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Original Research

Early life poly- and perfluoroalkyl substance levels and adiposity in the first 2 years of life

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Abstract

Importance: Poly- and perfluoroalkyl substances (PFASs) are nondegradable, man-made chemicals. They accumulate in humans with potential harmful effects, especially in susceptible periods of human development, such as the first months of life. We found that, in our cohort, exclusively breastfed (EBF) infants had 3 times higher PFAS plasma levels compared with exclusively formula-fed (EFF) infants at the age of 3 months. Thus, PFASs could potentially reduce the health benefits of breastfeeding.

Objective: We investigated the associations between PFAS levels at the age of 3 months and accelerated gain in fat mass during the first 6 months of life, body composition at 2 years, and whether these associations differ between EBF and EFF infants.

Setting: In 372 healthy term-born infants, we longitudinally assessed anthropometrics, body composition (by air-displacement plethysmography and dual-energy X-ray absorptiometry), and visceral and subcutaneous fat (by abdominal ultrasound) until the age of 2 years.

Measures: The plasma levels of 5 individual PFASs were determined by liquid chromatography–electrospray ionization–tandem mass spectrometry at the age of 3 months.

Main outcomes: We studied associations between PFAS levels and outcomes using multiple regression analyses.

Results: Higher early life plasma perfluorooctanoic acid and total PFAS levels were associated with an accelerated gain in fat mass percentage [FM%; >0.67 SD score (SDS)] during the first 6 months of life. Higher early life PFAS levels were associated with lower fat-free mass (FFM) SDS at the age of 2 years, but not with total FM% SDS at 2 years. Furthermore, we found opposite effects of PFAS levels (negative) and exclusive breastfeeding (positive) at the age of 3 months on FFM SDS at 2 years.

Conclusion: Higher PFAS levels in early life are associated with accelerated gains in FM% during the first 6 months of life and with lower FFM SDS at the age of 2 years, which have been associated with an unfavorable body composition and metabolic profile later in life. Our findings warrant further research with longer follow-up times.

Keywords: PFAS exposure, children, body composition, early life, rapid rise in fat mass

Significance

Poly- and perfluoroalkyl substances (PFASs) are an increasing burden for society, since they are nondegradable, man-made chemicals that can accumulate in humans with potential harmful effects. Our findings show that higher early life plasma PFAS levels are associated with accelerated gain in fat mass percentage (FM%) during the first 6 months of life, which is considered a critical window of adiposity programming. In addition, these higher PFAS levels are associated with lower fat-free mass (FFM) at the age of 2 years. As early accelerated gain in FM% and decreased FFM are associated with an unfavorable metabolic profile in young adults, our findings indicate that higher PFAS levels in early life could potentially lead to an unfavorable body composition and metabolic profile later in life.

Introduction

Poly- and perfluoroalkyl substances (PFASs) are a group of thousands of 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 such as food-packing materials and nonstick coating in pans. They are mostly nondegradable and can easily spread through the air and water. Poly- and perfluoroalkyl substances can be taken up by plants, animals, and humans. Because of their

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very long elimination half-life of up to 8.5 years, they have a tendency to accumulate in humans.[1,2] Poly- and perfluoroalkyl substances are considered to be "endocrine-disrupting chemicals", a group of chemicals that have been described to interfere with endocrine processes and cause adverse effects on perinatal, neurodevelopmental, metabolic, and reproductive outcomes, especially when exposure occurs during susceptible periods of human development, such as early life.[3] Rodent studies show concerning effects in offspring exposed to high levels of PFASs during pregnancy or in early life. These adverse effects consist of a wide range of developmental effects such as growth restriction, altered behavioral patterns, and endocrine disruption.[2,4] Except from a decreased vaccination response,[5] human studies to confirm or refute these findings are extremely scarce and have conflicting conclusions, partly because of small study populations and short or even a lack of follow-up periods.[6-8] Particularly, the potential association between PFAS exposure and adiposity development is of interest, since the period from which (childhood) obesity has become an increasing worldwide problem overlaps with the period from which production of PFASs and their presence in the environment have increased.

