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Impaired gross motor development in infants with higher PFAS concentrations

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ABSTRACT

Industrial produced perfluoroalkyl substances (PFAS) are environmentally persistent and found in humans around the globe. PFAS is transferred from mother to child during pregnancy and lactation and PFAS concentrations are high in infants. PFAS exposure in early life has been linked to a range of negative health effects.

In the present study we have investigated PFAS concentrations in mothers (pregnancy week 18, 28 and 36 and six weeks, four and six months postpartum, n = 114) and in infants at six months age (n = 94), and studied the effects of PFAS status on infant gross motor development by Alberta Infant Motor Scale (AIMS) at age six months.

PFAS concentrations declined in the mothers during pregnancy and postpartum period, and the highest concentrations were seen in infants aged six months. Parity was a strong negative predictor and fish intake a strong positive predictor of maternal PFAS status, while maternal concentrations of PFAS in pregnancy week 18 and months of exclusive breastfeeding determined the PFAS concentrations in infants at six months.

Infants who scored below the median on gross motor development had higher PFAS concentrations than infants with a better gross motor development. Ninety percent of the women reported having fish for dinner at least once a week, with fatty fish as the most popular choice (72%). A higher maternal fish intake in pregnancy week 18 was associated with a poorer gross motor development in the infants at six months.

Infant gross motor development is a marker of later cognitive outcome and our findings indicate that higher PFAS concentrations in young infants and maternal fatty fish intake may impair neurodevelopment.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic carbon fluorine compounds with water- and fat repelling features. The group of perfluoroalkyl acids (PFAAs) with functional moiety of carboxylate or sulfonate are environmentally persistent and bioaccumulate and biomagnify in the food chain. PFAS precursor compounds, as for example alcohols or amides, can be degraded and metabolised to PFAAs. Measurable amounts of these persistent PFAS are found ubiquitous in humans around the globe (Sunderland et al., 2019).

PFAS pass the placenta and can be detected in fetal blood and tissues already from gestational week seven (Mamsen et al., 2019). After birth, breastmilk is an important source of PFAS to the infant (Mogensen et al., 2015). Studies suggest that peak concentrations of PFAS; like perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), occur in infants before age 20 months (Winkens et al., 2017), which is utterly worrying. Epidemiological data have related PFAS exposure in early life to a range of negative health effects on reproduction, hormonal and immune system, as well as metabolic status (Liew et al., 2018). Detrimental effects of PFAS on neurodevelopment have additionally been suggested, but published data have been conflicting (Liew et al., 2018; Fei and Olsen, 2011; Niu et al., 2019; Oulhote et al., 2016; Stein et al., 2013; Rappazzo et al., 2017).

In order to protect the next generation against harmful environmental toxins, we need to increase our knowledge on what factors contribute to a higher PFAS status, and the related health hazards in

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children. We have investigated determinants and time trends of PFAS concentrations in pregnant and postpartum women, and in their infants at six months age. Additionally, we have studied the effects of PFAS on infant gross motor development at age six months.

2. Material and methods

2.1. Study population and design

The study population included 140 healthy women with a singleton pregnancy, who were recruited in pregnancy week 18 at routine ultrasound examination at the Obstetrical Department at Haukeland University Hospital, Bergen, Norway. The women were followed up at pregnancy week 28 and 36, 6 weeks, 4 and 6 months postpartum. The final visit also included the infant. Women with pregnancy related or chronic disease were excluded, except those with well-regulated hypothyroidism (n = 7). Of the 140 pregnant women initially recruited, 114 met the inclusion criteria, attended all visits, and were included in the present study.

The first and last author were responsible for the recruitment, interviews and data registry of all participants.

Ethical approval of the protocol was granted by the Regional Committee on Medical Research Ethics, REK 2011/2447, and written informed consent was obtained from all women.

2.2. Clinical data

At each visit, the participants completed a questionnaire concerning age, years of completed education, parity, body weight, health status, diet, including fish consumption and use of micronutrient supplements, alcohol and tobacco. Regular use of supplements was defined as use more than three days per week. The postpartum visits included additional information about infant nutrition and growth parameters.

At age six months, infant gross motor development was assessed by a pediatrician (IKT) using Alberta Infant Motor Scale (AIMS) (Darrah et al., 1998). The pediatrician was blinded to other results of the study. The AIMS test is a norm-referenced observational tool designed for evaluating gross motor development in infants from birth to 18 months (Darrah et al., 1998). The assessment is based on free observation of the child in different positions according to the age of the child (prone, supine, sitting and standing). The obtained score, 0 to 60 points, is converted to a normative age-dependent percentile rank (5th to 90th percentile). A score below the 10th percentile is classified as possibly delayed motor development (Darrah et al., 1998). AIMS percentiles were further categorized into four groups (<25, 25–50, 51–75 and > 75 percentile).

2.3. Blood sampling and analysis

At each visit, non-fasting blood samples were obtained by antecubital venipuncture into vacutainer tubes without additives (Terumo), including blood samples from the infants at age six months. For analysis of PFAS, frozen serum samples stored at − 80 °C in Sarstedt tubes without additives, were shipped to the Environmental Pollutant Laboratory, Department of Laboratory Medicine, University Hospital of North Norway (Tromsø, Norway) were they were stored at −30 °C prior to analysis. The sampling equipment underwent testing for background contamination, which was not present.

