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Longitudinal poly- and perfluoroalkyl substances (PFAS) levels in Dutch infants

Inge A.L.P. van Beijsterveldt [a,*], Bertrand D. van Zelst [b], Sjoerd A.A. van den Berg [b,c], Kirsten S. de Fluiter [a], Manouk van der Steen [d], Anita C.S. Hokken-Koelega [a,d]

[a] Department of Pediatrics, Subdivision of Endocrinology, Erasmus University Medical Center /Sophia Children's Hospital, Rotterdam, the Netherlands
[b] Diagnostic Laboratory of Endocrinology, Erasmus University Medical Center, Rotterdam, the Netherlands
[c] Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, the Netherlands
[d] Dutch Growth Research Foundation, Rotterdam, the Netherlands

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ABSTRACT

Background and aims: Per- and polyfluoroalkyl substances (PFAS) are a potential hazard for public health. These man-made-chemicals are non-degradable with an elimination half-life of multiple years, causing accumulation in the environment and humans. Rodent studies demonstrated that PFAS are harmful, especially when present during the critical window in the first months of life. Because longitudinal data during infancy are limited, we investigated longitudinal plasma levels in infants aged 3 months and 2 years and its most important determinants.

Methods: In 369 healthy term-born Dutch infants, we determined plasma PFOS, PFOA, PFHxS, PFNA and PFDA levels at age 3 months and 2 years, using liquid chromatography-electrospray-ionization-tandem-mass-spectrometry (LC-ESI-MS/MS). We studied the associations with maternal and child characteristics by multiple regression models.

Results: At age 3 months, median plasma levels of PFOS, PFOA, PFHxS, PFNA and PFDA were 1.48, 2.40, 0.43, 0.23 and 0.07 ng/mL, resp. Levels decreased slightly until age 2 years to 1.30, 1.81, 0.40, 0.21 and 0.08 ng/mL, resp. Maternal age, first born, Caucasian ethnicity and exclusive breastfeeding were associated with higher infant's plasma levels at age 3 months. Levels at 3 months were the most important predictor for PFAS levels at age 2 years. Infants with exclusive breastfeeding during the first 3 months of life (EBF) had 2–3 fold higher levels throughout infancy compared to infants with exclusive formula feeding (EFF), with PFOA levels at 3 months 3.72 ng/mL versus 1.26 ng/mL and at 2 years 3.15 ng/mL versus 1.22 ng/mL, respectively.

Conclusion: Plasma PFAS levels decreased only slightly during infancy. Higher levels at age 3 months were found in Caucasian, first-born infants from older mothers and throughout infancy in EBF-infants. Our findings indicate that trans-placental transmission and breastfeeding are the most important determinants of PFAS exposure in early life.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) consist of > 3000 man-made chemicals, produced since the 1950s. Because of their water-, dirt- and grease-repellent quality, they are used in a variety of consumer and industrial products, like outdoor clothing, baking paper, food-packing materials, non-stick coating in pans and fire-fighting foam, among other things (Bokkers et al., 2019; Zeilmaker et al., 2018). PFAS can simply migrate into the environment. Most PFAS are not biodegradable, can easily spread through the air and water and can be taken up by plants and animals. PFAS are hydrophobic and have an increased affinity with proteins in the human body. They have a tendency to accumulate in humans, because of their very long elimination half-life up to 8.5 years (Bokkers et al., 2019; Zeilmaker et al., 2018).

A wide range of adverse effects of PFAS has been described in adults, like liver damage, increased risk of testicular and kidney cancer, thyroid

* Corresponding author at: Erasmus University Medical Center/ Sophia Children's hospital Room number: Sk-0150, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands.
E-mail address: i.vanbeijsterveldt@erasmusmc.nl (I.A.L.P. van Beijsterveldt).

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disorders and changes in plasma lipid concentrations (Bokkers et al., 2019; Zeilmaker et al., 2018; Schrenk et al., 2020). The most sensitive effect identified in children is a decreased response to tetanus, hepatitis B and diphtheria vaccinations due to prenatal PFAS exposure (Abraham et al., 2020; Grandjean et al., 2017). Rodent studies show concerning developmental effects in offspring that was exposed to high levels of PFAS during pregnancy or in early life. Effects consist of a wide range of developmental effects, such as growth restriction, altered behavioral patterns and endocrine disruption (Zeilmaker et al., 2018; Johansson et al., 2009). However, the very limited studies in infants to confirm or refute these findings show conflicting conclusions, mainly because of small study populations, cross-sectional designs or short follow-up periods (Starling et al., 2019; Braun, 2017; Averina et al., 2021).

PFAS exposure predominately takes place by inhalation of dust in air or by ingestion of PFAS in drinking water and food, particularly from fish, fruit and eggs (Schrenk et al., 2020). PFAS have been detected in human plasma, cord blood and breast milk, so PFAS can migrate from a mother to her child during the pre- and postnatal period, potentially through trans-placental transmission and breastfeeding (Pérez et al., 2013; Yang et al., 2016). However, knowledge about longitudinal exposure in infants and its most important determinants is limited. Based on data from cord blood and in infants aged $>$ 6 months, it has been postulated that PFOS and PFOA levels increase during infancy and decrease thereafter to 1.5 ng/ml at age 10.5 years (Mogensen et al., 2015; Fromme et al., 2010; Koponen et al., 2018). However, knowledge about longitudinal plasma levels and its effects in infants is lacking.