The first months of life are thought to be a critical window of adiposity programming, in which especially accelerated weight gain in childhood leads to a disproportionately high fat mass (FM) and lower muscle mass later in life, subsequently causing insulin resistance, disturbances in lipid metabolism, and cardiovascular diseases in adulthood.[9,10] Earlier, we and others presented that accelerated weight gain for height in the first 3 months of life was associated with a higher body fat percentage, higher blood pressure, and unfavorable metabolic health in young adults.[11-14] Furthermore, we found that infants with accelerated gains in FM during the first 6 months of life had higher FM trajectories until 2 years of age.[15] Poly- and perfluoroalkyl substance exposure during this susceptible period in early life could potentially lead to alterations in adiposity programming and subsequently to more overweight and obesity later in life.

Some studies have investigated the associations between prenatal PFAS exposure and outcomes in childhood. Associations have been found between maternal serum PFAS levels during pregnancy and a higher body mass index (BMI) during childhood[1,16-18] and a higher risk of being overweight or obese in childhood and adolescence.[8,19,20] However, it is well known that children with comparable weight or BMI have a highly variable body composition.[21,22] Only a few research groups have studied the association between prenatal PFAS exposure and measured body composition during childhood, but findings have been inconsistent.[6,23-25] Furthermore, maternal serum PFAS levels might be unreliable proxies for early life PFAS exposure of the infant, as trans-placental transmission of PFASs differs per PFAS subtype.[26] Until now, the effects of early postnatal PFAS exposure are unknown.

In the area around Rotterdam, PFASs, especially perfluorooctanoic acid (PFOA), can be measured in higher concentrations in the environment compared with other Dutch areas.[27] Particularly around the chemical company "DuPont/Chemours" in Dordrecht, local residents have considerably higher than average concentrations of PFOA in their serum.[28] Recently, we reported longitudinal plasma PFAS levels in a large group of healthy term-born children aged 3-24 months, participating in the Sophia Pluto cohort, and living in the Rotterdam area, The Netherlands.[29] We found that plasma PFAS levels barely decreased between the ages of 3 and 24 months and that first-borns of older mothers, and those who lived closer to the abovementioned PFAS-producing company, had the highest plasma PFAS levels. Furthermore, we studied PFAS levels in infant feeding and found that PFASs could be detected in human milk but not in infant formula.[30] In fact, in this cohort, exclusively breastfed (EBF) infants had persistently 2-3 times higher plasma PFAS levels compared with exclusively formula-fed (EFF) infants, which were comparable with levels in Dutch adults,[29] indicating that not only transplacental transmission but also human milk is an important PFAS exposure pathway in early life. Breastfeeding is known for its health benefits such as protection against obesity and adverse metabolic profile.[31-33] Thus, PFAS exposure through human milk could potentially diminish these protective effects of human milk, but the differences in the effects of PFASs between EBF and EFF infants have never been reported.

We, therefore, investigated the association between 5 individual plasma PFAS levels and the sum of these PFAS levels at the age of 3 months and accelerated gain in FM during the first 6 months of life. Furthermore, we studied the associations between plasma PFAS levels at the age of 3 months and anthropometrics, adiposity outcomes measured as the total FM, abdominal subcutaneous and visceral fat, and total fat-free mass (FFM) at the age of 2 years. Lastly, we studied whether these associations were different for infants with exclusive breastfeeding and exclusive formula feeding, in order to evaluate whether PFAS exposure through human milk diminishes the known health benefits of breastfeeding. We hypothesized that higher plasma PFAS levels in early life are associated with accelerated gain in FM during the first 6 months of life.

Methods

Subjects

This study was embedded in the Sophia Pluto study, a large prospective birth cohort study in healthy term-born infants, aiming to provide detailed data on body composition trajectories from early life to childhood.[15,34] Infants were recruited from several maternity wards in the Rotterdam area, The Netherlands. All participants met the following inclusion criteria: born term (≥37 weeks of gestation), with an uncomplicated neonatal period. Exclusion criteria were severe asphyxia (defined as an Apgar score below 3 at 5 min), sepsis or the need for respiratory ventilation, and any known genetic, congenital, or postnatal disease, intrauterine infection and maternal disease, or medication use that could interfere with fetal growth, including maternal corticosteroids and diabetes mellitus. For this study, infants who completed the study visit at the age of 2 years and had their blood sampled were included. The Medical Ethics Committee of Erasmus Medical Centre approved the study, and written informed consent was given by all parents or caregivers with parental authority.

