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Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women

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Abstract

Background—Perfluoroalkyl substances (PFASs) are widespread pollutants that have been associated with adverse health effects although not on a consistent basis. Diet has been considered the main source of exposure. The aim of the present study was to identify determinants of four plasma PFASs in pregnant Norwegian women.

Methods—This study is based in the Norwegian Mother and Child Cohort Study (MoBa) conducted by the Norwegian Institute of Public Health. Our sample included 487 women who enrolled in MoBa from 2003–2004. A questionnaire regarding sociodemographic, medical, and reproductive history was completed at 17 weeks gestation and a dietary questionnaire was completed at 22 weeks gestation. Maternal plasma samples were obtained around 17 weeks of gestation. Plasma concentrations of four PFASs (perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA)) were examined in relation to demographic, lifestyle, dietary, and pregnancy-related covariates. Predictors were identified by optimizing multiple linear regression models using Akaike's information criterion (AIC).

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Results—Parity was the determinant with the largest influence on plasma PFAS concentrations, with r^2 between 0.09 and 0.32 in simple regression models. In optimal multivariate models, when compared to nulliparous women, parous women had 46%, 70%, 19%, and 62% lower concentrations of PFOS, PFOA, PFHxS, and PFNA respectively ($p < 0.001$ except for PFHxS, $p < 0.01$). In all these models, duration of breastfeeding was associated with reduced PFAS levels. PFOA showed the largest reduction from breastfeeding, with a 2–3% reduction per month of breastfeeding in typical cases. Levels of PFOS, PFOA, and PFNA increased with time since most recent pregnancy. While pregnancy-related factors were the most important predictors, diet was a significant factor explaining up to 4% of the variance. One quartile increase in estimated dietary PFAS intake was associated with plasma PFOS, PFOA, PFHxS, and PFNA concentration increases of 7.2%, 3.3%, 5.8% and 9.8%, respectively, resulting in small, although non-trivial absolute changes in PFAS concentrations.

Conclusion—The history of previous pregnancies and breastfeeding were the most important determinants of PFASs in this sample of pregnant women.

Keywords

Perfluoroalkyl substances; reproductive history; pregnancy; dietary exposure

1. Introduction

Perfluoroalkyl substances (PFASs) have been manufactured for over 50 years for use in a variety of consumer products such as stain repellents, leather and fabric coatings, paper coatings, and in fire-fighting foams (Kovarova and Svobodova, 2008). These substances are characterized by a fully fluorinated carbon chain of varying length attached to a functional end group. Most PFASs are highly persistent and have long half lives in the environment and humans. For example, two of the most common PFASs, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), have median half-lives in human blood of approximately 2–5 years (Bartell et al., 2010; Olsen et al., 2007). These substances have a high binding affinity for albumin and are thus found primarily in blood, liver, and kidney (Jones et al., 2003; Zhang et al., 2009). Since the year 2000, data from Europe and the US indicate a clear decline in blood levels of PFOS, but a less dramatic decline in PFOA levels (Glynn et al., 2012; Haug et al., 2009b; Kato et al., 2009; Schröter-Kermani et al., 2012). These data are consistent with a phase-out of PFOS by the major manufacturer between 2000 and 2002 and a more gradual phase-out plan for PFOA (United States Environmental Protection Agency, 2010).

Although plasma levels of PFASs are decreasing in human populations, whether they are related to health outcomes in humans is under active investigation (Halldorsson et al., 2012; Melzer et al., 2010; Shankar et al., 2012). Of particular relevance here are the many epidemiologic studies among reproductive-aged women (Fei et al., 2010; Maisonet et al., 2012; Vestergaard et al., 2012; Whitworth et al., 2012a; Whitworth et al., 2012b), in which a complete understanding of factors affecting plasma levels could help reduce bias.

PFOS and PFOA have been detected in numerous food items (Haug et al., 2010a; Schecter et al., 2010) and diet is considered an important determinant of PFASs body burden (Domingo, 2012; Trudel et al., 2008). Recent studies of estimated PFOS and PFOA exposure demonstrated that consumption of food is likely to be an important source among background exposed adult populations (Haug et al., 2011a; Trudel et al., 2008). Previous studies of determinants of PFAS concentrations have focused primarily on the contribution from diet, and the results have been inconsistent. Halldorsson et al (2008) found that red meat, animal fats, and snack foods were the most important determinants of plasma PFAS levels in a sample of Danish pregnant women, while Rylander et al. (2009) and Haug et al.

(2010b) both identified seafood as the most important dietary predictor in separate samples of the Norwegian population.

Few previous studies have included a thorough investigation of the contribution of factors other than diet to PFAS plasma levels. Because PFOS and PFOA cross the placenta (Fromme et al., 2010; Gützkow et al., 2012) and are found in breast milk (Haug et al., 2011a; Thomsen et al., 2010), the impact of pregnancy-related variables on women's PFASs body burden are of particular interest. Several studies report on the importance of breast milk consumption in determining infants' PFASs exposure (Haug et al., 2011a; Liu et al., 2011; Thomsen et al., 2010; Trudel et al., 2008). Therefore, one must consider the impact of breastfeeding on reducing the maternal body burden. As the epidemiologic literature surrounding potential adverse health effects of PFASs expands, it becomes increasingly important to carry out more comprehensive studies of determinants of PFAS levels. Such knowledge will provide a better understanding of important routes of PFASs exposure, but will also provide insight into the most important covariates for inclusion in epidemiologic investigations of associations between exposure and disease.

The aim of the present study was to identify determinants of plasma PFAS concentrations in a sample of pregnant Norwegian women.

2. Materials and methods

2.1 Study population and design

The present study is based on the Norwegian Mother and Child Cohort study (MoBa), initiated by and maintained at the Norwegian Institute of Public Health (Magnus et al., 2006). In brief, MoBa is a nation-wide pregnancy cohort that included 108,000 pregnancies enrolled from 1999 to 2008. Pregnant women were recruited through a postal invitation in connection with a routine ultrasound examination offered to all women around 17 weeks of gestation. Overall, 38.5% of invited women consented to participate. The women were asked to provide biological samples and to answer questionnaires during pregnancy. Pregnancy and birth records from the Medical Birth Registry of Norway (MBRN) were linked with the MoBa database (Magnus et al., 2006). The study was approved by the Regional Committee for Ethics in Medical Research and the Norwegian Data Inspectorate, and informed consent was obtained from each participant before the study.

The present study was based on MoBa data file version 4.301 and used data from MoBa subjects included in a prior case-base study of PFASs and subfecundity (Whitworth et al., 2012b). In the previous study, two groups were selected from all MoBa subjects who: enrolled in 2003–2004, delivered a live born child, provided a plasma sample at enrolment, and reported time to pregnancy. The first group (the base group) consisted of a random sample (n=550), while the second group (the case group) was a random sample of subfecund (time-to-pregnancy >12 months) women (n=400). Only the subjects selected in the base group are included in the primary analyses of the present study. Further, we also excluded women who did not complete the MoBa food frequency questionnaire (FFQ) (n=5) and women who reported energy intakes outside the valid range (n=4) of 4.5 to 20 MJ/day, previously established for MoBa (Meltzer et al., 2008). Of the remaining 541 women, 487 had complete data on all covariates and were included in the analysis. Among the 63 excluded women, the proportion of women with low educational attainment was larger than among the included women (50% versus 33% in the two lower education categories), but there were no differences between the groups with regard to other sociodemographic, life style or pregnancy related variables, or PFAS concentrations (data not shown).