Twenty different PFAS were analyzed according to Huber and Brox (2015) (Huber and Brox, 2015) by an automated fully validated high-throughput sample preparation method and analysis by ultrahigh pressure liquid chromatography tandem mass-spectrometry (UHPLC-MS/MS, Waters, Milford, MA, USA). Analyzed PFAS consist of perfluorobutanoate (PFBA), perfluoropentanoate (PFPA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA) and perfluorotetradecanoate (PFTeDA) perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and PFOS, perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorododecane sulfonate (PFDoDS) and perfluorooctane sulfonamide (PFOSA). Sum of perfluorocarboxylic acids (PFCA), sum of perfluorosulfonic acids (PFSA) and sum of all quantified perfluoroalkyl substances (PFAS= PFCA + PFSA) were calculated. (Huber and Brox, 2015).

Plasma total homocysteine (tHcy) were analyzed by a GC-MS method based on methylchloroformate derivatization (Windelberg et al., 2005).

2.4. Statistical analysis

Results for age and BMI are presented as mean and standard deviations (SD). Categorical data like parity and educational levels, are expressed as percentages. PFAS concentrations were not normally distributed, and are presented as median, interquartile range (IQR) and total range, and comparison between groups are done by Mann-Whitney *U* Test. Spearman correlation and multiple linear regression models were used to explore relationships between parameters. PFAS status in adult women have been associated with fish intake and parity (Brantsaeter et al., 2013) and these factors were included in the multiple linear regression models in addition to age and BMI. PFAS status in infants have been associated with maternal PFAS status during pregnancy and months of breastfeeding (Mamsen et al., 2019; Mogensen et al., 2015). Additionally, gender, gestational age, weight at birth and at six months, factors known to modify serum concentrations of substances which are transferred from mother to child during pregnancy and lactation (Allen, 2012; Bjorke Monsen et al., 2001), were included in multiple linear regression models.

Logistic regression was used to assess influence of infant PFAS concentrations on gross motor development at age six months. As infant gross motor development is affected by gender, infant weight (Slining et al., 2010) and cobalamin status, assessed by plasma tHcy concentrations, as shown in several publications (Torsvik et al., 2013, 2015), these factors were included in the regression model. When assessing the effect of maternal fish intake in pregnancy week 18 on infant gross motor development at age six month, we additionally included maternal education, parity, months of exclusive breastfeeding, plasma tHcy and infant weight at six months, in the regression model.

Graphical illustrations of the relationships between different PFAS concentrations in the mother in pregnancy week 18 and infants at six months were adjusted for gender, weight at six months and months of exclusive breastfeeding. Graphical illustrations of the relationships between sum PFCA and sum PFSA concentrations in infants at six months and infant gross motor development (AIMS percentiles), were adjusted for gender, weight at six months and infant plasma tHcy concentration. Adjustment for birthweight and gestational age did not change the associations, and were excluded from the analyses.

Limits of detection (LODs) were set as concentrations calculated by the Targetlynx-software for each individual sample (LOD*i*) and each individual analyte with a signal to noise ratio of 3 divided by the related sample amount. Where blank contamination was detected (background contribution during sample preparation), LOD was calculated as an average of the blanks multiplied by three times of their standard deviation. If the LOD calculated from the blank contamination was higher than the LOD*i* of the sample, the LOD calculated based on the blank samples, was used. Limit of quantification (LOQ) was defined as three times the LOD. To reduce possible bias of left censored data analyses we have used the actual values between LOQ and LOD. PFAS concentrations below the LOD were not quantified, and these data were replaced by LOD*i* divided by 2.

Statistical analyses were performed only for PFAS with detection rate ≥90% (i.e. PFOA, PFNA, PFDA, PFUnDA, PFHxS, PFHpS and PFOS).

PFAS with lower detection rates were included in the PFAS sum concentrations (sum PFAS), as well as sum PFCA and sum PFSA (i.e. PFHxA, PFHpA, PFDoDA, PFTeDA, PFBS, PFPS and PFNS) and these showed generally detection frequencies <50%.

The SPSS statistical program (version 26) and the packages "mgcv" in R, version 4.0.4. (The R Foundation for Statistical Computing) were used for the statistical analyses. Two-sided *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Demographics

The pregnant women were healthy with a normal prepregnancy BMI. The majority (55%) was pregnant with their first child, all were living together with the father, and two thirds had higher education (≥12 years). None of the women reported any occupational exposure of pollutants. All women had an omnivore diet, and 41% were regular users of micronutrient supplements. They all had public water supply. There was a strong positive correlation between education and fish intake for dinner (rho: 0.32, p < 0.001). Ninety percent of the women reported having fish for dinner at least once a week (Table 1) and farmed salmon was the most frequently used type of fish (63%), followed by lean fish (25%) and other types of fatty fish (9%).

All infants were healthy, born at term (mean gestational age 39.9 (SD 0.8), total range 38–42 weeks), with an appropriate for gestational age weight, mean 3573 (SD 418) grams and 53% (60/114) were males. At age six months, the weight had increased by mean 125% (SD 30) to a mean weight of 7969 (SD 987) grams. Mean duration of exclusive breastfeeding was 3.8 (SD 1.5) months, but 98 infants (86%) were still breastfed at six months. At four months, 50% (70/114) of the infants had been introduced to solid food, and four infants (3.5%) were exclusively breastfed at six months.