Data collection and measurements

Study visits were scheduled at 1, 3, 6, 9, 12, 18 months, and 2 years. Birth data were taken from hospital and midwife records. Parental characteristics were obtained by interviews and questionnaires. Information about feeding type was recorded during every outpatient clinic visit and through questionnaires. Exclusive breastfeeding was defined as receiving

only breastfeeding until at least the age of 3 months. Exclusive formula feeding was defined as starting exclusive formula feeding before the age of 1 month. Mixed feeding (Mix) was defined as starting with formula feeding after breastfeeding between 1 and 3 months of age.

Anthropometrics

Weight was measured to the nearest gram by using an electronic infant scale (Seca 717, Hamburg, Germany). Length was measured in the supine position to the nearest 0.1 cm by using an infantometer (Seca 416). Head, waist, and hip circumference were measured to the nearest 0.1 cm by using a circumference measuring tape (Seca 201). Hip-to-waist ratio was calculated as waist circumference (cm) divided by hip circumference (cm).

Body composition

Total body FM and FFM were measured with air-displacement plethysmography by PEA POD, Infant Body Composition System (COSMED) during the visits at 1, 3, and 6 months of age, and by a dual-energy X-ray absorptiometry (DXA) scan (GE Prodigy Advance R000279) with encore 14.1 software during the visits from the age of 9 months onward. During the DXA scan, children wore only a diaper or underwear. To prevent unsuccessful DXA scan due to movement artifacts, children were swaddled in a vacuum cushion (465 75100, Schmidt, Germany).[35] During the study, the same machines were used, which were calibrated daily and used according to the protocol recommended by the suppliers. Fat mass percentage (FM%) was calculated as FM divided by total body weight (kg) times 100%.

Abdominal fat

Visceral and subcutaneous fat were determined at every visit starting from 3 months onward, using ultrasound [Prosound 2 ultrasound with a UST-9137 convex transducer (both Hitachi Aloka Medical, Zug, Switzerland)]. Both were measured in the supine position, with the transducer on the intercept of the xiphoid line and the waist circumference measurement plane. Visceral fat was measured in the longitudinal plane from the peritoneal boundary to the corpus of the lumbar vertebra with a probe depth of 9 cm and subcutaneous fat in the transverse plane from the cutaneous boundary to the linea alba with a probe depth of 4 cm. Minimal pressure was applied. To adjust for body size, all measurements were corrected for length SD score (SDS). Reproducibility of the measurements was confirmed in a previous study.[36] The relative interobserver technical error of measurement was 3.2% for visceral fat and 3.6% for subcutaneous fat. The visceral-to-subcutaneous ratio was calculated as visceral fat divided by subcutaneous fat.

Sample collection

Between 2013 and 2018, a total of 276 blood samples at the age of 3 months and between 2015 and 2020, 310 blood samples at the age of 2 years were collected in 372 infants. The first blood sample was taken at the age of 3 months, since at this age, both exposure through pregnancy and infant feeding affect the plasma PFAS level. Capillary blood was collected in EDTA microtubes (BD Microtainer®) from the infants' toe or finger at the study location. Blood was then centrifuged at 4 °C, after which plasma was separated and frozen immediately. All samples were stored at −80 °C until analysis.

PFAS analysis

Poly- and perfluoroalkyl substance analyses were performed at the Diagnostic Laboratory of Endocrinology, an ISO-15189 certified laboratory, at the Erasmus University Medical Center in Rotterdam, The Netherlands.

The different PFASs were measured using liquid chromatography–electrospray ionization–tandem mass spectrometry (Acquity UPLC liquid chromatograph and a Xevo-TQ-S Mass Spectrometer; Waters™, The Netherlands). The targeted PFASs were total perfluorooctane sulfonic acid (PFOS), total PFOA, total perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA). We have described the process in more detail previously.[29] In summary, 2 mass transitions were used for each PFAS, whereby the results of samples with an ion ratio deviating more than 10% from the mean ion ratio were discarded. Quantification of the PFASs was performed using separate calibration curves, and a ^{13}C-labeled internal standard for each PFAS and Masslynx software was used to determine the different PFAS concentrations. Between-run precision of the assay was ensured by using quality control samples in each batch of samples. The coefficient of variation was between 1.9% and 5.1% for all tested PFASs. Absolute and relative matrix effects were negligible (<5%), and the 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 the PFAS level was below the specific LLoQ, the plasma level was considered to be LLoQ/$\sqrt{2}$.