2.2 Blood sampling and PFAS analyses

Maternal plasma samples were collected at the time of enrolment and shipped from the collection site to Oslo by mail at ambient temperature. Because PFASs are chemically stable, changes in PFAS plasma concentrations while in transit are believed to be negligible (Frömel and Knepper, 2010). At the Biorepository, plasma was separated, aliquoted and stored at -80 degrees Celsius. Although most of the existing epidemiologic literature regarding PFASs focuses on PFOS and PFOA, our analytical method is sensitive and can detect as many as 19 PFASs. Concentrations of PFOS, PFOA, perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA) and nine other PFASs were detected in 150 μ l of plasma using high performance liquid chromatography/tandem mass spectrometry at the Norwegian Institute of Public Health. Details of the analytical method have been published elsewhere (Haug et al., 2009a). A total of 50 quality assurance/quality control (QA/QC) plasma samples from a single pool were analysed in 17 sample batches alongside the specimens. The laboratory technicians were blinded to their identity, and the QA/QC samples were indistinguishable from the plasma samples from MoBa subjects. The median concentrations of PFOS, PFOA, PFHxS and PFNA in the (QA/QC) plasma samples in the 50 pooled specimens were 19.3 ng/mL and 3.7 ng/mL, 2.4 and 0.62, respectively. For PFOS, the within-batch correlation of variation was 4.5% and the between-batch correlation of variation was 11.3%. For PFOA, the within-batch correlation of variation was 3.5% and the between-batch correlation of variation was 6.7%. For PFNA, the within-batch correlation of variation was 6.0% and the between-batch correlation of variation was 15.6%. For PFHxS, the within-batch correlation of variation was 3.9% and the between-batch correlation of variation was 13.2%. Values of PFOS, PFOA and PFNA were found in all plasma samples, while two values of PFHxS were below the limit of quantification (0.05 ng/ml). We chose to include in our analyses the four PFASs most frequently detected.

2.3 Questionnaires

Data on individual characteristics and dietary intakes were obtained from detailed self-administered questionnaires (available online at <http://www.fhi.no/eway/?pid=238>). The first questionnaire (Q1), completed at enrolment around gestational week 17, covered information about sociodemographic, lifestyle and health factors, including data regarding previous pregnancies. The second questionnaire (Q2), completed around gestational week 22, was a FFQ designed to capture dietary habits and intake of dietary supplements during the first four to five months of pregnancy, and has been described in detail elsewhere (Meltzer et al., 2008).

2.4 Dietary variables

The FFQ included questions about intake of 255 food items or dishes, with a special emphasis on seafood items. For each food item, the frequency of consumption was reported by selecting one out of 8–10 frequencies, ranging from never to several times monthly, weekly or daily. Consumption frequencies were converted into food amounts (g/day) by the use of standard Norwegian portion sizes. Food and nutrient calculations were performed with the use of FoodCalc software (Lauritsen, 2005) and the Norwegian food composition table (Norwegian Food Safety Authority et al., 2006). The FFQ has been previously validated (Brantsæter et al., 2008a; Brantsæter et al., 2010). The food items in the FFQ were first combined into 100 non-overlapping food groups based on structure, nutrient profile or culinary usage as in previous MoBa studies (Brantsæter et al., 2008b; Brantsæter et al., 2012). Food groups were further combined into 13 non-overlapping groups based on relevance and previous knowledge about potential dietary sources of PFASs (Halldorsson et al., 2008; Haug et al., 2010b). Estimated maternal dietary intakes of PFOS (ng/day) and PFOA (ng/day) were calculated using values of PFOS and PFOA concentrations previously measured in samples of Norwegian food items and drinking water (Haug et al., 2010a).

Although values for PFHxS and PFNA have been measured in some Norwegian food items and drinking water, these substances were present in much lower concentrations than FPOS and PFOA and dietary intakes were not calculated.

2.5 Other variables

We examined the influence of numerous non-dietary variables on PFAS concentrations. Sociodemographic and lifestyle variables included: age at delivery (<25, 25–29, 30–34, 35+ years), marital status (married/cohabitating vs. single), length of education (<12, 12, 13–16, 17+ years), urban vs. rural residence as defined according to definitions obtained from Statistics Norway, smoking prior to pregnancy (non-smokers, occasional smokers and daily smokers), alcohol use during pregnancy (yes vs. no), type of housing (flat, farm, or detached), and native language (Norwegian vs. other). Additionally, self-reported height and pre-pregnancy weight were used to calculate body mass index (BMI; kg/m²). BMI was then divided into four categories defined by the World Health Organization (<18.5, 18.5–24.9, 25–29.9, 30+ kg/m²). Maternal and paternal combined annual income was also included (both <300,000, one 300,000, and both 300,000 NOK). In addition, we examined several pregnancy-related variables including: parity (nulliparous, 1, 2, 3+), pregnancy weight gain at 17 weeks (decreased weight, no change, 1–3, 4+ kg gain), gestational week of blood draw (<16, 17–18, 19–20, 21+ weeks), and marine fatty acids (n-3) supplement use (yes vs. no). For parous women, the duration of breastfeeding was reported in Q1 for each child born prior to the present pregnancy, and the total number of months spent breastfeeding all previous children (referred to as *total breastfeeding* duration) was calculated. Birth dates for older children were obtained from the MBRN and the time interval since the most recent pregnancy, defined as the number of months from the birth of the previous child to conception of the index child, was calculated for each woman.

2.6 Statistical methods

All results reported are based on a complete case analysis. Because the distributions of PFAS concentrations were skewed, all analyses were based on the natural log transformations (ln) of the PFAS variables. In univariate analyses, the examination of linear trend for ordered categorical variables was based on the Wald test for the categorical variable included as an ordinal term in the regression model, while for nominal categorical variables, we examined differences between categories using the Kruskal-Wallis test.

Dietary data were explored using both continuous variables as well as ranked categories. All dietary variables were energy adjusted by the residual method (Willett et al., 1997).

Determinants of PFAS concentrations were identified separately for PFOS, PFOA, PFHxS, and PFNA. We applied a multiple regression approach starting with all covariates (16–20, including candidates for interaction terms) in the model. We then applied stepwise backward variable exclusion using Akaike's information criterion (AIC) for (Akaike-) optimal model fit. AIC is defined by the following formula $AIC=2k-\ln(L)$, where k is the number of parameters in the model, and L is the value of the likelihood function for the estimated model. Minimizing the AIC value provides a means for model selection that is optimal, where the reward for goodness of fit is balanced with a penalty for increasing the number of parameters (Claeskens and Hjort, 2008). Typically, with several hundreds of cases, the AIC-optimal model will include terms that are not significantly different from zero in the ordinary $p<0.05$ sense. However, with our data, the AIC approach was similar in effect to selecting optimal models with candidate variables having p values < 0.10, and occasionally the standard penalty for including variables was increased to exclude factors having little influence. Model fit was additionally assessed using several diagnostic plots.

We also performed two sensitivity analyses. In the first, we repeated the main analyses using the multiple imputations option in SPSS software to deal with missing data on covariates (n=54). Also, to examine whether subjects' selection for the previous study of subfecundity (case vs. base) affected our results, we repeated the analyses including the 400 subjects selected in the case group and controlled for subject selection (case vs. base).

For the multiple regression models with ln(PFAS) as the dependent variable, beta values correspond to the expected geometric mean of the non-transformed outcome variable associated with the independent variable, relative to the mean in the referent category of the independent variable, where $[(\exp(\beta)) - 1] * 100$ is the % change in PFAS for a one unit change in the independent variable.