3.2. Serum PFAS concentrations in pregnant and postpartum women and infants

PFAS data were available for 94 of the 114 infants (82%) at age six months and all mothers (n = 114) during pregnancy and postpartum, except for pregnancy week 18 (n = 107). Data are summarized in Table 2.

PFOA, PFNA, PFDA and PFUnDA were detected in serum samples of all women in pregnancy week 18 and in all infants at six months, and account for more than 92% of sum PFCA. PFHxS and PFOS were detected in all pregnant women and infants, while PFHpS was detected in all infants and in 96% of pregnant women, and account for more than 90% of sum PFSA. The remaining PFAS were detected in ≤36% of the samples from pregnant women and infants: In infants at six months, PFHpA was detected in 36% of the samples, PFBS 36%, PFTriDA 15%, PFNS 4%, PFHxA 3%, PFDoDA 1%, while PFTeDA, PFPS, PFDS, PFDoDS and PFOSA were not detected in any samples. The detection rate in pregnant women resembled the findings in the infants. Sum PFAS represents the sum of all PFAS detected, regardless of detection rate.

In all groups, PFAS with a detection rate above 90% showed a very large total range, with maximum concentrations more than 20 times the lowest concentrations (Table 2).

Maternal serum concentrations of all PFAS, decreased from pregnancy week 18 to six months postpartum (from −8% for PFDA to −42% for PFOA, all p < 0.001) (Table 2). From pregnancy week 18–36, sum PFCA decreased by 15% and an additional 16% to six months postpartum (total 31%), while sum PFSA decreased by 15% during pregnancy and an additional 6% to six months postpartum (total 21%) (Fig. 1).

Infant serum concentrations of all PFAS were strongly correlated to maternal levels throughout pregnancy and the postpartum period (rho>0.7, p < 0.001). Fig. 2 shows the near linear relations between maternal PFAS concentrations in pregnancy week 18 and infant concentrations at age six months adjusted for birth weight and months of exclusive breastfeeding. The highest PFAS concentrations were seen in the infants (Table 2, Fig. 1). Infant sum PFCA and sum PFAS concentrations were significantly higher than in the mothers already from pregnancy week 18 (p < 0.001), while infant sum PFSA were significantly higher from pregnancy week 28 (p = 0.01). The most pronounced difference between infant and maternal concentrations was seen for PFOA, where median infant concentration was 4.9 times higher than the maternal level at six months postpartum.

3.3. Determinants of serum PFAS concentrations in mothers and infants

Parity, followed by fish intake, were the strongest determinants of maternal PFAS concentrations in pregnancy week 18, in multiple linear regression models, which additionally included age and BMI in week 18 (Table 3). While a higher parity reduced, high maternal fish intake increased maternal PFAS concentrations.

Maternal concentrations of PFAS in pregnancy week 18 and months of exclusive breastfeeding were strong positive determinants of PFAS concentrations in infants at six months, in multiple linear regression models, which additionally included gender, gestational age, birth weight and weight at six months (Table 4).

3.4. Determinants of infant gross motor development at six months

AIMS data, available for 112 infants at six months, were negatively correlated to all individual maternal PFAS concentrations in pregnancy week 18, significant for PFOA (rho: 0.21, p = 0.04), PFDA (rho: 0.19, p = 0.05), PFUnDA (rho: 0.24, p = 0.01) and sum PFCA (rho: 0.27, p = 0.005), and additionally significantly negatively correlated to maternal sum PFCA throughout pregnancy and postpartum period (ranging from rho: −0.25 to −0.21, with p < 0.01). AIMS percentiles were also significantly negatively correlated to all individual infant PFAS concentrations, ranging from rho: −0.30 to −0.22, with p < 0.04, except for PFDA and sum PFOS.

Serum concentrations of all PFAS (except for PFDA and PFUnDA) were higher in infants with a gross motor development below (n = 36) versus above (n = 58) the median AIMS percentile (Table 5). Using logistic regression adjusted for gender, weight at six months and infant plasma tHcy concentration, we assessed the influence of quartiles of infant sum PFAS status on superior (above median) versus inferior (below median AIMS percentile) gross motor development. Infants who had lower infant sum PFCA, PFSA and PFAS had overall a significantly better gross motor development (Table 6). A visual presentation of the relationship between AIMS percentiles and infant concentrations of sum PFCA and sum PFSA, adjusted for gender, weight at six months and infant plasma tHcy concentration, is given in Fig. 3.

Maternal fish intake in pregnancy week 18 was the only significant negative predictor of AIMS percentile at six months (B = −0.53, p = 0.03) in a multiple linear regression model, which additionally included

Table 1
Baseline characteristics of pregnant women in pregnancy week 18 (n = 114).

Parameters	
Age, y, mean (SD)	31.5 (4.3)
Prepregnancy BMI, kg/m^2, mean (SD)	22.8 (3.1)
Education ≥12 years, n (%)	67 (59)
Para 0, n (%)	63 (55)
Regular smoking, n (%)	2 (2)
Alcohol units per week, median (IQR)	0 (0)
Regular use of micronutrient supplements (≥3 days/week), n (%)	47 (41)
Fish for dinner, n (%)	
<3 days/month	12 (11)
1–2 days/week	85 (75)
3–7 days/week	17 (15)

Table 2
Serum PFAS concentrations in infants at 6 months, women during pregnancy and postpartum period.