Because of the life-long negative effects of PFAS and the very limited data on longitudinal plasma PFAS levels in infants, we evaluated plasma PFOS, PFOA, PFHxS, PFNA and PFDA levels in a large cohort of healthy infants at 3 months and 2 years of age. Secondly, we determined which maternal and infant characteristics were associated with infant PFAS plasma levels and if infant feeding type was associated with PFAS plasma levels. We hypothesized that infant PFAS plasma levels would decrease during the first 2 years of life and would be associated with maternal characteristics and infant feeding.

2. Material and Methods

2.1. Subjects

This study was embedded in the Sophia Pluto study, a birth cohort study in healthy infants aiming to provide detailed data on body composition trajectories and determinants from early life to childhood (Breij et al., 2015; de Fluiter et al., 2020). Infants were recruited from several maternity wards in the Rotterdam area, The Netherlands. All participants met the following inclusion criteria: born singleton and term ($\geq$37 weeks of gestation), with an uncomplicated neonatal period. Exclusion criteria were severe asphyxia (defined as an Apgar-score below 3 after 5 min), sepsis or the need for respiratory ventilation, and any maternal disease or medication that could interfere with fetal growth, including maternal corticosteroids and diabetes mellitus, or known congenital or postnatal disease or intrauterine infection that could interfere with growth. For this study, infants with blood samples at age 3 months and/or 2 years were included. The Medical Ethics Committee of Erasmus Medical Center approved the study and written informed consent was given by all parents or caregivers with parental authority.

2.2. Data collection and measurements

Outpatient clinic visits were scheduled at age 1, 3, 6, 9, 12, 18 months and 2 years. Birth data were taken from hospital and midwife records. Maternal characteristics were obtained by interviews and questionnaires. Information about feeding type was recorded during every outpatient clinic visit and through questionnaires. Exclusive breast feeding (EBF) was defined as receiving only breastfeeding until at least the age of 3 months. Exclusive formula feeding (EFF) was defined as starting exclusive formula feeding before the age of 1 month. Mixed feeding (Mix) was defined as starting with formula feeding next to breastfeeding between 1 and 3 months of age. Living area at birth was based on zip codes and subsequently arranged as east and west Rotterdam.

2.3. Blood samples

Between 2013 and 2018 a total of 276 blood samples at age 3 months and between 2015 and 2020 310 blood samples at age 2 years were collected in 369 infants. Capillary blood was collected in EDTA microtubes (BD Microtainer®) from the infant's finger or toe at the study location. Blood was then centrifuged at 4 °C, after which plasma was separated and frozen immediately. Samples were stored at −80 °C until analysis.

2.4. PFAS analysis

PFAS analysis took place at the Diagnostic Laboratory of Endocrinology, an ISO-15189 certified laboratory, at the Erasmus University Medical Center in Rotterdam, The Netherlands.

2.4.1. Sample preparation

The PFAS plasma levels were measured in EDTA-plasma. 50 µl of plasma, calibrator or QC was mixed with 100 µl of methanol including the internal standard of each PFAS and 150 µl of 50% formic acid. Thereafter, the samples were subjected to an offline solid phase extraction using a WAX µ-elution plate (Waters™, the Netherlands) which was conditioned with methanol and water. After loading of the samples the plate was washed with 50% formic acid and 5% methanol. The PFAS were eluted with 1% of ammonia in methanol and subsequently 1:1 diluted with 10 mM ammonium acetate before analysis.

2.4.2. Lc-Esi-Ms/Ms

After the sample preparation, the different PFAS were measured using liquid chromatography-electrospray-ionization tandem mass spectrometry (LC-ESI-MS/MS) (Acquity UPLC liquid chromatograph and a Xevo-TQ-S Mass Spectrometer (Waters™, the Netherlands)). An Acquity CSH Phenyl-Hexyl column was used for separation with a gradient utilizing 10 mM ammonium-acetate as solvent A and 10 mM ammonium acetate in methanol as solvent B. A 10-minute linear gradient was used with 50%A:50%B as initial condition, leading to 37% A:63%B after 10 min before re-equilibrating the column for the next injection. Mass spectrometer setting were: capillary voltage 1.00 kV in the negative mode, cone voltage 40 V, desolvation temperature 450 °C at a gasflow of 750 L/hr and cone gasflow of 200 L/hr. Argon was used as collison gas at a flowrate of 0.19 ml/min. The targeted PFAS were total and linear Perfluorooctane sulfonic acid (PFOS & LinPFOS), total and linear Perfluorooctanoic acid (PFOA & LinPFOA), total and linear Perfluorohexane sulfonic acid (PFHxS & LinPFHxS), Perfluorononanoic acid (PFNA) and Perfluorodecanoic acid (PFDA). Two mass transitions were used for each PFAS, whereby the result of samples with an ion-ratio deviating more than 10% from the mean ion-ratio were discarded. Quantification of the PFAS was performed using separate calibration curves and a ^{13}C-labeled internal standard for each PFAS and Masslynx software was used to determine the levels. Between run precision of the assay was ensured by using quality control samples in each batch of samples along with reagent blanks. Between run precision was between 1.9% and 5.1% for all tested PFAS. Absolute- and relative matrix effects were negligible ($<$5%) and lower limit of quantification (LLoQ) was set at 0.05 ng/mL for PFNA and PFDA, 0.10 ng/mL for PFHxS and 0.15 ng/mL for PFOS and PFOA. If PFAS level was below the specific LLoQ, the plasma level was considered to be LLoQ/$\sqrt{2}$.

