Environment International 160 (2022) 107065

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Quantitation of linear and branched perfluoroalkane sulfonic acids (PFSAs) in women and infants during pregnancy and lactation

Kristin Varsi [a], Sandra Huber [b], Maria Averina [b,c], Jan Brox [b], Anne-Lise Bjørke-Monsen [a,d,e,*]

[a] Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway
[b] Department of Laboratory Medicine, University Hospital of North Norway, Tromsø, Norway
[c] Department of Community Medicine, The Arctic University of Norway, Tromsø, Norway
[d] Laboratory of Medical Biochemistry, Innlandet Hospital Trust, 2609 Lillehammer, Norway
[e] Unilabs, Oslo, Norway

ARTICLE INFO

Handling Editor: Marc-André Verner

Keywords:
PFSA
Branched
Linear
Pregnancy
Infant
Women

ABSTRACT

Background: Per- and polyfluoroalkyl substances (PFAS) are associated with negative health effects, and exposure during fetal life and infancy are of concern. A subgroup of PFAS, linear and branched perfluoroalkane sulfonic acids (PFSA), have significant differences in biochemical reactions, bioaccumulation and potential toxic exposure effects, and data on transfer of PFSA isomers from mother to baby through placenta or in breastmilk are scarce.
Objectives: The objective was to investigate differences in branched and linear PFSA isomers in never-pregnant, pregnant and postpartum women and infants.
Methods: Serum concentrations of branched and linear, perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS) and perfluorooctane sulfonate (PFOS) were measured in never-pregnant women (n = 158), pregnant and postpartum women (n = 114) and their infants (n = 94) at age six months.
Results: There was a linear relation between maternal PFSA concentrations in pregnancy week 18 and the infant at age six months. The PFSA concentrations in maternal and infant serum varied with a factor up to 20. The maternal branched/ linear PFHxS ratio increased in the latter part of pregnancy (+45%) and remained high postpartum, and was substantially lower in the infants. Branched/linear PFHpS ratio increased during pregnancy and was highest in the infants, while the branched/linear PFOS ratio decreased in the mothers and was high in the infants.
Discussion: The linear relations between PFSA concentrations in infants aged six months and mothers in pregnancy week 18 confirm that pregnancy and lactation are major excretion routes for PFSA, but accumulate in the infant. The observed great variability in PFSA burden among mothers and infants, as well as the reduced maternal transfer of branched PFHxS isoforms and increased transfer of branched PFOS isoforms compared to the respective linear isoforms to the infant, might impact adverse health effects associated with PFSA exposure, but this should be confirmed in future studies.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic carbon fluorine compounds that have been widely used in many industrial and consumer products for more than 50 years due to their water- and fat repelling features (European Chemicals Agency (ECHA) 2018). PFAS are known to be persistent to environmental and biological degradation, and several of these substances bioaccumulate and biomagnify in the food chain and also demonstrate toxic properties (Conder et al., 2008). There are currently two production methods giving rise to isomeric forms with different physicochemical properties, generating a subcategory of PFAS, perfluoroalkane sulfonic acids (PFSAs). Telomerisation gives exclusively linear PFSAs, while electrochemical fluorination (ECF) produces both linear and branched PFSAs (Buck et al., 2011; Schulz et al., 2020). The ratio of branched versus linear isomers measured in biological matrices, depend on exposure to PFSAs from different production methods. Additionally, degradation and metabolism of PFSA precursors in the environment and organisms, together with bioaccumulation and biomagnification in the food chain, will affect the ratio of branched versus linear isomers. Linear and branched PFSAs

* Corresponding author at: Department of Medical Biochemistry and Pharmacology, Laboratoriebygget, Haukeland University Hospital, Bergen, Norway.
E-mail address: almo@helse-bergen.no (A.-L. Bjørke-Monsen).

https://doi.org/10.1016/j.envint.2021.107065
Received 19 August 2021; Received in revised form 20 December 2021; Accepted 22 December 2021
Available online 24 December 2021

are considered to have significant differences in biochemical reactions, bioaccumulation and also potential toxic exposure effects.

PFASs are considered to be potentially carcinogenic and hormone disrupting substances, associated with IQ loss, autism, obesity, diabetes and male infertility, among others (Sunderland et al., 2019). Humans are particularly vulnerable to toxic substances during periods of rapid growth and development, and studies indicate that PFASs, including PFSAs, may cause a range of negative health effects on the developing child (Liew et al., 2018), and more data on transfer of linear and branched PFSA isomers from the mother to the baby through placenta or in breastmilk are needed (Schulz et al.,2020).

The present study has investigated changes in linear and branched PFSAs in Norwegian healthy never-pregnant women of fertile age, in pregnant women from week 18 to six months postpartum and in their infants at six months age. The aim of the study was to define and compare the ratios of linear and branched PFSA isomers in the blood of never-pregnant, pregnant and lactating women and their infants.

2. Material and methods

2.1. Study population and design

Between June 2012 to March 2015, 140 healthy women with a singleton pregnancy were recruited in pregnancy week 18 at routine ultrasound examination at the Obstetrical Department at Haukeland University Hospital, Bergen, Norway. The women were invited for follow 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 study.