Statistical analysis

The SD scores were calculated to correct for (gestational) age and sex. The scores for (birth-)length, (birth-)weight, and weight for length were calculated based on Dutch references, using Growth Analyser software (http://www.growthanalyser.org)[37,38] and for FM, FM%, and FFM, using reference values obtained by the same machines.[35] Accelerated gain in FM% during the first 6 months of life was defined as an increase of FM% > 0.67 SDS between 1 and 6 months of age.[15]

Clinical characteristics are expressed as mean (SD) and non-normally distributed values as median (interquartile range). Plasma PFAS levels were analyzed as individual levels and as total plasma PFAS levels. One-way analysis of variance and χ^2 tests were used to determine differences in the baseline characteristics between exclusively breastfed, mixed fed, and EFF infants.

In order to obtain normal distribution, plasma PFAS levels were ^{10}log-transformed. In our primary analyses, logistic regression analyses and multiple regression analyses were used to determine the association between plasma PFAS levels at the age of 3 months (independent variable) and accelerated gain in FM% between 1 and 6 months of age and several anthropometric and body composition measures at the age of 2 years, as dependent variables, respectively. Dependent variables were accelerated gain in FM% (yes = 1/no = 0), weight-for-length SDS, length SDS, head circumference SDS, FM% SDS, FFM SDS, waist circumference (cm), subcutaneous abdominal fat (cm), and visceral fat (cm). All analyses were corrected for sex, infant feeding type, and birth weight SDS, and additionally for change in length SDS between 1

Table 1. Clinical characteristics.

	Exclusive breast feeding	Mixed feeding	Exclusive formula feeding	*P*-value
N	150	125	97	
Birth				
Boys (%)	83 (55.3%)	74 (59.2%)	58 (59.8%)	.729
Gestational age (weeks)	39.9 (1.2)	39.6 (1.2)	39.4 (1.3)	**.011**
Ethnicity				.656
White	101 (67.3%)	82 (65.6%)	72 (74.2%)	
Black	10 (6.7%)	11 (8.8%)	6 (6.2%)	
Other and mixed	35 (23.4%)	28 (22.4%)	15 (15.4%)	
Missing	4 (2.6%)	4 (3.2%)	4 (4.1%)	
Birth weight SDS	0.26 (1.1)	0.16 (1.2)	0.32 (1.2)	.569
Birth length SDS[a]	0.78 (1.1)	0.51 (1.3)	0.54 (1.2)	.322
Age 3 months				
Weight SDS	0.10 (1.0)	0.15 (0.9)	0.24 (1.0)	.316
Length SDS	0.09 (0.8)	0.28 (1.1)	0.21 (0.9)	.531
Sum plasma PFAS level (ng/mL)	7.35 (5.2-9.4)	4.71 (3.2-6.7)	2.37 (1.95-3.04)	**<.001**
PFOA (ng/mL)	3.41 (2.5-4.9)	2.45 (1.7-3.8)	1.07 (0.8-1.3)	**<.001**
PFOS (ng/mL)	2.20 (1.4-3.2)	1.52 (1.0-2.1)	0.87 (0.6-1.2)	**<.001**
PFHxS (ng/mL)	0.62 (0.4-0.8)	0.41 (0.3-0.7)	0.26 (0.2-0.3)	**<.001**
PFDA (ng/mL)	0.11 (0.07-0.14)	0.07 (0.06-0.11)	0.05 (0.04-0.07)	**<.001**
PFNA (ng/mL)	0.30 (0.2-0.4)	0.22 (0.2-0.3)	0.11 (0.09-0.15)	**<.001**
Age 2 years				
Weight SDS	−0.29 (1.0)	−0.12 (1.1)	−0.26 (1.1)	.385
Length SDS	0.19 (0.9)	0.36 (1.0)	0.21 (0.9)	.313
Sum plasma PFAS level (ng/mL)	6.14 (4.7-7.5)	3.57 (2.7-5.2)	2.73 (2.2-3.3)	**<.001**
PFOA (ng/mL)	2.94 (2.1-3.9)	1.69 (1.4-2.4)	1.19 (1.0-1.5)	**<.001**
PFOS (ng/mL)	2.02 (1.4-2.7)	1.16 (0.8-1.8)	0.92 (0.7-1.2)	**<.001**
PFHxS (ng/mL)	0.61 (0.5-0.8)	0.37 (0.3-0.5)	0.26 (0.2-0.3)	**<.001**
PFDA (ng/mL)	0.11 (0.07-0.14)	0.08 (0.06-0.11)	0.07 (0.06-0.09)	**<.001**
PFNA (ng/mL)	0.29 (0.2-0.4)	0.19 (0.1-0.3)	0.16 (0.1-0.2)	**<.001**