2.5. Statistical analysis

Of all 369 infants participating in the Sophia Pluto study, 276 had a blood sample at age 3 months and 310 at age 2 years, with 267 infants having blood samples at both time points. Reason for absent blood samples was no parental permission for blood sampling or child's resistance. SD scores for birth length and birth weight and for length, weight and BMI were calculated at every visit were calculated using Dutch references (Schönbeck et al., 2013) by Growth Analyser RCT software (http://www.growthanalyser.org). Baseline characteristics and body composition measurements are expressed as mean (SD). Not normally distributed values are expressed as median [interquartile range]. Independent student's *t*-test was used to determine differences in the baseline characteristics between boys and girls and Wilcoxon signed rank test for differences in plasma PFAS levels between age 3 months and 2 years. Correlations between plasma PFAS levels were determined with Spearman's correlation coefficient.

Multiple linear regression analysis was used to determine associated maternal and child characteristics. PFAS plasma levels at 3 months and 2 years were the dependent variables. Maternal characteristics were ethnicity, education level, living area at delivery, fertility treatment, amenorrhea duration (AD), delivery mode, first born baby, age at delivery, BMI before pregnancy, breastfeeding of any previous children and food intake during pregnancy (amount of vegetables, fruit, meat, fish and eggs per week). Infant variables were sex and infant feeding mode until age 3 months. For PFAS levels at 2 years, ethnicity of the infant, total duration of breastfeeding and PFAS plasma level at 3 were added. Using backward elimination, non-significant characteristics were removed from the model. Since PFAS accumulation changes with time, all multiple linear regression analysis were corrected for blood collection date. Repeated measures ANOVA analysis and Mann-Whitney U tests were used to determine differences between infant feeding types.

All statistical tests were performed with SPSS statistical package version 25.0 (SPSS Inc. Chicago, Illinois). Tests were performed two-sided and results were regarded statistically significant if the p-value was < 0.05.

3. Results

Child characteristics are presented in Table 1. Of all infants, 57.7 % was male and 68.8% Caucasian. Characteristics were not different between boys and girls.

3.1. PFAS plasma levels during infancy

Median [IQR] plasma levels of PFOS, PFOA, PFHxS, PFNA and PFDA are presented in Table 2 and Fig. 1. PFOS, PFOA and PFHxS levels decreased from age 3 months to 2 years, while levels of PFNA and PFDA remained similar (Table 2 and Fig. 1). There were no differences in PFAS levels between boys and girls. Because total PFOA and PFHxS levels consisted mostly of the linear isomer, only analyses with total levels are presented from this point onwards.

3.2. Association with maternal and child characteristics

The results of multiple linear regression analysis are shown in Table 3. At 3 months of age, the models explained 14.2% – 42.1% of variance in PFAS plasma levels. All PFAS levels were higher with increasing maternal age and when the child was first born. For example, when maternal age increased with 1 year, PFOS levels increased with 0.059 ng/mL. Additionally, PFOS levels were higher in case the pregnancy was conceived spontaneously instead of with fertility treatment. Besides, PFOS and PFHxS levels were higher in children of Caucasian mothers. PFOA levels were higher in children of mothers with lower pre-pregnancy BMI. Also, PFOA levels were associated with living area, with infants born in the east of the Rotterdam area having the highest plasma PFOA levels. Lastly, PFDA levels were higher with increasing gestational age. As determinants of linPFOS were similar to those of PFOS, data of only the latter were presented. Maternal education level, breastfeeding of a previous child, delivery mode and infant sex were no determinants of PFAS plasma levels. When studying maternal dietary habits, with correction of the aforementioned characteristics, only the PFDA levels at age 3 months were higher when the mother consumed more meat (β:0.121, $p = 0.037$). Other PFAS levels were not influenced by maternal dietary habits.

Table 1
Clinical characteristics in 369 Dutch infants.

	Boys	Girls	p-value
N	213	156	
Child characteristics			
Ethnicity N(%)			0.158
Caucasian	142 (70.3%)	98 (63.2%)	
Non-Caucasian	60 (29.7%)	57 (36.8%)	
Birth weight SDS	0.33 (1.06)	0.13 (1.20)	0.087
Birth length SDS *	0.70 (1.17)	0.58 (1.21)	0.455
Infant feeding			0.628
EBF	81 (38.0%)	67 (42.9%)	
Mix	74 (34.7%)	49 (31.4%)	
EFF	58 (27.2%)	40 (25.6%)	
Total breastfeeding duration (months)	6.35 (8.3)	7.37 (9.0)	0.270
Pregnancy and delivery characteristics			
Fertility treatment	27 (13.2%)	19 (13.3%)	0.989
Parity	1.58 (1.6)	1.54 (0.77)	0.674
AD (weeks)	39.63 (1.3)	39.73 (1.2)	0.434
Delivery mode N(%)			0.908
Vaginal	148 (69.5%)	111 (71.2%)	
Caesarian section	63 (29.6%)	44 (28.2%)	
Missing	2 (0.9%)	1 (0.6%)	
Maternal characteristics			
Age (years)	32.80 (4.6)	32.49 (4.7)	0.527
Pre-pregnancy BMI	24.41 (4.6)	24.13 (4.2)	0.569
Ethnicity N(%)			0.515
Caucasian	162 (77.1%)	115 (74.2%)	
Non-Caucasian	48 (22.9%)	40 (25.8%)	
Living area			0.210
East Rotterdam	132 (62.3%)	87 (55.8%)	
West Rotterdam	80 (37.7%)	69 (44.2%)	
Dietary habits (≥3 times/ week)			
Vegetables	173 (89.6%)	122 (89.1%)	0.954
Fruit	175 (91.2%)	124 (90.5%)	0.593
Meat	170 (88.5%)	121 (87.7%)	0.259
Fish	5 (2.6%)	9 (6.7%)	0.165
Eggs	24 (12.5%)	24 (17.6%)	

Data expressed as mean (SD). *Available in 122 boys and 86 girls. Abbreviations: AD = amenorrhea duration BMI = body mass index, N = number of subjects, SDS = standard deviation score.

