3.64	1.96	−0.39,	4.31	−3.23	−9.28,	2.83	0.00	−0.06,	0.07
	Q3	3.06	−4.93,	11.05	2.49	0.00,	4.97	2.60	−4.49,	9.70	−0.03	−0.10,	0.05
	Q4	7.59	−0.42,	15.60	4.45	2.04,	6.86	5.51	−1.62,	12.64	0.00	−0.07,	0.07
	Per ln-ng/mL	8.96	1.70,	16.22	4.39	2.37,	6.42	6.48	−0.07,	13.03	−0.02	−0.09,	0.04
	Per IQR	4.25	0.81,	7.69	2.08	1.12,	3.04	3.07	−0.03,	6.18	−0.01	−0.04,	0.02

[a] Weighted for prior selection by subfecundity.

[b] Adjusted for age, pre-pregnant body mass index, nulliparous or interpregnancy interval, duration of breastfeeding previous child, education completed, current smoking at mid-pregnancy, gestational weeks at blood draw, oily fish consumed daily.

[c] Least-squares mean is presented for Quartile 1, which serves as the reference category for quartile analyses.

[d] 95% confidence interval.

[e] Coefficient represents the change in lipid outcome for each 1 ln-(ng/mL) increase in PFAS concentration.

[f] Coefficient represents the change in lipid outcome for a shift in PFAS concentration from the 25th percentile to the 75th percentile of the observed exposure distribution.

[g] IQR of ln(PFDA) estimated as 2*(75th percentile-median) because the 25th percentile of PFDA was below the limit of quantification.