Data expressed as mean (SD) or median (interquartile range) if not normally distributed. Significant differences are boldfaced.
Abbreviations: EBF, exclusively breastfed; PFAS, poly- and perfluoroalkyl substance; PFDA, perfluorodecanoic acid; PFHxS, total perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, total perfluorooctanoic acid; PFOS, total perfluorooctane sulfonic acid; SDS, SD score; Sum, sum of PFAS levels.
[a]Birth length available in 92 EBF infants, 62 mix, and 56 EFF infants.

and 6 months of age and length SDS at the age of 2 years for the logistic regression analyses and the multiple regression analyses for body composition and abdominal fat outcomes at 2 years, respectively. Using forward elimination, we found that ethnicity, gestational age, maternal parity, education level, and prepregnancy BMI were not associated with any outcome at 2 years, and were, therefore, not included in the analyses. Secondly, we analyzed whether the associations between plasma PFAS levels at the age of 3 months and body composition at the age of 2 years were different in children with exclusive breastfeeding compared with those with exclusive formula feeding, by repeating the abovementioned analyses without mixed fed infants (EFF = 1, EBF = 2).

All statistical tests were performed using SPSS statistical package version 25.0 (SPSS Inc., Chicago, IL, USA). Missing data were deleted listwise. Two-sided tests were performed, and the results were considered statistically significant if the *P*-value was <.05.

Results

Child characteristics are presented in Table 1. A total of 372 children were included in this study. One hundred and fifty children (40.3%) were exclusively breastfed for at least 3 months. Of all children, 57.8% were males and 68.5% Caucasian. Data on plasma PFAS levels were described in detail previously.[29] In short, the median sum of plasma PFAS levels was highest in exclusively breast fed infants and lowest in EFF infants ($P < .001$). In EBF and mixed fed infants, total PFAS levels decreased between ages 3 months and 2 years ($P < .001$). In EFF infants, PFAS levels remained similar during this period ($P = .382$).

Accelerated gain in FM percentage in early life

Previously, our research group reported that infants with accelerated gain in FM% (>0.67 SDS) during the first 6 months of life had higher FM trajectories until the age of 2 years.[15] For infants included in the present study, we also found that those with an accelerated gain in FM% SDS during the first 6 months of life had a higher mean FM% SDS at the age of 2 years [mean FM% SDS (SD): 0.38 (0.8) SDS], compared with those without accelerated gain in FM% SDS [mean FM % SDS (SD): 0.04 (1.0) SDS, $P = .05$]. The occurrence of accelerated gain in FM% was significantly different between EBF and EFF infants.

We found that infants with higher total PFAS levels at the age of 3 months had 4.10 higher odds ($P = .034$) for an accelerated gain in FM% during the first 6 months of life (Table 2). This was predominantly driven by plasma PFOA levels, since infants with higher PFOA levels at the age of 3 months had 3.86 higher odds ($P = .021$) for an accelerated gain in FM% during the first 6 months of life. For the other individual plasma PFAS levels, we found no association with an accelerated gain in FM% SDS.

Age 2 years

Anthropometrics

We found no associations of plasma PFAS levels at the age of 3 months with height or head circumference SDS at the age of

2 years. Plasma PFAS levels at the age of 3 months were negatively associated with weight-for-height SDS at the age of 2 years (Table 3). There was no difference between boys and girls (data not shown).

Adiposity and abdominal fat

Total and individual plasma PFAS levels at the age of 3 months were not associated with FM% SDS at the age of 2 years (Table 3). Only PFNA levels at the age of 3 months showed a weak inverse association with FM% SDS at the age of 2 years ($P = .035$).

Total and individual plasma PFAS levels at the age of 3 months, except PFHxS and PFDA, were inversely associated with abdominal subcutaneous fat (Table 3). We found no associations between plasma PFAS levels at the age of 3 months and visceral fat at the age of 2 years. There was also no association between plasma PFAS levels at the age of 3 months and fat distribution, measured as waist-to-hip ratio and abdominal visceral-to-subcutaneous fat ratio at the age of 2 years (data not available). No sex-specific differences were found (data not available).