We used predictive analytics software (PASW) for Windows, version 17.0 (IBM® SPSS®, USA) for descriptive statistical analyses and the statistical software R, version 2.11.0 to fit optimized multiple regression models. A significance level of $p=0.05$ was used if not otherwise stated.

3. Results

3.1 Descriptive statistics

PFOS, PFOA and PFNA were detectable in all women, while two women had PFHxS values below the limit of quantification (0.05 ng/ml) and were excluded. The median plasma concentrations were as follows: PFOS: 12.8 ng/ml (mean, 13.7 ng/ml; interquartile range (IQR), 10.1–16.6 ng/ml), PFOA: 2.11 ng/ml (mean, 2.35 ng/ml; IQR, 1.54–2.93 ng/ml), PFHxS: 0.60 ng/ml (mean, 0.76 ng/ml; IQR, 0.43–0.86 ng/ml), and PFNA: 0.39 ng/ml (mean, 0.42 ng/ml; IQR, 0.28–0.51 ng/ml).

Plasma concentrations of all four PFAS increased with maternal educational attainment and household income, while the direction of the correlation with other demographic and lifestyle variables differed by substance (Table 1). Concentrations of PFOS and PFOA increased with age. Plasma PFOA concentration was higher in smokers than in non-smokers. The median concentration of each PFAS was highest in the obese group, but the increase with BMI was statistically significant only for PFHxS. The concentrations of PFOA, PFHxS, and PFNA were lower in women living in rural vs. urban areas, and in women living in farmhouses vs. other housing. All plasma PFAS concentrations were strongly correlated with the pregnancy related variables: parity, time interval since the most recent pregnancy, and total breastfeeding duration (Table 1).

The median estimated dietary intakes of PFOS and PFOA were 44.6 ng/day (0.66 ng/kg bw/day) and 34.2 ng/day (0.52 ng/kg bw/day), respectively. Estimated intakes of both PFOS and PFOA were statistically significantly correlated with plasma PFOS and PFOA as well as with PFHxS and PFNA concentrations, indicating that the four PFASs share similar dietary sources (Table 2).

In unadjusted analyses, all plasma PFAS concentrations were associated with intakes of shellfish and oily fish, but the associations were relatively weak, and differed according to parity. Associations between plasma PFASs and the other food groups varied even more (Table 3). Plasma PFOS levels were not correlated with any food group other than seafood. Plasma PFOA levels correlated positively with the intake of oils in nulliparous subjects. Plasma PFHxS correlated positively with oils in nulliparous subjects, and plasma PFNA correlated positively with eggs, vegetables (nulliparous subjects), and oils. In most cases, with positive associations, the relative differences between medians of PFAS levels in the highest and lowest intake quartiles in a food group were 20–30% or less.

3.2 Regression analyses

Table 4 summarizes AIC-optimised linear model fits for natural log-transformed plasma levels of PFOS, PFOA, PFHxS, and PFNA. Models include dietary exposure in the form of the combined estimated dietary intake of PFOS and PFOA. The total variance explained by the models for PFOS, PFOA, PFHxS, and PFNA was: 21%, 47.9%, 17.5%, and 24.6%, respectively. Parity (nulliparous vs. parous) was the most important covariate for all PFASs. In comparison to parous women, on average, nulliparous women had 70% higher PFOA concentration. Similarly, nulliparous women had 47% higher PFOS, 19% higher PFHxS, and 62% higher PFNA concentration. We considered the parameterization of parity using three categories (nulliparous, primiparous, and multiparous), but the coefficient representing the decrease in PFAS concentrations among multiparous (compared with primiparous) women was small (data not shown). Therefore, for parsimony, we used only two categories (parous vs nulliparous).

Total breastfeeding duration was correlated with reduced levels of all PFASs examined in this study. The largest reduction from breastfeeding was seen for PFOA, with a 2.4% reduction in plasma PFOA levels per month of breastfeeding. Time since the most recent pregnancy was associated with increased concentrations of PFOS, PFOA, and PFNA. Total breastfeeding duration and time since the most recent pregnancy were more strongly associated with PFOA than with the other PFAS concentrations. Figure 1 shows the unadjusted changes in plasma PFOA concentration associated with these variables. Plasma PFOA concentration is higher in nulliparous than in parous women, the concentration in parous women increases with time since the most recent pregnancy (Figure 1a), and decreases with longer duration of total breastfeeding (Figure 1b).

Maternal education and household income both reflect socioeconomic status, and were not selected in the same models. Maternal education was positively associated with models for plasma PFOS, PFHxS and PFNA. Women in the highest education category (17 years or more) had 33% higher PFOS, 40% higher PFHxS and 45% higher PFNA than women in the lowest category (<12 years). Household income was positively associated with the model for PFOA. In comparison with rural residence, urban residence was associated with 7% higher levels of PFOA and 14% higher levels of PFHxS. Smoking was associated with reduced levels of PFOS. Women who reported daily smoking prior to pregnancy had 9% lower PFOS concentration than non-smokers.

Because the estimated dietary intakes of PFOS and PFOA were strongly correlated ($r=0.70$), they were summed, and the summed dietary intake was associated with increasing concentration of all PFASs in all the multivariate models. One quartile increase in estimated dietary PFAS intake was associated with plasma PFOS, PFOA, PFHxS, and PFNA concentration increases of 7.2%, 3.3%, 5.8% and 9.8%, respectively, resulting in small, although non-trivial absolute changes in PFAS concentrations. Even though diet was a significant predictor, it contributed relatively little to the explained variance of PFAS plasma levels regardless of how it was parameterized.

When we evaluated the effect on the model of including intake from specific food groups in addition to or instead of the estimated summed PFOS and PFOA intake, only the model for PFHxS was clearly improved ($R^2=0.189$ vs 0.175); in this case, including beef (negatively correlated), pork, oils and butter/margarine (positively correlated) improved the fit. Furthermore, the summed PFOS and PFOA intake estimate was always selected instead of the food groups upon optimization when present in the initial model, indicating that the food groups proved little additional information relative to total exposure. Shellfish and oily fish were the most influential food groups in the food groups-only PFAS models. Compared with

the other PFASs, a greater number of food groups were associated with PFHxS, but the influence of each food group was small.

In each of the sensitivity analyses, with imputation of missing values on covariates and with inclusion of participants in the subfecundity group, we obtained results that were comparable to our primary analyses (Supplementary Tables 1–4).

4. Discussion

In this study of pregnant women, variables representing reproductive history (i.e. parity, the time interval since the most recent pregnancy, and total breastfeeding duration) were the major determinants of PFASs. In comparison to these variables, demographic and lifestyle variables including diet, contributed much less to the explained variance in plasma PFAS concentrations.

Parity was the determinant with the largest influence in all PFAS models. Three factors may account for this finding. First, PFASs have the ability to cross the placental barrier (Apelberg et al., 2007; Fromme et al., 2010), making direct foetal transfer a likely mechanism through which parity affects PFAS plasma concentrations. A recent study from MoBa reported PFAS concentrations in cord blood ranging from 30 to 79% of the maternal concentrations (Gützkow et al., 2012). However, the reduction in PFAS concentrations by parity apparent in our models is quantitatively larger than the reduction likely due to foetal transfer alone. A possible additional explanation could be accumulation of PFASs in the placenta, resulting in lower plasma concentrations when the placenta is removed after delivery.