At age 2 years, the models explained 32.6%–73.4% of the variance in PFAS plasma levels (Table 3). PFAS levels were highly correlated with plasma levels at age 3 months, with correlation coefficients for PFOS, PFOA, PFHxS, PFNA and PFDA being 0.76, 0.82, 0.82, 0.68 and 0.46, resp. (all $p < 0.001$). PFOA, PFHxS and PFNA levels were higher in Caucasian infants.

3.3. Infant feeding

Infants with exclusive breastfeeding until age 3 months (EBF) had 2–3 fold higher PFAS levels throughout infancy compared to infants with exclusive formula feeding (EFF), with PFOA levels at 3 months being 3.72 ng/mL versus 1.26 ng/mL and PFOS levels being 2.44 ng/mL versus 0.97 ng/mL. At 2 years, PFOA levels were 3.15 ng/mL versus 1.22 ng/mL and PFOS levels were 2.08 ng/mL versus 0.94 ng/mL (Table 4). Even when corrected for other characteristics by multiple linear regression analysis, EBF-infants had 1.35 ng/mL higher PFOA level compared to mix-fed infants and 2.70 ng/mL compared to EFF-

Table 2
PFAS plasma levels during infancy.

	Age 3 months					Age 24 months					Δ PFAS levels between age 3–24 months				
	N	% detected	Total group	Boys	Girls	N	% detected	Total group	Boys	Girls	N	Total group	P-value
PFOS (ng/mL)	276	100%	1.484 [0.91–2.42]	1.448 [0.90–2.36]	1.569 [0.92–2.46]	310	100%	1.302 [0.89–2.03]	1.348 [0.90–2.03]	1.298 [0.88–2.03]	208	−0.148 [-0.71–0.14]	**<0.001**
LinPFOS (ng/mL)	276	99.3%	0.749 [0.48–1.31]	0.736 [0.45–1.33]	0.786 [0.48–1.29]	310	100%	0.640 [0.43–1.00]	0.654 [0.43–0.95]	0.624 [0.44–1.05]	208	−0.097 [-0.36–0.10]	**<0.001**
PFOA (ng/mL)	276	100%	2.400 [1.30–3.89]	2.382 [1.21–4.08]	2.405 [1.39–3.77]	310	100%	1.801 [1.25–2.93]	1.811 [1.33–2.94]	1.710 [1.21–2.73]	208	−0.329 [-1.27–0.16]	**<0.001**
LinPFOA (ng/mL)	276	100%	2.350 [1.27–3.84]	2.347 [1.18–4.03]	2.350 [1.36–3.72]	310	100%	1.747 [1.20–2.87]	1.770 [1.28–2.89]	1.645 [1.17–2.68]	208	−0.330 [-1.25–0.15]	**<0.001**
PFHxS (ng/mL)	272	98.2%	0.431 [0.26–0.70]	0.415 [0.26–0.74]	0.441 [0.26–0.64]	307	98.7%	0.402 [0.27–0.61]	0.409 [0.27–0.63]	0.383 [0.26–0.58]	208	−0.004 [-0.14–0.06]	**0.047**
LinPFHxS (ng/mL)	272	98.2%	0.411 [0.25–0.68]	0.396 [0.24–0.70]	0.425 [0.25–0.63]	307	98.7%	0.381 [0.25–0.58]	0.385 [0.25–0.60]	0.367 [0.24–0.56]	208	−0.008 [-0.14–0.05]	**0.017**
PFNA (ng/mL)	276	96.7%	0.225 [0.14–0.32]	0.225 [0.13–0.31]	0.226 [0.14–0.34]	309	99.0%	0.207 [0.15–0.30]	0.212 [0.15–0.29]	0.203 [0.15–0.32]	208	0.006 [-0.02–0.03]	0.478
PFDA (ng/mL)	275	72.5%	0.071 [0.05–0.11]	0.071 [0.05–0.12]	0.072 [0.05–0.11]	309	87.4%	0.084 [0.07–0.12]	0.086 [0.07–0.12]	0.082 [0.07–0.13]	208	0.006 [-0.02–0.03]	0.080

Data are presented as median [IQR]. N = number of measurements. % detected = amount of samples with PFAS level above the lower limit of detection. Difference between PFAS levels at age 3 months and 2 years were determined with Wilcoxon signed rank test Significant p-values are boldfaced. Abbreviations: PFOS = Total Perfluorooctane sulfonic acid, LinPFOS = linear Perfluorooctane sulfonic acid, PFOA = Total Perfluorooctanoic acid, LinPFOA = linear Perfluorooctanoic acid, PFHxS = Total Perfluorohexane sulfonic acid, LinPFHxS = Linear Perfluorohexane sulfonic acid, PFNA = Perfluorononanoic acid, PFDA = Perfluorodecanoic acid. Δ PFAS levels between age 3–24 months = difference in infants PFAS plasma levels between 3 and 24 months.

infants (Table 3) and at age 2 years, PFOA levels were 0.065 ng/mL higher with each month longer duration of breastfeeding (Table 3).

All PFAS plasma levels at age 3 months were highest in EBF-infants and lowest in EFF-infants (all $p < 0.001$) (Fig. 1 and Table 4). At age 2 years, this difference had persisted (all $p < 0.04$) (Fig. 1 and Table 4). The PFAS trajectories were different between infant feeding types (Fig. 1 and Table 4). Plasma PFOA and PFOS levels decreased between age 3 months and 2 years in infants with EBF and mix feeding, but remained low and similar in EFF infants, while PFNA and PFDA levels remained higher and similar in infants with EBF and increased in infants with EFF.