Total body FFM

All individual and total plasma PFAS levels at the age of 3 months were inversely associated with total body FFM SDS, corrected for height, at the age of 2 years (Table 3), which meant that higher plasma PFAS levels at the age of 3 months were associated with lower FFM SDS at the age of 2 years. There were no sex-specific differences (data not available).

Influence of infant feeding type

Using multiple regression analyses, we also investigated whether the associations between plasma PFAS levels at the age of 3 months and outcomes at the age of 2 years were different for children with exclusive breastfeeding during 3 months, compared with those with exclusive formula feeding (Table 4). We found that the regression coefficients for the associations between plasma PFAS levels at the age of 3 months and FM% SDS at the age of 2 years were significantly different for children with exclusive breastfeeding compared with those with exclusive formula feeding ($P = .010$) and that the effect of PFASs on FM% SDS at the age of 2 years was different in EBF infants compared with that in EFF infants, with a lower FM% SDS at the age of 2 years for every increase in PFAS level at the age of 3 months in EBF infants compared with that in EFF infants

Feeding type and plasma PFAS levels at the age of 3 months were significantly associated with FFM SDS at the age of 2 years (Table 4). Exclusive breastfeeding had a positive regression coefficient, indicating that FFM SDS at the age of 2 years was higher in EBF infants than in EFF infants. In contrast, plasma PFAS levels at the age of 3 months had an inverse association, indicating that higher plasma PFAS levels at the age of 3 months were associated with lower FFM SDS at the age of 2 years. This meant that higher PFAS levels, compared with breastfeeding, in early life had an opposite association on FFM at the age of 2 years.

Discussion

To our knowledge, we are the first to show that higher individual PFOA and total plasma PFAS levels at the age of 3 months

Table 2. Logistic regression analysis for PFAS plasma levels at the age of 3 months and accelerated gain in FM% during the first 6 months of life.

	PFOA		PFOS		PFHxS		PFDA		PFNA		Sum	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Accelerated gain in FM%	**3.863 (1.23-12.15)**	**.021**	2.304 (0.72-7.37)	.159	2.461 (0.76-8.02)	.135	1.285 (0.30-5.51)	.735	1.619 (0.43-6.1)0	.476	**4.102 (1.12-15.08)**	**.034**

The results of multiple logistic regression analysis, corrected for infants' sex birthweight SDS, feeding type, and rise in length SDS in the same period. Data are presented as odds ratio and 95% CIs. Accelerated gain in FM% is defined as an increase of >0.67 SDS in FM% during the first 6 months of life. Significant results are boldfaced.
Abbreviations: FM%, fat mass percentage; PFAS, poly- and perfluoroalkyl substance; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, SD score; Sum, sum of PFAS levels.

Table 3. Regression coefficients for PFAS plasma levels at the age of 3 months and outcomes at the age of 2 years.

Plasma PFAS levels at 3 months		Anthropometrics			Adiposity				Fat-free mass
		Height SDS	Weight-for-height SDS	Head circumference SDS	FM% SDS	Waist circumference	Visceral fat	Subcutaneous fat	FFM SDS
PFOA	B ± SE	0.087 ± 0.21	−0.987 ± 0.22	− 0.103 ± 0.18	−0.363 ± 0.26	−2.546 ± 0.70	−0.129 ± 0.15	−0.059 ± 0.03	−0.634 ± 0.17
	P-value	.679	**<.001**	.575	.167	**<.001**	.379	**.019**	**<.001**
PFOS	B ± SE	−0.085 ± 0.22	−0.855 ± 0.23	0.059 ± 0.19	−0.482 ± 0.26	−1.938 ± 0.69	−0.181 ± 0.15	−0.057 ± 0.03	−0.598 ± 0.17
	P-value	.693	**<.001**	.751	.070	**.006**	.223	**.025**	**<.001**
PFHxS	B ± SE	−0.083 ± 0.22	−0.856 ± 0.23	−0.111 ± 0.19	−0.185 ± 0.27	−1.891 ± 0.70	−0.201 ± 0.15	−0.041 ± 0.03	−0.621 ± 0.17
	P-value	.703	**<.001**	.557	.486	**.007**	.184	.118	**<.001**
PFDA	B ± SE	−0.246 ± 0.28	−1.009 ± 0.30	−0.040 ± 0.24	−0.540 ± 1.30	−2.386 ± 0.91	−0.315 ± 0.20	−0.048 ± 0.03	−0.763 ± 0.22
	P-value	.383	**<.001**	.870	.677	**.009**	.109	.160	**<.001**
PFNA	B ± SE	−0.138 ± 0.25	−1.023 ± 0.27	0.031 ± 0.22	−0.625 ± 0.30	−2.740 ± 0.81	−0.219 ± 0.17	−0.061 ± 0.03	−0.694 ± 0.19
	P-value	.582	**<.001**	.887	**.035**	**<.001**	.208	**.043**	**<.001**
Sum	B ± SE	0.046 ± 0.24	−1.171 ± 0.25	−0.072 ± 0.21	−0.485 ± 0.29	− 2.897 ± 0.75	−0.218 ± 0.16	−0.069 ± 0.03	−0.756 ± 0.19
	P-value	.846	**<.001**	.729	.099	**<.001**	.187	**.016**	**<.001**