Breastfeeding is another mechanism through which parous women may achieve lower PFAS concentrations compared with nulliparous women. Both PFOS and PFOA have been measured in breast milk (Haug et al., 2011a; Kärman et al., 2007; Liu et al., 2011; Thomsen et al., 2010). A study of matched maternal serum, cord serum, and breast milk samples indicated that the postnatal exposure through human milk was higher than the prenatal exposure, particularly for PFOA (Liu et al., 2011). The authors of a recent study examined elimination rates of several compounds in breast milk during twelve months of lactation in 10 Norwegian mothers. The reduction of PFOA concentration in breast milk during the lactation period was more than twice that of PFOS (94% versus 37% in one year) (Thomsen et al., 2010).

Lastly, because a major elimination route of PFASs may be urine (Andersen et al., 2006; Harada et al., 2004; Loccisano et al., 2013), pregnant women may experience greater reduction in PFASs body burden due to an increase in the glomerular filtration rate. In healthy pregnancies, the glomerular filtration rate may increase by 40% (Dunlop, 1981; Gibson, 1973; Krutzen et al., 1992; Sims and Kranz, 1958).

It was also of note that the time interval since the most recent pregnancy was associated with increased concentrations of PFOS, PFOA and PFNA, probably reflecting re-accumulation of the chemicals with increasing time between pregnancies. Apelberg et al (2007) previously reported that parity was a determinant of PFAS concentrations in cord blood and epidemiologic studies have reported lower maternal plasma PFAS levels among parous women and women with a longer breastfeeding history (Fei et al., 2007; Fei et al., 2008). These analyses, however, were univariate, and the present analyses provide a clearer indication of the relative importance of the factors determining levels. Because of the relatively large effect of reproduction-related events on PFAS concentrations, the findings in epidemiologic studies of PFAS levels and health outcomes can easily be biased if the effects are not adequately considered in the analysis. For example, associations between PFASs and

duration of breastfeeding and associations between PFASs and fecundability disappeared when the analyses were restricted to nulliparous women (Fei et al., 2010; Whitworth et al., 2012b).

The relative contribution of different exposure pathways to human exposure to PFASs has been difficult to define. Diet has been considered the most important non-occupational source of exposure to PFASs, particularly for PFOS and PFOA (Domingo, 2012; Trudel et al., 2008). Seafood has been identified as a major route of dietary exposure to PFASs in other studies from Norway (Haug et al., 2010b; Rylander et al., 2010). Haug et al., evaluated concentrations of ten PFASs in serum and food consumption in 175 men and women in the Norwegian Fish and Game Study. The study distinguished between lean fish, fish liver and shrimp, and consumption of one or more of the seafood items were associated with all PFASs (Haug et al., 2010b). Rylander et al., examined associations between the four PFASs examined in the present study and dietary habits and lifestyle in a representative group of middle-aged Norwegian women (n=315). Increased plasma concentrations of PFOS, PFHxS, and PFNA were observed in “fish eaters”. Lower concentrations of the same substances were seen in women with a “western” diet consisting of rice, pasta, water, white and red meat, chocolate, snacks, and pastries; no specific food pattern was associated with increased PFOA concentrations. In Sweden, eleven PFASs were measured in a set of archived food market basket samples from 1999, 2005, and 2010. The highest concentration was observed for PFOS in fish, meat, and egg homogenates. Although present at lower concentrations, similar contamination patterns were seen for PFHxS and PFNA, while PFOA was quantified at relatively low concentrations in food homogenates of both animal and non-animal origin (Vestergren et al., 2012). A recent report from the European Food Safety Authority summarised occurrence data for PFASs collected in 13 European countries during the period 2006 to 2012. Across food groups, PFASs were found more frequently in fish and other seafood and in meat and meat products (EFSA, 2012). Seafood items were also the main food groups associated with PFAS levels in the current study. However, the dietary variables contributed little to the overall explained variance in our models. A possible explanation could be the relatively low dietary exposure in our study population. A much higher influence of estimated intakes were seen in the previously mentioned study among men and women with much higher consumption of fish and seafood (Haug et al., 2010b). Although diet has been consistently correlated with PFASs body burden, studies across countries reveal large variation in the foods associated with their body burden. It is possible that some of this variation may be explained by drinking water, food packaging, cooking procedures (Domingo, 2012), as well as exposure to household dust (D’Hollander et al., 2010; Haug et al., 2011a; Haug et al., 2011b; Kato et al., 2009; Knobeloch et al., 2012).

Haug et al (2011a) showed that although food was the major exposure source in a group of Norwegian women, the indoor environment (air and dust) accounted for up to 50% of the total PFASs intake for several women, highlighting the importance of these exposures in determining PFASs body burden. Unfortunately, we did not have measures of indoor air or dust exposure in the present study. We found that variables related to socioeconomic status (i.e. maternal education and household income), were among the important determinants of various PFASs. We are not aware of other studies that have reported similar associations. No association between maternal socio-occupational status and PFOS or PFOA levels were observed in Danish pregnant (Fei et al., 2009). A possible explanation of this finding can be that households with women who are better educated or have higher income purchase more textiles and sports equipment containing PFASs (Herzke et al., 2012). However, the PFASs content in consumer products and possible emissions from such products remains unclear.

Women who were daily smokers had lower plasma PFOS concentration than non-smokers. This association has been inconsistently reported in previous studies. Although smoking status was not associated with plasma PFAS concentrations in either the Norwegian Fish and Game study (Haug et al., 2010b) or a study among Japanese men (Harada et al., 2004), lower levels of both PFOS and PFOA in active smokers were reported among two Danish populations (Eriksen et al., 2011; Halldorsson et al., 2008). Although Eriksen et al., (2011) reported on an inverse association between ever smoking and PFOA and PFOS levels, the study did not find an association between smoking intensity and PFAS levels, suggesting that the association may be explained by different lifestyle patterns in smokers and non-smokers.

The present study benefited from a large sample size and detailed information about a broad range of demographic, lifestyle, and pregnancy related factors, which were likely to capture a large proportion of variability in socioeconomic background and behaviour. Information about the time interval since the most recent previous pregnancy was obtained from the medical birth registry, while the other information was self-reported and may have led to misclassification with regard to, for instance, education, household income and smoking. Although the food frequency questionnaire has been thoroughly validated, the estimated intake of PFOS and PFOA were based on a relatively small database of concentrations measured in Norwegian food and drinking water, which may not be representative of all Norwegian foods. Imprecision in estimates of diet may have caused this determinant to appear less important than it is. The food group registration, on the other hand, is relatively precise and complete. Therefore, the lack of explanatory power of food groups in the multivariate models might indicate that either the dietary exposure route is not very important, or the PFOS/PFOA load varies widely with sample or food item. Another potential concern is selection bias. MoBa participants include a higher proportion of non-smokers and are older and better educated than non-participants. However, the relationships between variables were not necessarily distorted by exclusion of nonparticipants. For example, for eight exposure-disease relationships examined in the MoBa data, the associations were essentially the same as those found in an analysis of data for the entire pregnant population in Norway (Nilsen et al., 2009).