4. Discussion

We present longitudinal PFAS plasma levels at 3 months and 2 years of age in a large group of healthy Dutch infants. Older maternal age, first born, Caucasian ethnicity, exclusive breastfeeding and longer total breastfeeding duration were associated with higher infant's PFAS plasma levels at age 3 months. PFAS plasma levels decreased during infancy, but levels at 3 months of age were an important predictor for PFAS levels at age 2 years. Infants with exclusive breastfeeding during the first 3 months of life had the highest PFAS concentrations throughout infancy.

Our longitudinal PFAS levels until age 2 years show a decrease, which is in line with the very limited reported longitudinal data (Mogensen et al., 2015; Fromme et al., 2010). In The Netherlands, PFAS plasma levels have only been reported for adults. The median PFOA level of an average Dutch adult was 3.4 ng/mL in 2016 (van Poll et al., 2017), which is almost two-fold higher compared to the median level we measured in infants at age 2 years. Our findings show that PFAS plasma levels decrease during infancy, but might eventually increase later in life.

At 3 months of age, PFAS plasma levels were associated with several maternal characteristics. To our knowledge, we are the first to describe the association between maternal characteristics and infant PFAS plasma levels in early life. Infant plasma levels were higher with increasing maternal age and in first-born infants. Additionally, we found that PFOS and PFHxS levels at age 3 months were higher in children from Caucasian mothers. It has been described that gestational PFAS levels increase with maternal age (Kingsley et al., 2018) and that ethnicity was a major determinant of PFAS levels in midlife women (Park et al., 2019). Also, PFAS levels were reported to be lower in multiparous women, suggesting child-birth is an important PFAS elimination route (Kingsley et al., 2018). We now show that these factors are determinants of PFAS levels in early life, potentially because older primiparous Caucasian mothers had more PFAS accumulation and subsequently more trans-placental transmission, resulting in higher PFAS levels in their infants.

In children, adolescents and adults, associations between PFAS plasma levels and high BMI have been inconsistent, with some describing positive associations and others no association (Qi et al., 2020; Averina et al., 2021; Blake et al., 2018). However, the association between maternal BMI and infant PFOA levels have not yet been reported. Our findings show that infant PFOA levels were lower with increasing maternal pre-pregnancy BMI. High levels of several PFAS were thought to influence male and female fertility, but evidence has been inconsistent (Bach et al., 2016). Our findings suggest that those who required fertility treatment, did not have infants with higher PFOS levels. Although, pregnancy duration was previously not associated with PFAS plasma levels in mothers (Manzano-Salgado et al., 2017), our findings show that a longer pregnancy duration (AD) was slightly associated with a higher PFDA level in infants. This could be the result of a longer trans-placental transmission period, from mother to her child during pregnancy, with eventually more accumulation in the infant, resulting in higher infant plasma levels. It is, however, unclear why this association was only found for PFDA. It warrants further research.