Median (IQR) Total range Ng/mL	Infants 6 months N = 94	Women during pregnancy, N = 114			Women during postpartum, N = 114		
		Week 18	Week 28	Week 36	6 weeks	4 months	6 months
PFOA	3.27 (2.14, 4.23) 0.73, 8.87	1.15 (0.79, 1.49) 0.26, 3.18	1.04 (0.77, 1.35) 0.28, 3.39	0.96 (0.70, 1.24) 0.30, 3.05	0.93 (0.67, 1.11) 0.26, 2.50	0.76 (0.56, 0.96) 0.22, 2.21	0.67 (0.50, 0.87) 0.23, 4.19
PFNA	0.71 (0.56, 0.91) 0.20, 2.03	0.45 (0.37, 0.55) 0.07, 1.83	0.41 (0.33, 0.52) 0.08, 2.06	0.38 (0.31, 0.48) 0.10, 1.67	0.39 (0.30, 0.48) 0.10, 1.80	0.36 (0.28, 0.48) 0.13, 1.56	0.35 (0.27, 0.46) 0.10, 1.45
PFDA	0.23 (0.16, 0.30) 0.06, 0.67	0.22 (0.17, 0.29) 0.05, 0.62	0.21 (0.16, 0.26) 0.05, 0.73	0.19 (0.15, 0.26) 0.05, 0.63	0.21 (0.16, 0.25) 0.07, 0.65	0.19 (0.15, 0.24) 0.05, 0.60	0.20 (0.15, 0.25) 0.07, 0.64
PFUnDA	0.16 (0.10, 0.21) 0.03, 0.67	0.26 (0.18, 0.34) 0.06, 0.88	0.26 (0.18, 0.35) 0.02, 0.87	0.24 (0.18, 0.34) 0.02, 0.85	0.22 (0.14, 0.28) 0.03, 0.81	0.21 (0.14, 0.30) 0.02, 1.29	0.22 (0.14, 0.30) 0.02, 0.87
Sum PFHxS	0.68 (0.53, 0.92) 0.20, 2.26	0.54 (0.39, 0.67) 0.17, 1.34	0.51 (0.37, 0.65) 0.11, 1.24	0.54 (0.38, 0.69) 0.14, 1.28	0.52 (0.38, 0.67) 0.14, 1.34	0.44 (0.36, 0.66) 0.15, 1.85	0.45 (0.35, 0.66) 0.15, 1.58
Sum PFHpS	0.12 (0.08, 0.16) 0.03, 0.46	0.08 (0.05, 0.10) 0, 0.25	0.07 (0.05, 0.10) 0, 0.25	0.07 (0.05, 0.09) 0, 0.23	0.06 (0.05, 0.09) 0, 0.24	0.06 (0.04, 0.08) 0, 0.23	0.06 (0.04, 0.08) 0, 0.19
Sum PFOS	4.71 (3.53, 6.23) 0.94, 10.99	4.30 (3.23, 5.94) 0.70, 11.64	4.00 (3.03, 5.58) 0.68, 9.98	3.86 2.96, 5.21) 0.68, 10.93	3.76 (2.70, 5.00) 0.84, 8.51	3.55 (2.64, 4.68) 0.80, 8.22	3.53 (2.42, 4.37) 0.71, 8.56
Sum PFCA	4.70 (3.27, 5.95) 1.19, 11.78	2.17 (1.73, 2.67) 0.47, 5.11	2.00 (1.56, 2.53) 0.47, 5.84	1.85 (1.46, 2.35) 0.51, 5.40	1.71 (1.38, 2.23) 0.48, 4.97	1.52 (1.24, 2.08) 0.46, 4.37	1.50 (1.16, 1.98) 0.50, 5.62
Sum PFSA	5.48 (4.15, 7.30) 1.16, 13.34	5.18 (3.67, 6.69) 0.87, 12.84	4.68 (3.55, 6.32) 0.80, 11.55	4.42 (3.48, 6.05) 0.83, 12.47	4.37 (3.22, 5.70) 0.99, 9.70	4.08 (3.05, 5.40) 0.96, 9.30	4.10 (2.95, 5.21) 0.89, 9.67
Sum PFAS	10.14 (7.80, 13.67) 2.34, 21.42	7.30 (5.72, 9.76) 1.34, 16.81	6.65 (5.15, 9.23) 1.26, 14.61	6.49 (5.08, 8.63) 1.33, 15.81	6.33 (4.79, 8.18) 1.47, 12.02	5.81 (4.31, 7.51) 1.42, 12.01	5.75 (4.28, 7.01) 1.39, 12.25