In conclusion, the history of previous pregnancies and breastfeeding were important determinants of PFAS plasma concentrations in these reproductive-aged women. The results of the present study indicate that increased elimination of PFASs occur during and after pregnancy and provide insight into the most important covariates for inclusion in epidemiologic investigations of associations between exposure and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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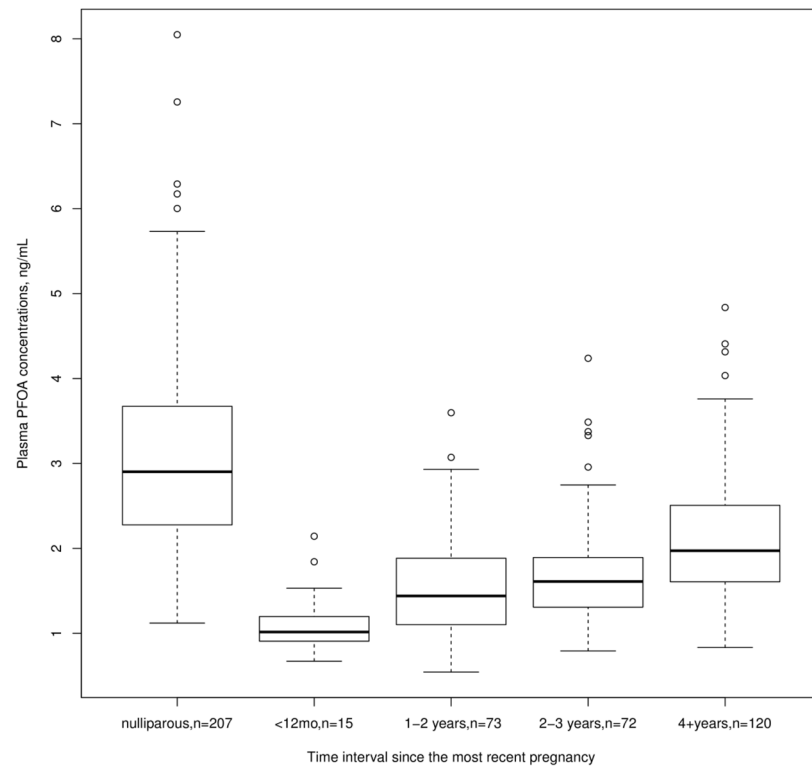
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Highlights

- The history of previous pregnancies and breastfeeding were the most important determinants of PFASs in pregnant Norwegian women.
- Women having given birth one or more times had 46%, 70%, 19%, and 62% lower concentrations of PFOS, PFOA, PFHxS, and PFNA respectively.
- The time interval since the most recent pregnancy was associated with increased concentrations of PFOS, PFOA and PFNA, probably reflecting re-accumulation of the chemicals with increasing time between pregnancies
- The duration of breastfeeding was associated with reduced levels of all PFASs, with the largest reduction in PFOA.
- Diet was a significant factor explaining up to 4% of the variance in plasma PFAS concentrations.

Figure 1a



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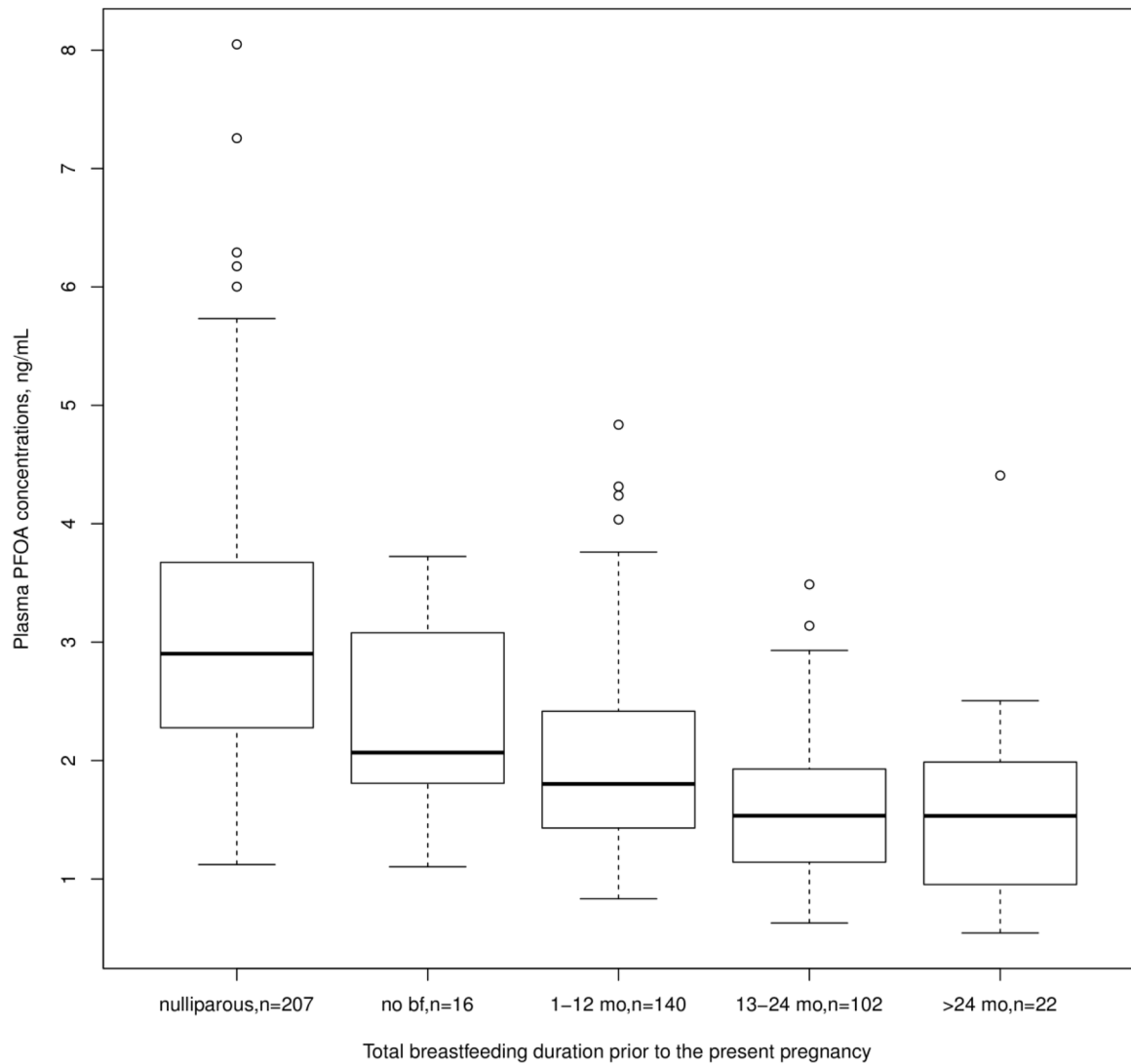
Figure 1b

Figure 1. Unadjusted plasma PFOA concentration (ng/mL) by categories representing time intervals since the most recent pregnancy (Figure 1a) and by categories representing total breastfeeding duration (Figure 1b). Box plot details: the *horizontal* lines indicate the median PFOA concentration; the *box* indicates the interquartile range (IQR) (25th percentile to 75th percentile); the *whiskers* represent observations within 1.5 times the IQR; and the *circles* indicate observations more than 1.5 times the IQR away from the box, considered outliers.

Table 1

Unadjusted plasma PFAS concentrations (ng/ml) by maternal demographic, lifestyle, and pregnancy related characteristics.