We found that infant sex and maternal education levels or dietary

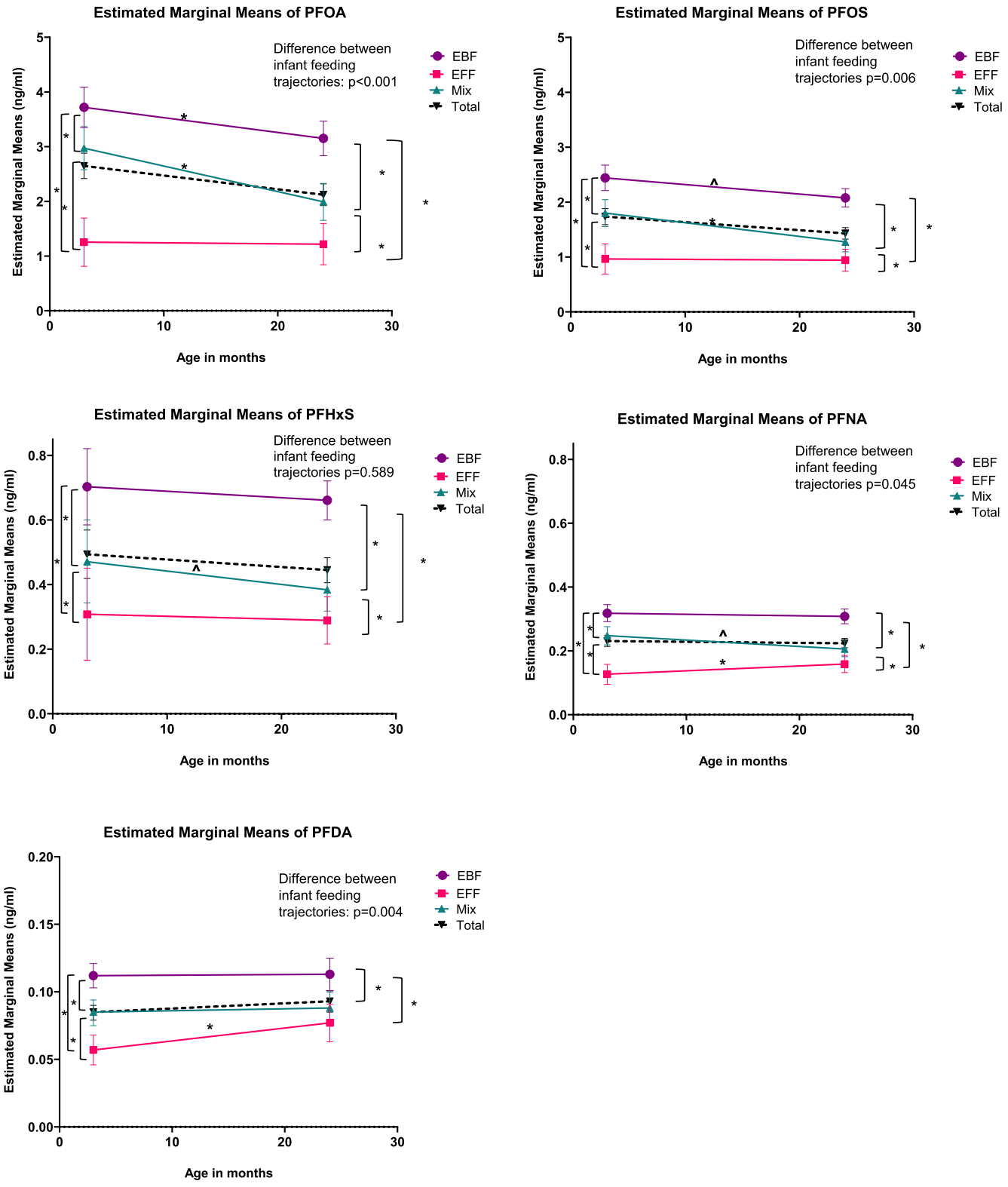

Fig. 1. PFAS plasma levels during infancy. Estimated marginal means with 95% confidence interval of infant plasma level of PFOS = Total Perfluorooctane sulfonic acid, PFOA = Total Perfluorooctanoic acid, PFHxS = Total Perfluorohexane sulfonic acid, PFNA = Perfluorononanoic acid, PFDA = Perfluorodecanoic acid at 3 and 24 months for the total group and categorized by infant feeding type., * $p < 0.001$, differences between infant feeding types at 3, 24 and Δ 3–24 months. ^ $p < 0.05$, differences between infant feeding types at 3, 24 and Δ 3–24 months. Difference between infant feeding trajectories is the differences between feeding types in PFAS trajectories between age 3 months and 2 years.

Table 3
Multiple linear regression analysis for PFAS plasma levels at age 3 months and 2 years.

	PFOS			PFOA			PFHxS			PFNA			PFDA		
	B ± SE	β	p-value	B ± SE	β	p-value	B ± SE	β	p-value	B ± SE	β	p-value	B ± SE	β	p-value
Age 3 months															
EBF_{3mo}	0.805 ± 0.08	0.52	<0.001	1.352 ± 0.13	0.51	<0.001	0.205 ± 0.04	0.32	0.008	0.104 ± 0.01	0.53	<0.001	0.029 ± 0.00	0.44	<0.001
First born	0.531 ± 0.12	0.21	<0.001	1.333 ± 0.21	0.31	<0.001	0.171 ± 0.06	0.16	0.019	0.065 ± 0.02	0.20	<0.001	0.013 ± 0.01	0.12	0.031
Maternal age	0.059 ± 0.01	0.22	<0.001	0.065 ± 0.02	0.14	0.005	0.015 ± 0.01	0.14	0.019	0.004 ± 0.00	0.13	0.010	0.002 ± 0.00	0.14	0.011
Maternal BMI				−0.055 ± 0.02	−0.12	0.020									
Mother Caucasian	0.644 ± 0.14	0.21	<0.001				0.187 ± 0.07	0.15	0.008						
Living area				0.53 ± 0.21	0.12	0.013									
Fertility treatment	−0.373 ± 0.18	−0.10	0.040												
AD													0.005 ± 0.00	0.11	0.041
Constant	84.13 ± 17.4			101.1 ± 30.0			8.27 ± 8.74			10.6 ± 2.26			3.02 ± 0.83		
Adjusted R^2 (p-value)	0.421 (<0.001)			0.413 (<0.001)			0.142 (<0.001)			0.357 (<0.001)			0.262 (<0.001)		
Age 2 years															
$PFAS_{3mo}$	0.435 ± 0.04	0.58	<0.001	0.570 ± 0.03	0.68	<0.001	0.388 ± 0.02	0.69	<0.001	0.392 ± 0.04	0.47	<0.001	0.254 ± 0.07	0.21	<0.001
EBF_{3mo}							0.071 ± 0.02	0.18	0.001						
Breastfeeding duration	0.033 ± 0.05	0.33	<0.001	0.065 ± 0.01	0.36	<0.001	0.008 ± 0.00	0.23	<0.001	0.006 ± 0.00	0.47	<0.001	0.003 ± 0.00	0.41	<0.001
Child Caucasian				0.392 ± 0.13	0.11	0.003	0.082 ± 0.03	0.12	0.003	0.027 ± 0.01	0.11	0.021			
Constant	45.90 ± 12.4			40.4 ± 18.3			10.2 ± 4.73			7.54 ± 1.66			5.07 ± 0.95		
Adjusted R^2 (p-value)	0.573 (<0.001)			0.734 (<0.001)			0.713 (<0.001)			0.579 (<0.001)			0.326 (<0.001)		

Results of Multiple linear regression analysis, corrected for sample collection date. B ± SE = Unstandardized coefficient B and Standard Error. β = standardized coefficient Beta Abbreviations: PFOS = Total Perfluorooctane sulfonic acid, PFOA = Total Perfluorooctanoic acid, PFHxS = Total Perfluorohexane sulfonic acid, PFNA = Perfluorononanoic acid, PFDA = Perfluorodecanoic acid.
EBF_{3mo}: 1 = EFF, 2 = mix, 3 = EBF. First born: 0 = no, 1 = yes, Maternal age in years, Maternal BMI in kg/m^2, Mother Caucasian: 0 = no, 1 = yes, Living area: 1 = West Rotterdam, 2 = East Rotterdam, Fertility treatment: 0 = no, 1 = yes. AD = amenorrhea duration in weeks, Breastfeeding duration: total duration of breastfeeding in months (with 2 decimals), Child Caucasian: 0 = no, 1 = yes.