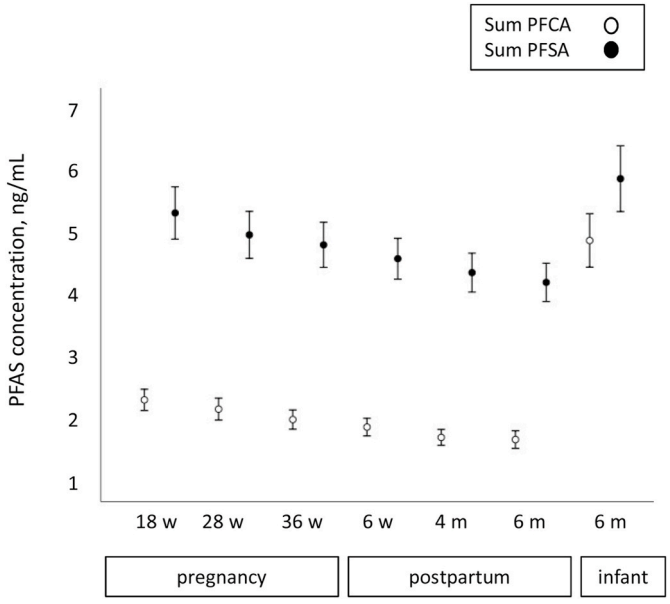

Fig. 1. Serum sum PFCA and sum PFSA concentrations in pregnant and postpartum women (n = 114) and their infants (n = 94).

maternal education, parity, months of exclusive breastfeeding, plasma tHcy and infant weight at six months.

4. Discussion

Five PFAS (PFOA, PFNA, PFDA, PFHxS and PFOS) were detected in all Norwegian pregnant and postpartum women and infants. There were near linear relations between maternal concentrations in pregnancy week 18 and infant concentrations at age six months, and while the concentrations declined in the mothers during pregnancy and postpartum period, the highest concentrations were seen in infants aged six months.

We were able to demonstrate that infants with a poorer gross motor development had higher PFAS concentrations than infants with a better gross motor development. A higher maternal fish intake in pregnancy week 18 was also associated with a poorer gross motor development in the infants at six months.

Infant concentration of dietary substances, such as micronutrients, depend on maternal status in pregnancy, gestational age and birth weight, in addition to months of breastfeeding (Allen, 2012; Bjorke Monsen et al., 2001; Bjørke-Monsen and Ueland, 2011). As PFAS is easily transferred over the placenta and mammary glands, infant PFAS status depend on the same factors. PFAS concentrations in infants at six months were determined by maternal PFAS concentrations in pregnancy week 18 and by months of exclusive breastfeeding, maternal PFAS concentrations were determined by parity and fish intake. Ninety percent of the women reported having fish for dinner at least once a week, and fatty fish was the most popular choice.

4.1. PFAS status in the mothers

PFOA and PFOS in humans have declined over the past decade in most countries, while other PFAS, like PFNA, PFDA and PFHxS have increased (Glynn et al., 2012). Direct emissions of PFOA and PFOS may be declining, but they are still detected in drinking water samples around the world, including Norway (Kabore et al., 2018). PFOA was the dominating PFAS in drinking water collected from the Oslo region with levels ranging from 0.7 to 2.5 ng/L in 2008 (Haug et al., 2010). There is currently no data on PFAS concentrations in drinking water from Bergen, where our participants lived. A limit of 1 ng/L for combined PFOS and PFOA in drinking water has been proposed as a benchmark dose for immunotoxicity in children (Grandjean and Budtz-Jorgensen, 2013), however a recent risk assessment approach suggested that the concentrations of PFOA and PFOS analyzed in tap water around the world should not pose a health risk for drinking water consumers (Kabore et al., 2018).

Maternal PFAS concentrations in our study are comparable to pregnant Swedish and Norwegian mothers recruited during the same period 2007–2010 (Wikstrom et al., 2020; Berg et al., 2014). We observed a gradual decline of maternal sum PFCA and sum PFSA from pregnancy week 18 to six months postpartum, as documented by others (Brantsaeter et al., 2013). The decline of sum PFCA was equal during pregnancy and the postpartum period, while sum PFSA declined more during pregnancy than postpartum.

Parity was a strong negative determinant of maternal PFAS status in pregnancy week 18. Parity is a confirmed negative predictor of concentration of several persistent organic pollutants in women, including PFAS (Brantsaeter et al., 2013; Fernandez-Rodriguez et al., 2015). In the