	N	%	PFOS	PFOA	PFHxS ³	PFNA
	Median (ng/mL)					
<i>Demographic variables</i>						
Maternal age at delivery						
< 25 years	32	6.6	12.9	2.70	0.60	0.39
25 – 29 years	161	33.1	13.9	2.40	0.62	0.40
30 – 34 years	232	47.6	11.9	1.91	0.56	0.38
35+ years	62	12.7	12.8	1.93	0.60	0.44
<i>P-value 1</i>			0.015	<0.01	0.27	0.77
<i>Marital status</i>						
Married/cohabitant	480	98.6	12.8	2.11	0.60	0.40
Single	7	1.4	10.3	2.12	0.38	0.24
<i>P-value 2</i>			0.15	0.88	0.40	0.12
<i>Maternal education</i>						
<12 years	31	6.4	9.9	1.87	0.43	0.28
12 years	127	26.1	12.1	2.06	0.55	0.35
13 – 16 years	228	46.8	13.2	2.14	0.62	0.42
17 + years	101	20.7	13.8	2.32	0.65	0.42
<i>P-value 2</i>			<0.01	0.02	<0.01	<0.01
<i>Residence</i>						
Rural	254	52.2	12.4	1.95	0.54	0.37
Urban	233	47.8	13.3	2.28	0.65	0.41
<i>P-value 2</i>			0.16	0.01	<0.001	0.01
<i>Native language</i>						
Norwegian	445	91.4	12.8	2.14	0.60	0.40
Other	42	8.6	12.2	1.91	0.62	0.39
<i>P-value 2</i>			0.84	0.78	0.47	0.68
<i>Lifestyle variables</i>						

	N	%	PFOS	PFOA	PFHxS ³	PFNA
			Median (ng/mL)			
Smoking prior to pregnancy						
Non smoker	351	72.1	12.8	1.99	0.60	0.40
Occasional smoker	49	10.1	13.8	2.12	0.60	0.43
Daily smoker	87	17.9	12.0	2.30	0.64	0.37
<i>P</i> -value ²			0.23	0.02	0.42	0.27
Alcohol during pregnancy						
No	389	79.9	12.8	2.11	0.59	0.39
Yes	98	20.1	13.0	2.04	0.66	0.40
<i>P</i> -value ²			0.74	0.73	0.08	0.09
Pre-pregnancy BMI,						
<18.5 kg/m ²	10	2.1	10.7	1.88	0.45	0.29
18.5 – 24.9 kg/m ²	311	63.9	12.3	2.06	0.60	0.39
25 – 29.9 kg/m ²	113	23.2	13.1	2.08	0.60	0.38
30+ kg/m ²	53	10.9	14.2	2.40	0.67	0.43
<i>P</i> -value ¹			0.13	0.30	0.01	0.22
Income						
Both <300 000 NOK	129	26.5	12.5	2.16	0.59	0.36
One 300 000 NOK	233	47.8	12.8	1.99	0.57	0.38
Both 300 000 NOK	125	25.7	13.3	2.41	0.67	0.44
<i>P</i> -value ¹			0.02	0.02	0.04	<0.01
House						
Flat, semidetached	191	39.2	13.7	2.38	0.67	0.41
Farm	28	5.7	12.4	1.68	0.48	0.34
Detached	268	55.0	12.6	1.94	0.56	0.39
<i>P</i> -value ²			0.38	<0.001	<0.001	0.06
Pregnancy related variables						
Parity						
Nulliparous	207	42.5	14.8	2.90	0.71	0.45
1	194	39.8	11.6	1.67	0.52	0.34

	N	%	PFOA	PFHxS ³	PFNA	
2	69	14.2	11.3	1.82	0.47	0.34
3+	17	3.5	12.4	1.66	0.49	0.30
<i>P</i> -value ¹			<0.001	<0.001	<0.001	<0.001
Time interval since most recent pregnancy						
<12 months	15	3.1	9.3	1.02	0.46	0.20
12 – 24 months	73	15.0	10.7	1.44	0.48	0.30
2 – 4 years	72	14.8	11.4	1.61	0.49	0.34
4+ years	120	24.6	12.4	2.97	0.54	0.40
Nulliparous	207	42.5	14.8	2.90	0.71	0.45
<i>P</i> -value ¹			<0.001	<0.001	<0.001	<0.001
Total breastfeeding duration						
Nulliparous	207	42.5	14.8	2.90	0.71	0.45
No	16	3.3	13.4	2.07	0.62	0.40
1 – 12 months	140	28.7	12.1	1.80	0.54	0.36
13 – 24 months	102	20.9	10.7	1.53	0.49	0.30
>24 mo	22	4.5	10.9	1.53	0.42	0.32
<i>P</i> -value ¹			<0.001	<0.001	<0.001	<0.001
Weight change week at 17						
Decreased weight	48	9.9	12.8	2.20	0.53	0.42
No change	34	7.0	12.8	2.46	0.70	0.37
1–3 kg gain	213	43.7	13.4	2.00	0.61	0.39
4+ kg gain	192	39.4	12.2	2.08	0.60	0.40
<i>P</i> -value ¹			0.35	0.47	0.98	0.87
Gestational age blood draw						
<16 w	98	20.1	13.8	2.06	0.65	0.40
17–18 w	188	38.6	12.3	2.07	0.60	0.39
19–20 w	162	33.3	13.0	2.17	0.59	0.40
21+	39	8.0	12.9	2.06	0.60	0.39
<i>P</i> -value ^{1, 2}			0.63	0.99	0.58	0.71

	N	%	PFOS	PFOA	PFHxS ³	PFNA
Nausea with vomiting (week 22)						
No	357	73.3	12.8	2.11	0.61	0.39
Yes	130	26.7	12.7	2.11	0.60	0.40
<i>P</i> -value ²			<i>0.81</i>	<i>0.81</i>	<i>0.40</i>	<i>0.99</i>
Marine fatty acids supplement use (fish oil/fish liver oil)						
No	229	47.0	12.5	1.99	0.59	0.37
Yes	258	53.0	13.1	2.28	0.62	0.41
<i>P</i> -value ²			<i>0.18</i>	<i>0.02</i>	<i>0.12</i>	<i><0.01</i>

¹ *P*-value represents the linear trend from an ordinary linear model (Wald test) and ln(PFAS)

² *P*-value represents the difference between groups based on the Kruskal-Wallis test

³ For PFHxS n=485

Table 2

Spearman correlations between estimated dietary intake of PFOS and PFOA and PFOA and PFAS plasma concentrations, n=487

	Estimated intakes ¹	Median	Plasma concentrations			
			PFOS	PFOA	PFHxS ²	PFNA
			Spearman correlation coefficients r (95% CI)			
PFOS	44.6 ng/day	0.25 (0.16, 0.33)	0.13 (0.04, 0.22)	0.16 (0.07, 0.25)	0.26 (0.17, 0.34)	
PFOA	34.2 ng/day	0.23 (0.14, 0.31)	0.17 (0.09, 0.26)	0.22 (0.13, 0.30)	0.28 (0.20, 0.36)	

¹ PFOS and PFOA estimated from food intakes by FFQ and a database of PFAS concentrations in Norwegian food items

² For PFHxS n=485

Table 3

Unadjusted plasma PFAS concentrations (median, ng/ml) in nulliparous and parous women, by quartiles of food consumption. N= 487