The results of multiple linear regression analysis. Anthropometrics are corrected for infants' sex, birth weight SDS, and feeding type. Adiposity and FFM outcomes are additionally corrected for length SDS at the age of 2 years. Poly- and perfluoroalkyl substance levels are ^{10}log-transformed. Feeding type: 1 = EFF, 2 = mix, 3 = EBF. Sex: 0 = male, 1 = female. Significant results are boldfaced.
Abbreviations: FM, fat mass; FFM, fat-free mass; PFAS, poly- and perfluoroalkyl substance; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SDS, SD score; Sum, sum of PFAS levels.

are associated with an accelerated gain (>0.67 SDS) in FM% during the first 6 months of life, which is known to be related with unfavorable body composition and metabolic outcomes in young adults. Furthermore, we found that higher individual and total plasma PFAS levels at the age of 3 months are associated with lower FFM SDS but not (yet) with increased adiposity at the age of 2 years. Lastly, we found opposite associations of plasma PFAS levels (negative) and exclusive breastfeeding (positive) at the age of 3 months with FFM SDS at the age of 2 years.

We showed that plasma PFOA and total PFAS levels at the age of 3 months were associated with an accelerated gain in FM% during the first 6 months of life but not with increased FM% at the age of 2 years. We and others showed that accelerated gain in weight for length during the first months of life was associated with higher FM%, higher waist circumference, and more central adiposity, as well as decreased insulin sensitivity during adolescence and at the age of 21 years.[11-14] Children with accelerated weight gain in early life had a significantly higher weight for length at the age of 6 months but not anymore at the age of 1 and 3-6 years.[11,39] However, we also showed that higher weight for length reappeared later in life, together with an unfavorable body composition in early adulthood.[11] Altogether, these findings suggest that accelerated gain in FM% during the first months of life is associated with increased adiposity and an unfavorable metabolic profile in young adulthood but do not yet manifest in young children. Therefore, our finding that higher plasma PFAS levels at the age of 3 months are associated with accelerated gain in FM% during the first 6 months of life might suggest that high PFAS levels in early life could have adverse effects on FM%, not yet at the age of 2 years, but later in life, which warrants further research.

Our findings also show an association between higher early life plasma PFAS levels and lower FFM SDS at the age of 2 years. Previously, we reported that FFM tracks from infancy into childhood.[40] Fat-free mass is important for metabolic processes such as resting energy expenditure and glucose uptake. Low muscle mass in infants has been associated with metabolic syndrome, type 2 diabetes mellitus, and cardiovascular events later in life.[41,42] Altogether, our findings might suggest that the inverse association between early life plasma PFAS levels and FFM SDS at the age of 2 years could contribute to an unfavorable body composition with lower lean body mass and unfavorable metabolic outcomes later in life. This also warrants further research with a long-term follow-up.