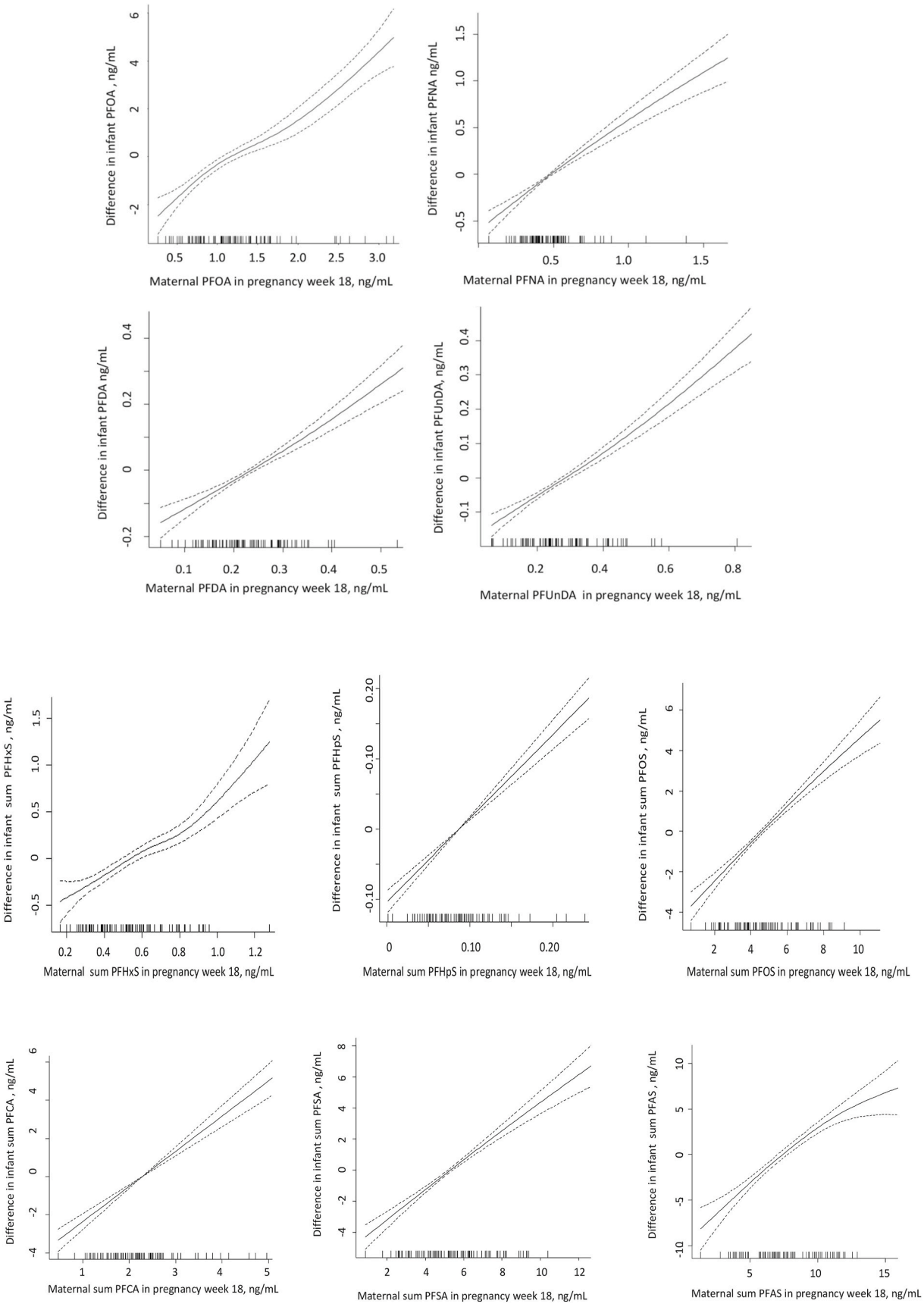

Fig. 2. Infant PFASs concentrations at age six months (n = 94) in relation to the respective maternal serum concentration in pregnancy week 18 by generalized additive models (GAM) adjusted for gender, weight at six months and months of exclusive breastfeeding. The values on the y-axes represents the difference from the respective mean infant PFASs values.

Table 3
Determinants of serum PFAS concentrations in pregnant women in week 18 by multiple linear regression, n = 107.

Variables included in the model	Maternal serum concentrations in pregnancy week 18, ng/mL					
	sum PFCA		sum PFSA		sum PFAS	
	B [a]	P value	B [a]	P value	B [a]	P value
Parity[b]	−0.42	<0.001	−0.68	0.02	−1.10	0.002
Fish consumption[c]	0.39	0.03	0.69	0.14	1.08	0.06

The model included age and BMI in pregnancy week 18, in addition to the parameters listed in the table.
[a] Unstandardized coefficient.
[b] Parity (the percentage of mothers in each category) was categorized as: Para 0 (55%), Para 1 (29%), Para 2 (12%), Para 3 (4%).
[c] Fish for dinner: Less than 3 times/month (11%), 1–2 times/week (74%) and 3–7 times/week (15%).

Table 4
Determinants of serum PFAS concentrations in infants aged six months by multiple linear regression, n = 94.

Variables included in the model	Infant PFAS status at age 6 months, ng/mL					
	sum PFCA		sum PFSA		sum PFAS	
	B [a]	P value	B [a]	P value	B [a]	P value
Maternal serum PFAS in pregnancy week 18[b]	1.84	<0.001	0.97	<0.001	1.16	<0.001
Exclusive breastfeeding, months[c]	0.36	<0.001	0.43	<0.001	0.74	<0.001

The model included gender, gestational age, birth weight and weight at six months, in addition to the parameters listed in the table.
[a] Unstandardized coefficient.
[b] Corresponding maternal serum PFAS in pregnancy week 18.
[c] Months of exclusive breastfeeding given as a continuous variable: range 0–6 months.

Table 5
Serum PFAS concentrations in relation to gross motor development (AIMS test) in infants aged 6 months.

Infant serum PFAS, median (25,75), ng/mL	AIMS test score percentile		P value[a]
	<50 percentile N = 36	>50 percentile N = 58	
PFOA	3.89 (2.94, 4.75)	2.84 (2.05, 3.86)	0.004
PFNA	0.80 (0.65, 0.98)	0.63 (0.50, 0.85)	0.016
PFDA	0.24 (0.20, 0.30)	0.23 (0.15, 0.29)	0.380
PFUnDA	0.17 (0.13, 0.25)	0.15 (0.08, 0.20)	0.069
Sum PFHxS	0.73 (0.64, 1.07)	0.62 (0.48, 0.91)	0.014
Sum PFHpS	0.13 (0.12, 0.18)	0.11 (0.07, 0.16)	0.004
SumPFOS	5.15 (3.95, 7.24)	4.24 (3.08, 5.46)	0.025
SumPFCA	5.55 (4.47, 6.81)	4.03 (3.11, 5.45)	0.003
SumPFSA	5.94 (4.74, 8.63)	4.93 (3.81, 6.64)	0.016
SumPFAS	11.91 (9.44, 15.03)	8.96 (6.95, 12.12)	0.004

[a] Serum PFAS concentrations in infants according to AIMS score were compared by the Mann-Whitney test.