Food group ¹	PFOS		PFOA		PFHxS		PFNA	
	pari=0	pari>0	pari=0	pari>0	pari=0	pari>0	pari=0	pari>0
Shellfish, g/day								
Quartile 1 (0, 0.1)	12.9	10.8	2.84	1.56	0.67	0.49	0.39	0.33
Quartile 2 (0.1, 2.7)	13.4	10.8	2.99	1.71	0.64	0.54	0.44	0.31
Quartile 3 (2.7, 5.4)	15.6	12.0	2.90	1.75	0.78	0.50	0.48	0.34
Quartile 4 (5.4+)	16.6	12.1	3.02	1.83	0.92	0.51	0.50	0.37
<i>P</i> -value ³	<i>0.001</i>	<i>0.07</i>	<i>0.08</i>	<i>0.05</i>	<i><0.001</i>	<i>0.39</i>	<i><0.001</i>	<i>0.02</i>
Oily fish, g/day								
Quartile 1 (0, 4.0)	14.1	10.0	2.98	1.69	0.69	0.48	0.41	0.29
Quartile 2 (4.0, 8.8)	13.4	10.9	2.98	1.62	0.66	0.50	0.45	0.34
Quartile 3 (8.8, 16)	15.9	12.0	2.90	1.73	0.87	0.51	0.53	0.33
Quartile 4 (16+)	15.2	12.6	2.87	1.87	0.74	0.57	0.45	0.39
<i>P</i> -value ³	<i>0.08</i>	<i>0.001</i>	<i>0.69</i>	<i>0.13</i>	<i>0.14</i>	<i>0.02</i>	<i>0.04</i>	<i><0.001</i>
Lean fish, g/day								
Quartile 1 (0, 10)	13.4	11.5	2.88	1.84	0.70	0.48	0.45	0.33
Quartile 2 (10, 18)	15.2	12.5	3.11	1.63	0.71	0.50	0.45	0.36
Quartile 3 (19, 29)	16.1	11.8	2.90	1.70	0.70	0.55	0.47	0.33
Quartile 4 (29+)	15.8	10.8	2.62	1.62	0.83	0.49	0.43	0.33
<i>P</i> -value ³	<i>0.09</i>	<i>0.20</i>	<i>0.42</i>	<i>0.14</i>	<i>0.98</i>	<i>0.96</i>	<i>0.50</i>	<i>0.62</i>
Eggs, g/day								
Quartile 1 (0, 4.5)	13.9	10.1	2.91	1.63	0.66	0.51	0.42	0.32
Quartile 2 (4.5, 7.4)	16.1	11.3	3.09	1.74	0.85	0.53	0.44	0.31
Quartile 3 (7.5, 10)	14.0	11.9	2.69	1.64	0.68	0.48	0.46	0.33
Quartile 4 (10+)	15.9	11.4	3.02	1.82	0.83	0.52	0.50	0.37
<i>P</i> -value ³	<i>0.74</i>	<i>0.10</i>	<i>0.75</i>	<i>0.38</i>	<i>0.19</i>	<i>0.18</i>	<i>0.10</i>	<i>0.01</i>
Poultry, g/day								
Quartile 1 (0, 12)	14.5	12.2	2.92	1.71	0.74	0.48	0.48	0.30

Food group ^f	PFOS		PFOA		PFHxS		PFNA	
	pari=0	pari>0	pari=0	pari>0	pari=0	pari>0	pari=0	pari>0
Quartile 2 (12, 18)	13.9	11.3	2.81	1.71	0.66	0.47	0.42	0.33
Quartile 3 (18, 30)	16.1	11.9	2.98	1.80	0.85	0.55	0.47	0.35
Quartile 4 (30+)	14.8	10.9	2.97	1.63	0.71	0.55	0.44	0.34
<i>P</i> -value ³	<i>0.91</i>	<i>0.91</i>	<i>0.90</i>	<i>0.59</i>	<i>0.52</i>	<i>0.04</i>	<i>0.82</i>	<i>0.15</i>
Beef, g/day								
Quartile 1 (0, 14)	14.5	10.9	2.95	1.69	0.85	0.60	0.44	0.37
Quartile 2 (14, 21)	15.7	11.6	2.90	1.64	0.73	0.51	0.46	0.33
Quartile 3 (22, 29)	14.7	12.1	2.95	1.70	0.81	0.49	0.45	0.34
Quartile 4 (30+)	13.7	11.2	2.87	1.78	0.67	0.47	0.47	0.31
<i>P</i> -value ³	<i>0.31</i>	<i>0.71</i>	<i>0.70</i>	<i>0.23</i>	<i>0.23</i>	<i>0.01</i>	<i>0.82</i>	<i>0.66</i>
Pork, g/day								
Quartile 1 (0, 7.5)	14.4	11.3	2.85	1.60	0.66	0.46	0.43	0.32
Quartile 2 (7.6, 13)	14.8	11.9	3.06	1.69	0.68	0.53	0.44	0.34
Quartile 3 (13, 20)	15.5	11.8	2.86	1.76	0.77	0.52	0.46	0.34
Quartile 4 (21+)	14.4	11.2	3.05	1.77	0.80	0.53	0.47	0.35
<i>P</i> -value ³	<i>0.64</i>	<i>0.91</i>	<i>0.91</i>	<i>0.22</i>	<i>0.10</i>	<i>0.13</i>	<i>0.82</i>	<i>0.16</i>
Milk/dairy, g/day								
Quartile 1 (0, 145)	13.5	11.7	3.04	1.84	0.67	0.59	0.44	0.36
Quartile 2 (146, 310)	15.7	10.7	2.92	1.81	0.84	0.47	0.50	0.31
Quartile 3 (312, 488)	14.8	11.7	2.97	1.69	0.71	0.50	0.48	0.34
Quartile 4 (489+)	15.3	12.1	2.81	1.54	0.68	0.49	0.41	0.33
<i>P</i> -value ³	<i>0.63</i>	<i>0.48</i>	<i>0.52</i>	<i>0.05</i>	<i>0.16</i>	<i>0.58</i>	<i>0.72</i>	<i>0.99</i>
Bread, g/day								
Quartile 1 (0, 178)	13.8	10.7	3.05	1.58	0.64	0.50	0.41	0.31
Quartile 2 (180, 220)	15.2	11.9	3.06	1.82	0.82	0.51	0.48	0.36
Quartile 3 (220, 265)	16.9	11.9	2.87	1.66	0.77	0.55	0.48	0.33
Quartile 4 (266+)	13.6	11.8	2.68	1.84	0.74	0.48	0.42	0.35
<i>P</i> -value ³	<i>0.80</i>	<i>0.37</i>	<i>0.17</i>	<i>0.50</i>	<i>0.38</i>	<i>0.28</i>	<i>0.78</i>	<i>0.10</i>
Fruit/berries, g/day								

Food group ¹	PFOS		PFOA		PFHxS		PFNA	
	pari=0	pari>0	pari=0	pari>0	pari=0	pari>0	pari=0	pari>0
Quartile 1 (0, 158)	14.6	11.2	2.82	1.56	0.68	0.49	0.39	0.33
Quartile 2 (158, 244)	15.2	11.5	3.10	1.63	0.78	0.50	0.46	0.33
Quartile 3 (245, 342)	14.5	12.0	2.87	1.80	0.82	0.55	0.48	0.34
Quartile 4 (343+)	14.3	11.9	2.92	1.88	0.71	0.49	0.47	0.34
<i>P</i> -value ³	0.79	0.66	0.49	0.08	0.10	0.31	0.10	0.52
Vegetables, g/day								
Quartile 1 (0, 97)	13.7	11.3	2.87	1.82	0.68	0.49	0.39	0.32
Quartile 2 (98, 143)	14.7	11.9	2.85	1.64	0.67	0.50	0.43	0.34
Quartile 3 (143, 203)	15.2	11.4	3.29	1.56	0.74	0.44	0.54	0.31
Quartile 4 (204+)	15.6	12.2	3.05	1.81	0.88	0.55	0.46	0.36
<i>P</i> -value ³	0.41	0.18	0.14	0.37	0.02	0.45	0.04	0.34
Oils, g/day								
Quartile 1 (0, 1.0)	14.7	10.5	2.78	1.59	0.62	0.43	0.42	0.27
Quartile 2 (1.0, 1.5)	15.4	11.7	2.80	1.66	0.74	0.49	0.42	0.33
Quartile 3 (1.5, 3.2)	14.5	11.9	3.14	1.74	0.70	0.53	0.45	0.39
Quartile 4 (3.2+)	14.7	11.8	3.08	1.81	0.90	0.55	0.51	0.36
<i>P</i> -value ³	0.46	0.11	0.07	0.48	0.001	0.05	0.02	0.04
Butter/margarine, g/day								
Quartile 1 (0, 5.1)	15.1	10.8	2.86	1.63	0.77	0.54	0.44	0.34
Quartile 2 (5.2, 13)	15.7	12.8	3.06	1.89	0.73	0.51	0.48	0.39
Quartile 3 (13, 22)	13.6	11.2	2.63	1.70	0.65	0.48	0.45	0.31
Quartile 4 (22+)	14.6	11.3	3.09	1.63	0.78	0.49	0.45	0.33
<i>P</i> -value ³	0.91	0.93	0.82	0.20	0.12	0.66	0.36	0.89

pari=0; Nulliparous, pari>0: Parous

¹ All food intakes are energy adjusted

² For PFHxS n=485

³ *P*-value represents the linear trend from an ordinary linear model (Wald test) and ln(PFAS)

Table 4

Determinants of plasma concentrations (ng/ml) of PFOS, PFOA, PFHxS and PFNA in 487 pregnant women the Norwegian Mother and Child Cohort Study (MoBa), 2003–2004.