In addition to the total group, we studied whether the associations were different between EBF and EFF infants. Higher plasma PFAS levels were associated with lower FM% SDS at the age of 2 years in EBF infants but not in EFF infants. The World Health Organization prescribes exclusive breastfeeding for at least the first 6 months,[31] because of its health benefits in terms of growth outcomes and protection against obesity, infections, and allergies.[31-33] In our cohort, EBF infants have 2-3 times higher plasma PFAS concentrations compared with EFF infants.[30] The higher PFAS exposure in early life could make EBF infants potentially more prone to not only decreased vaccination response,[5,43] but also other potential adverse developmental effects of PFAS exposure. In 2021, the Agency for Toxic Substances and Disease Registry stated that the benefits of breastfeeding most likely outweigh the potential risks of PFAS exposure through breastmilk.[44] Our present findings show that plasma PFAS levels in early

Table 4. Regression coefficients for PFAS plasma levels at the age of 3 months and exclusive infant feeding type.

		Age 2 years				
		Weight-for-height SDS	FM% SDS[a]	Waist circumference	Abdominal subcutaneous fat	FFM SDS
Sum plasma PFAS levels at age 3 months	B ± SE	−1.398 ± 0.31	−4.147 ± 1.46	−3.604 ± 1.04	−0.081 ± 0.04	−1.019 ± 0.24
	β	**−0.425**	**−1.456**	**−0.336**	**−0.234**	**−0.324**
	P-value	**<.001**	**.005**	**<.001**	**.036**	**<.001**
Feeding type (EFF = 1, EBF = 2)	B ± SE	0.702 ± 0.20	−0.934 ± 0.50	1.896 ± 0.65	0.047 ± 0.02	0.596 ± 0.15
	β	**0.342**	−0.499	**0.647**	0.218	0.596
	P-value	**<.001**	.064	**.004**	.052	**<.001**

The results of multiple linear regression analysis using the forward method. B = unstandardized coefficient B and *β* is the standardized coefficient beta. Poly- and perfluoroalkyl substance levels are ^{10}log-transformed. All analyses are corrected for sex and birth weight SDS. In addition, all analyses, expect weight-for-height SDS, are also corrected for height SDS at the age of 2 years. Significant results are boldfaced.
Abbreviations: FM%, fat mass percentage; FFM, fat-free mass; NA, not applicable; PFAS, poly- and perfluoroalkyl substance; SDS, SD score; Sum, sum of PFAS levels. [a]In the FM% SDS model, interaction term PFAS × feeding type was significant and was therefore included in the final model. Feeding type: 1 = EFF, 2 = EBF. Sex: 0 = male, 1 = female.

life do not seem to attenuate the health benefits of breastfeeding in terms of total body fat percentage during the first 2 years of life, but long-term research is needed before definite conclusions can be drawn.

We found opposite associations of higher PFAS levels (negative) and exclusive breastfeeding (positive) at the age of 3 months with FFM at the age of 2 years, since not only increased adiposity, but also decreased FFM is associated with an unfavorable cardiometabolic profile.[42] These opposite effects of PFAS exposure could potentially compromise the health benefits of breastfeeding later in life in terms of protection against insulin resistance or the development of metabolic syndrome in (young) adulthood, which again warrants further research.

The strength of our study is the large collection of infant blood samples in a prospective cohort of healthy Dutch infants in which plasma PFAS analyses were performed using a high-quality measuring method combined with detailed growth and body composition data in a 2-year follow-up period. We acknowledge some limitations, as we did not collect maternal blood samples during gestation, which could potentially have given a deeper insight into prenatal PFAS exposure. However, we measured the PFAS levels of infants who were already at 3 months of age, and as most PFASs have an elimination half-time of several years, we assume that our early life plasma levels also partly represent prenatal PFAS exposure.

Conclusions

Higher plasma PFAS levels at the age of 3 months are associated with an accelerated gain in FM% during the first 6 months of life and with decreased FFM at the age of 2 years, both of which are known to be associated with an unfavorable metabolic profile in young adults. Thus, higher PFAS levels could potentially lead to an unfavorable body composition and metabolic profile later in life. This warrants further research with a long-term follow-up.

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Authors' contributions

Inge van Beijsterveldt (Conceptualization [equal], Formal analysis [lead], Investigation [equal], Methodology [equal], Writing—original draft [lead]), Bertrand van Zelst (Investigation [equal], Methodology [equal], Writing—review and editing [equal]), Demi Dorrepaal (Investigation [equal], Writing—review and editing [equal]), Sjoerd van den Berg (Methodology [equal], Writing—review and editing [equal]), and Anita Hokken-Koelega (Conceptualization [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal])

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