Table 6
Association between PFAS concentrations and superior versus inferior gross motor development (AIMS test) in infants aged 6 months.

PFAS	Concentration, ng/mL Mean (SD)	Number of infants with superior/inferior gross motor development [a]	OR (95% CI) for superior gross motor development	P value
Sum PFCA				
Quartile 4[b]	7.69 (1.78)	9/14	1	
Quartile 1	2.60 (0.52)	18/6	5.1 (1.3, 19.2)	0.02
Quartile 2	4.01 (0.46)	18/5	5.0 (1.3, 20.0)	0.02
Quartile 3	5.29 (0.41)	13/11	1.7 (0.46, 6.0)	0.44
Sum PFSA				
Quartile 4[b]	9.55 (1.61)	10/13	1	
Quartile 1	2.99 (0.87)	20/4	6.3 (1.5, 26.2)	0.01
Quartile 2	4.79 (0.30)	12/11	1.1 (0.31, 3.8)	0.91
Quartile 3	6.27 (0.53)	16/8	1.9 (0.5, 6.9)	0.33
Sum PFAS				
Quartile 4[b]	16.31 (2.03)	10/14	1	
Quartile 1	5.82 (1.46)	20/4	7.6 (1.8, 32.1)	0.006
Quartile 2	9.02 (0.59)	15/8	3.4 (0.9, 12,3)	0.06
Quartile 3	11.82 (1.07)	13/10	1.4 (0.4, 5.1)	0.66

[c] Reference group.
[a] Number of infants with an AIMs percentile above the median (Superior) versus below the median (Inferior).
[b] Adjusted for gender, weight at six months and infant plasma tHcy concentration.

Norwegian Mother and Child Cohort Study (MoBa) multiparous women had 46%, 70%, 19%, and 62% lower concentrations of PFOS, PFOA, PFHxS, and PFNA, respectively, compared to nulliparous women, additionally, duration of breastfeeding was also associated with reduced PFAS levels (Brantsaeter et al., 2013).

In our population of well-educated women, almost all (90%) the mothers reported having fish for dinner at least once a week, and fatty fish was the most popular choice. Number of fish dinners per week was a strong positive determinant of maternal PFAS status in pregnancy week 18. Fish intake, and particularly fatty fish, is recognized as an important positive predictor not only for PFAS status, but also for other persistent organic pollutants, like dioxins and polychlorinated biphenyls (PCB) (Brantsaeter et al., 2013; Fernandez-Rodriguez et al., 2015; Tian et al., 2018; Shu et al., 2018; Bjorke-Monsen et al., 2020; Averina et al., 2018; Malisch and Kotz, 2014; Lee et al., 2021).

4.2. PFAS status in the infants

There were linear relationships between maternal PFAS concentrations in pregnancy week 18 and infant PFAS status at six months, and the PFAS concentrations were higher in infants at six months age than in the mothers in pregnancy week 18. This was most pronounced for PFOA, which was 4.9 times higher than maternal levels six months postpartum. Fromme et al. observed almost identical figures six months after birth, where infant PFOA concentrations were 4.6-fold higher compared to maternal serum (Fromme et al., 2010). These high PFAS concentrations in young infant give rise to concern for fetal development (Lau et al., 2004).

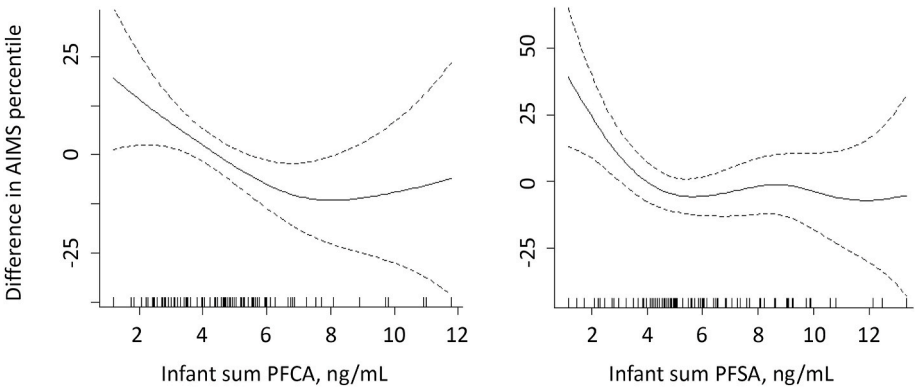

Fig. 3. Infant AIMs percentile (n = 94) in relation to infant serum sum PFCA and sum PFSA concentration at age six months, corrected for gender, weight at six months and plasma total homocysteine by generalized additive models (GAM). The values on the y-axes are given as difference from the respective mean values.