Dependent variable	Determinants	Beta (95% CI)	Percent change in outcome (ng/mL) per unit exposure (95% CI) ^I	Cumulative adjusted R squared (%)
<i>Ln(PFOS)</i>	Constant	2.65 (2.41, 2.89)		
	Nulliparity			
	Yes	Reference	Reference	
	No	-0.63 (-0.91, -0.34) ***	-46.7 (-59.9, -29.0)	9.8
	Ln(time since most recent pregnancy), years ²	0.14 (0.06, 0.21) ***	9.3 (4.3, 14.4)	12.8
	Total breastfeeding duration, months ³	-0.024 (-0.04, -0.006) **	-1.2 (-2.0, -0.28)	13.7
	Maternal education			
	<12 years	Reference	Reference	
	12 years	0.22 (0.08, 0.36) **	24.6 (8.1, 43.6)	
	13 – 16 years	0.21 (0.07, 0.35) **	23.5 (7.6, 41.7)	
	17 + years	0.29 (0.14, 0.43) ***	33.0 (14.9, 54.0)	16.3
	Smoking prior to pregnancy			
	Non smoker	Reference	Reference	
	Occasional smoker	-0.04 (-0.15, 0.07)	-3.7 (-13.5, 7.3)	
	Daily smoker	-0.10 (-0.19, -0.01) *	-9.4 (-17.0, -1.0)	16.9
	Marital status			
	Married/cohabitant	Reference	Reference	
	Single	-0.25 (-0.52, 0.02) †	-22.1 (-40.6, 2.1)	17.3
	Estimated summed PFOS+PFOA intake, Increase per quartile	0.07 (0.04, 0.10) ***	7.2 (4.2, 10.4)	20.8
<i>Ln(PFOA)</i>	Constant	1.53 (1.33, 1.72)		
	Nulliparity			
	Yes	Reference	Reference	
	No	-1.21 (-1.47, -0.94) ***	-70.1 (-77.0, -61.1)	31.8
	Ln(time since most recent pregnancy), years ²	0.25 (0.19, 0.32) ***	17.9 (13.0, 23.0)	42.5
	Total breastfeeding duration, months ³	-0.05 (-0.07, -0.03) ***	-2.4 (-3.2, -1.6)	46.2
	Urban residence			
	No	Reference	Reference	
	Yes	0.06 (0.003, 0.12) *	6.6 (0.35, 13.2)	46.6
	Household income			
	Both < 300 000 NOK	Reference	Reference	
	One 300 000 NOK	0.05 (-0.03, 0.12)	4.7 (-2.6, 12.6)	
Both 300 000 NOK	0.12 (0.04, 0.20) **	12.6 (3.6, 22.3)	47.2	
Gestational weight gain, up to week 17, kg	-0.01 (-0.02, 0.001) †	-0.9 (-1.9, 0.09)	47.4	

Dependent variable	Determinants	Beta (95% CI)	Percent change in outcome (ng/mL) per unit exposure (95% CI) ^I	Cumulative adjusted R squared (%)
<i>Ln(PFHxS)</i> ⁴	Estimated summed PFOS+PFOA intake, Increase per quartile	0.032 (0.006, 0.06) [*]	3.3 (0.6, 6.1)	47.9
	Intercept	-1.15 (-1.50, -0.79)		
	Nulliparity			
	Yes	Reference	Reference	
	No	-0.20 (-0.34, -0.07) ^{**}	-18.5 (-29.0, -6.4)	10.6
	Total breastfeeding duration, months ³	-0.04 (-0.06, -0.013) ^{**}	-1.9 (-3.1, -0.7)	12.2
	Maternal education			
	<12 years	Reference	Reference	
	12 years	0.19 (-0.02, 0.40)	20.19 (-1.9, 49.0)	
	13 – 16 years	0.24 (0.04, 0.44) [*]	27.0 (3.8, 55.3)	
	17 + years	0.34 (0.12, 0.55) ^{**}	40.2 (13.0, 74.0)	13.7
	Body mass index	0.02 (0.007, 0.029) ^{**}	1.8 (0.7, 2.9)	15.4
	Urban residence			
	No	Reference	Reference	
Yes	0.13 (0.04, 0.23) ^{**}	14.2 (3.7, 25.7)	16.5	
<i>Ln(PFNA)</i>	Estimated summed PFOS+PFOA intake, Increase per quartile	0.06 (0.014, 0.10) [*]	5.8 (1.4, 10.5)	17.5
	Intercept	-1.22 (-1.69, -0.75)		
	Nulliparity			
	Yes	Reference	Reference	
	No	-0.97 (-1.32, -0.62) ^{***}	-62.1 (-73.3, -46.1)	9.4
	Ln(Time since most recent pregnancy), years ²	0.21 (0.12, 0.30) ^{***}	14.7 (8.2, 21.5)	15.4
	Total breastfeeding duration, months ³	-0.02 (-0.05, -0.001) [*]	-1.1 (-2.2, -0.02)	15.8
	Maternal education			
	<12 years	Reference	Reference	
	12 years	0.31 (0.14, 0.49) ^{***}	36.6 (14.8, 62.7)	
	13 – 16 years	0.28 (0.12, 0.45) ^{***}	32.9 (12.4, 57.1)	
	17 + years	0.37 (0.19, 0.55) ^{***}	45.1 (21.4, 73.5)	18.7
	Marital status			
	Married/cohabitant	Reference		
Single	-0.36 (-0.70, -0.03) [*]	-30.5 (-50.1, -3.3)	19.1	
Maternal age at delivery	0.013 (0.002, 0.024) [*]	1.3 (0.2, 2.4)	20.2	
Marine fatty acids supplement use (fish oil/fish liver oil)				
No	Reference	Reference		
Yes	0.07 (-0.01, 0.15) [†]	7.1 (-1.2, 16.1)	20.6	
Estimated summed PFOS+PFOA intake, Increase per quartile	0.09 (0.06, 0.13) ^{***}	9.8 (5.9, 13.8)	24.6	

[†] 0.05 > *P*-value < 0.10

* *P*-value < 0.05

** *P*-value < 0.01

*** *P*-value < 0.001

¹ Percent change in the (untransformed) dependent variable for a one unit change in the independent variable $[(\exp(\beta)) - 1] * 100$

² For Ln (Time since most recent pregnancy, years) percent change per unit is computed for an interval increase from 1 to 2 years.

³ Total breastfeeding duration, months; adjusted for time since most recent pregnancy, percent change is computed for one extra month at the average pregnancy interval.

⁴ For PFHxS n=485