Table 4
PFAS plasma level and infant feeding.

	3 months			24 months			Difference between infant feeding trajectories from 3 to 24 months		
	EBF	mix	EFF	EBF	mix	EFF	EBF vs EFF	EBF vs mix	EFF vs mix
N	112	93	71	120	104	86			
PFOS (ng/mL)	2.44 (0.12)	1.80 (0.13)	0.97 (0.14)	2.08 (0.09)	1.27 (0.09)	0.94 (0.10)	**0.037**	0.326	**<0.001**
LinPFOS (ng/mL)	1.32 (0.06)	0.91 (0.07)	0.46 (0.08)	1.05 (0.05)	0.62 (0.05)	0.50 (0.06)	**0.001**	0.913	**<0.001**
PFOA (ng/mL)	3.72 (0.19)	2.97 (0.20)	1.26 (0.22)	3.15 (0.16)	1.99 (0.17)	1.22 (0.19)	**0.002**	**0.036**	**<0.001**
LinPFOA (ng/mL)	3.66 (0.18)	2.92 (0.20)	1.22 (0.22)	3.09 (0.16)	1.94 (0.17)	1.18 (0.19)	**0.001**	**0.033**	**<0.001**
PFHxS (ng/mL)	0.70 (0.06)	0.47 (0.07)	0.31 (0.07)	0.66 (0.03)	0.38 (0.03)	0.29 (0.04)	0.314	**0.008**	0.067
LinPFHxS (ng/mL)	0.68 (0.06)	0.45 (0.07)	0.29 (0.07)	0.64 (0.03)	0.36 (0.03)	0.27 (0.04)	0.313	**0.007**	0.074
PFNA (ng/mL)	0.32 (0.01)	0.25 (0.02)	0.13 (0.02)	0.31 (0.01)	0.21 (0.01)	0.16 (0.01)	**0.006**	0.138	**<0.001**
PFDA (ng/mL)	0.11 (0.01)	0.09 (0.01)	0.06 (0.01)	0.11 (0.01)	0.09 (0.01)	0.08 (0.01)	**0.002**	0.978	**<0.001**

Data are presented as estimated marginal means (SD). Levels are presented in ng/mL. N = number of infants. Difference between infant feeding trajectories is the differences between feeding types in PFAS trajectories between age 3 months and 2 years. Abbreviations: PFOS = Total Perfluorooctane sulfonic acid, LinPFOS = linear Perfluorooctane sulfonic acid, PFOA = Total Perfluorooctanoic acid, LinPFOA = linear Perfluorooctanoic acid, PFHxS = Total Perfluorohexane sulfonic acid, LinPFHxS = Linear Perfluorohexane sulfonic acid, PFNA = Perfluorononanoic acid, PFDA = Perfluorodecanoic acid. EBF = exclusive breastfeeding until 3 months of age, EFF = exclusive formula feeding until 3 months of age, mix = mixed feeding during the first 3 months of age.

habits were not associated with infant PFAS levels. In pregnant women, results about the influence about education level and dietary habits on their PFAS levels have been inconsistent. Some described an association between lower education levels and higher PFAS levels (Sagiv et al., 2015), while others reported the opposite, partly based on dietary habits (Brantsæter et al., 2013). PFAS can be ingested by the consumption of several products, especially fish, fruit, vegetables and eggs (Schrenk et al., 2020). However, in pregnant women the change in PFAS levels due to the consumption of these products resulted in only small and non-trivial changes in PFAS levels (Brantsæter et al., 2013), which might

have led to attenuated trans-placental transmission and could, therefore, explain why we did not find an association between maternal dietary habits and infant PFAS levels.

Lastly, infants born in the eastern area of Rotterdam had higher PFOA levels compared to infants born in the west. Rotterdam is a large city, located near a large industrial and harbor region in The Netherlands. In Dordrecht, about 20 km south-east from Rotterdam, adult residents have higher median plasma levels of PFOA (10.2 ng/mL), compared to those in other regions (3.4 ng/mL), because of a local PFAS-producing chemical company, which produced PFOA until 2012 (van Poll et al., 2017). In a radius of 50 km around this company PFOA remained present in higher levels in soil and groundwater compared to 150 other areas in The Netherlands (Wintersen et al., 2020; Gebbink and van Leeuwen, 2020). The higher PFOA levels in infants born in East Rotterdam might potentially be the result of more PFAS accumulation in mothers living closer to the chemical company with subsequently more trans-placental transmission, resulting in higher PFAS levels in their infants. Despite the discontinuation of PFOA production by this company in 2012, our findings suggest that PFOA levels in infants born closer to this company remain higher. As this could have potentially negative consequences throughout life, further research is mandatory.

At age 2 years, PFAS plasma levels were highly associated with levels at age 3 months. This can be explained by the very long elimination half-life in humans, ranging from 1.7 to 3.2 years for PFNA, 3.8 years for PFOA, 5.4 years for PFOS and up to 8.5 years for PFHxS (Olsen et al., 2007; Zhang et al., 2013). Our findings show that PFAS plasma levels are measurable in considerable amounts in early life.