Maternal PFAS concentrations in pregnancy week 18 together with months of exclusive breastfeeding determined infant PFAS status at six months age, as also shown by others (Fei et al., 2007). Increasing infant serum concentrations of PFOA, PFOS, and PFHxS have been associated with prolonged breastfeeding (Fromme et al., 2010). The maternal elimination of PFAS through breast milk is assumed to be greater than the placental transference to the fetus (Kim et al., 2011). Exclusive breastfeeding for several months is therefore associated with an increased burden of PFAS in the child. The majority of our infants (89%) were still breastfed at six months, although only four infants were exclusively breastfed at that age.

4.3. *Effects of PFASs on gross motor development in infants at six months age*

We were able to demonstrate poorer gross motor development with higher PFAS concentrations in very young infants. At age six months, infant serum PFAS concentrations were negatively correlated to AIMS percentiles, and infants with poorer gross motor development (AIMS score below the 50th percentile) had significantly higher PFAS concentrations, except for PFDA and PFUnDA, than infants who scored above the 50th percentile. Additionally, infants with sum PFCA, PFSA and PFAS in the lower quartile had significantly higher ORs for having a superior gross motor development at age six month. Our results are in line with animal studies, which have shown a decreased motor function in the litter of mice and rats after maternal PFOS and PFOA exposure during pregnancy (Butenhoff et al., 2009; Fuentes et al., 2007; Onishchenko et al., 2011). A dose-response relationship between cord blood PFOS concentrations and gross-motor development at age two years has been documented in children from Taiwan (Chen et al., 2013).

Published data on the associations between maternal PFAS exposure and neurodevelopment in children are however conflicting (Liew et al., 2018; Fei and Olsen, 2011; Niu et al., 2019; Oulhote et al., 2016; Stein et al., 2013; Rappazzo et al., 2017). In the Danish National Birth Cohort established between 1996 and 2002, maternal serum levels of PFOA and PFOS were not associated with behavioral and motor coordination problems in 7-year old children (Fei and Olsen, 2011).

Early motor development is reported to correlate with later cognitive outcome (Murray et al., 2006; Ghassabian et al., 2016), whereas gross motor dysfunction is associated with cognition deficits, such as autism spectrum disorder and attention deficit/hyperactivity disorder (Kaiser et al., 2015; Fournier et al., 2010). Neurodevelopment is however multifactorial and subject to genetic factors, growth (Slining et al., 2010), nutrition (Torsvik et al., 2013, 2015), cultural and socioeconomic factors (Ozal et al., 2020), as well as various environmental pollutants (De Felice et al., 2015), such as PCBs, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and polybrominated diphenyl ethers (Grandjean and Landrigan, 2014), something which makes it challenging to identify and evaluate the effect of one specific factor.

The observed wide concentration ranges and the right skewed distribution of many PFAS, indicate that there is a great variability in PFAS burden among people. In our study, some women and infants had a PFAS concentration, which was 10–20 times higher than the lowest concentration measured in our population, something which may influence the adverse health effects associated with PFAS exposure.

We observed strong intercorrelations between the different PFAS in both mothers and infants; whenever serum PFOA was high, so were other PFAS, indicating that these pollutants most likely have common sources (Schecter et al., 2010). To what extent the measured PFAS are coming from direct exposure to PFAAs or via transformation of precursor PFAS to PFAAs is uncertain. However, maternal fish intake in pregnancy week 18 was a significant negative predictor of gross motor development at six months in a multiple linear regression model, possibly reflecting the negative consequences of the mixture of toxins related to maternal fatty fish intake. When evaluating health effects of PFAS in infants and older children, it is important to remember that the fetus and the infant get a cocktail of toxins (Braun and Gray, 2017; Panseri et al., 2019), which most certainly will modify the observed health effects (Braun and Gray, 2017).

4.4. *Strength and limitations*

This study was designed with longitudinal measurements during pregnancy and postpartum period, including infants at six months, with no lost to follow up. Clinical data were collected by questionnaires, prone to recall bias, but the same two doctors did all the interviews throughout the study period. Despite the limitation of rather low number of participants in the present study, we were able to demonstrate significant associations between PFAS concentrations and gross motor development in infants at age six months.

We studied gross motor function, which is a major developmental function in early infancy, using the AIMS test, considered to be among the most reliable tests used (Darrah et al., 1998). Our analyses were adjusted for other factors known to influence CNS development, like cobalamin status, weight at six months, maternal educational level and gender (Torsvik et al., 2013), and the pediatrician who performed the test, was blinded to other study results, which are a strength to the study.

5. Conclusion

We have demonstrated near linear relations between maternal PFAS concentrations in pregnancy week 18 and infant PFAS concentrations at age six months. While the concentrations declined in the mothers during pregnancy and postpartum period, the highest concentrations were seen in infants aged six months. Parity and fish intake were strong predictors

of maternal PFAS status, while maternal concentrations of PFAS in pregnancy week 18 and months of exclusive breastfeeding determined the PFAS concentrations in infants at six months.

Infants who scored below the median on gross motor development had higher PFAS concentrations than infants with a better gross motor development. A higher maternal fish intake in pregnancy week 18 was additionally associated with a poorer gross motor score in the infants at six months.

Infant gross motor development is a marker of later cognitive outcome and our findings indicate that a high concentration of PFASs in young infants may be a risk factor for impaired neurodevelopment.

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Ethical approval

Ethical approval of the protocol was granted by the Regional Committee on Medical Research Ethics, REK 2011/2447, and written informed consent was obtained from all women.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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