Our multiple linear regression models, at the age of 3 months primarily consisting primarily of maternal characteristics and at age 2 years mainly of the PFAS plasma levels at 3 months, explained a maximum of 42.1% and 73.4% of the variance in infant's PFAS levels at age 3 months and 2 years, resp. These findings suggest that PFAS levels in infants are also determined by maternal PFAS levels during pregnancy and their vertical transmission from mother to her child by migrating through the placenta. It was postulated that linear isomers are less likely to cross the placental barrier (Schulz et al., 2020), but our findings show that PFOA and PFHxS levels in early life consist predominately of linear isomers. As these levels were not different between breastfed and formula-fed infants, this suggests that linear isomers are capable of crossing the placental barrier.

We found exclusive breastfeeding until age 3 months and total duration of breastfeeding to be important determinants for PFAS levels throughout infancy. This is an important finding, as exclusive breastfeeding is highly recommended by the World Health Organization, due to its health benefits, in terms of growth outcomes, protection against obesity, infections and allergies (World Health, 2001; Kramer and Kakuma, 2012; Victora et al., 2016). Exclusive breastfeeding has been associated with higher PFAS plasma levels in children aged 3 and 8 years (Kingsley et al., 2018) and a longer duration of breastfeeding was associated with higher PFAS levels at 5 years of age (Mogensen et al., 2015). We now add that PFAS plasma levels at 3 months are already 2–3 times higher in exclusively breastfed infants compared to mixed fed and EFF infants and that this remains present until at least 2 years of age. In fact, infants with EBF had similar PFAS plasma levels as Dutch adults (van Poll et al., 2017). These findings show that breastfeeding is an important PFAS exposure pathway in infants. This is particularly concerning because the first months of life are known to be a critical window for adiposity programming and an important period for the programming of growth, neurodevelopment and immune system. Our findings could, therefore, indicate that infants with exclusive breastfeeding in the first months of life and those with a longer total duration of breastfeeding are more prone to the potential adverse developmental effects of PFAS in early life, such as growth restriction, increased adiposity, altered behavioral patterns, endocrine disruption and decreased vaccination response (Zeilmaker et al., 2018; Abraham et al., 2020; Grandjean et al., 2017; Johansson et al., 2009), which could limit the health benefits of breastfeeding (World Health, 2001; Kramer and Kakuma, 2012; Victora et al., 2016). However, currently the knowledge about safe infant PFAS levels is lacking. Further research is, therefore, mandatory.

In contrast, EFF-infants had the lowest PFAS plasma levels, which remained low until 2 years of age. Only one research group measured PFAS in infant formula and reported low PFAS levels (Macheka et al., 2021). It has been calculated that approximately 5% of the total oral PFAS exposure can be attributed to drinking water in The Netherlands (van der Aa et al.). Our findings suggests that exposure to PFAS due to formula feeding is negligible during infancy and that exclusive formula feeding contributes to only minor PFAS accumulation between age 3 months and 2 years, especially compared with exclusive breastfeeding.

The PFOS and PFOA levels in our cohort were lower compared to 3 other studies conducted in Scandinavia and Germany, who reported these levels in infants aged 6 – 19 months, ranging from 3.0 to 24.0 ng/mL and 4.6 – 8.2 ng/mL, resp. (Mogensen et al., 2015; Fromme et al., 2010; Koponen et al., 2018). However, these study populations were smaller compared to ours, consisted predominantly of breastfed infants and were conducted between 1997 and 2010. Since several PFAS have been enlisted as Persistent Organic Pollutants (POPs) by the Stockholm convention in 2009, guidelines regarding the reduction of these chemicals have been installed and production of PFOS and PFOA have been phased out from industrial production lines in Europe ever since (Commision, 2020; Parliament, 2019). Also, the European Food Safety Authority (EFSA) adjusted the safety thresholds for PFAS intake through food (Schrenk et al., 2020). We collected blood samples between 2013 and 2020, which could explain why we measured lower plasma levels compared to other research groups.

The strength of our study is the large collection of longitudinal blood samples in a prospective cohort of healthy Dutch infants in which PFAS plasma analyses were performed, using a high quality measuring method. We acknowledge some limitations, the blood samples were collected during two 5-year periods between 2013 and 2020. Over the last years, health concerns with regard to PFAS have been risen, guidelines to phase out the production of several PFAS have been set up and, therefore, PFAS levels are likely to decrease with time. We, therefore, have adjusted the multiple linear regression analyses for collection date. Also, plasma level trajectories between age 3 months and 2 years were not different in infants born in the first and last year of the study period and the time between PFAS sampling in the same child is shorter than the elimination half-life. We, therefore, consider the influence of the sampling period on our findings on PFAS trajectories in infancy and its determinants to be small. Also, we did not collect maternal blood samples, which could have potentially given more detailed insight in the trans-placental transmission of PFAS. However, we measured the infant's PFAS levels already at 3 months of age.

In conclusion, Dutch infants aged 3 months have considerable PFAS plasma levels, which only slightly decrease until age 2 years. PFAS levels at age 3 months and 2 years were highly correlated. Higher PFAS levels were especially found in first-born infants of older primiparous Caucasian mothers. Infants with exclusive breastfeeding for the first 3 months of life had 2–3 fold higher longitudinal PFAS levels compared to exclusive formula fed infants. Our findings indicate that trans-placental and breastfeeding transmission are the most important determinants of PFAS exposure in early life, with potentially life-long effects, which require further research.

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Conflict of interest

The Sophia Pluto study is an investigator-initiated cohort study, for

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Author contributions

AHK was in charge of designing the study. IvB, KdF and AHK were in charge of the cohort, design, and collecting of the data and samples. BvZ and SvdB were in charge of the design of the laboratory method. BvZ conducted all PFAS analysis. IvB performed the statistical analysis. Drafting the manuscript was primarily done by IvB under supervision of AHK. All authors were involved in writing the manuscript and had final approval of the submitted version.

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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