During the same period, 158 healthy, never-pregnant women aged 18–40 years were recruited among students and employees at the University of Bergen and Haukeland University Hospital, Bergen, Norway.

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

The participants completed a questionnaire at each visit concerning age, years of completed education, parity, body weight, health status/pregnancy related disease, alcohol and tobacco. The postpartum questionnaires additionally included data on infant weight and months of exclusive breastfeeding versus use of formula and of solid food. The questionnaire for the never-pregnant women additionally included information about use of hormonal contraception, including oral contraceptives, hormone implants and injections.

2.3. Blood sampling and analysis

At each visit, non-fasting blood samples were obtained by antecubital venipuncture and collected into vacutainer tubes without additives (Terumo). Serum was transferred to Sarstedt tubes without additives with plastic pipettes and stored at −80 °C. Blood samples were drawn from the infants at age six months. For analysis of PFSA, the samples 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.

PFSA measurements were available for all 114 mothers at all six time points during pregnancy and postpartum, except for pregnancy week 18 (missing n = 7), for 94 of the 114 infants (82%) at age six months, and all 158 never-pregnant women.

The different serum PFSA concentrations were analyzed according to the method described by Huber and Brox (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). Briefly, 50 μL serum and quality control samples were extracted by solid phase micro-extraction after addition of an isotope labelled internal standard mixture (single standards provided from Wellington Laboratories, Ontario, Canada, mixed inhouse) and dilution. Instrumental analysis was performed in single analysis runs together with an eight-point calibration curve. The internal standard method was applied for quantification of the analytes.

Linear species (*lin*) and sum of linear and branched species (*sum*) were quantified for perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS), perfluorononane sulfonate (PFNS) and perfluorodecane sulfonate (PFDcS). Branched isomers of PFHxS, PFHpS and PFOS were calculated as follows: Branched = *sum* – *lin*. In the method used, branched PFOS, PFHxS and PFHpS represent a sum of different branched isomers of PFOS, PFHxSs and PFHpSs, respectively. For perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS) and perfluorododecane sulfonate (PFDoDS) only linear species were observed and quantified.

For quality assurance, four blank samples, four SRM 1958 (NIST, Gaithersburg, MD, USA) and four SRM 1957 (NIST, Gaithersburg, MD, USA) samples and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of 96 samples to control for background and carry-over effects. All the quality controls were within the acceptance limits. Analytical coefficients of variation (CVa) were $\leq$ 10% for all the measured PFSAs.

2.4. Statistical analysis

Results for age and BMI are presented as mean and standard deviation (SD), and compared by Student's *t*-test. The Chi-square test was used for categorical data like educational level and smoking status. PFSA 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 was used to explore relationships between parameters. General linear model repeated measures was used to test change in PFSA concentrations during pregnancy and postpartum period.

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.

We calculated a ratio between infant serum PFSA concentrations at age six months and maternal concentrations in pregnancy week 18, in order to give an estimate of the concentration one might expect to find in the infant based on maternal levels in early pregnancy.

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 (Supplementary Material Table S1). 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. PFSA concentrations below the LOD were not quantified, and these data were replaced by LOD*i* divided by 2.

Statistical analyses were performed only for PFSAs with detection rate >90%. For details regarding detection frequencies of our study group see Supplementary Material Table S2.

The SPSS statistical program (version 24) and the packages "mgcv"

in R, version 3.3 (The R Foundation for Statistical Computing) were used for the statistical analyses. Two-tailed *P*-values < 0.05 were considered statistically significant.

3. Results

3.1. Demographics

Baseline demographic characteristics of the never-pregnant (n = 158) and pregnant (n = 114) women are shown in Table 1. Compared to the never-pregnant women, the pregnant women were significantly older ($p < 0.001$), they did not use alcohol ($p < 0.001$) and were more often regular users of micronutrient supplements ($p = 0.001$). There were no significant differences in body mass index (BMI) ($p = 0.38$), educational level ($p = 0.96$) and smoking habits ($p = 0.65$).

All infants were healthy, born at term (mean gestational age 40 (SD 0.8) 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. At four months, 50% (70/114) of the infants had been introduced to solid food. At six months, 98 infants (86%) were breastfed in addition to solid food, while four infants (3.5%) were still exclusively breastfed.

3.2. Serum PFSA concentrations in never pregnant, pregnant and postpartum women and infants

Linear PFHxS and linear and branched PFOS were detected in serum of all infants at age six months, pregnant women in pregnancy week 18, and never-pregnant women. Branched PFHxS was detected in serum of all infants and in 99% of pregnant and never-pregnant women. Linear and branched PFHpS were detected in serum of all infants, in 90% of the pregnant women and 99% of the never-pregnant women. In all groups, PFSAs had a very large total range, with maximum concentrations more than 20 times the lowest observed concentration (Table 2).

Fig. 1 and Table 2 show PFHxS, PFHpS and PFOS serum concentrations in never-pregnant women, during pregnancy and postpartum period and in infants at six months.

The highest levels were seen in the infants. Compared to never-pregnant women, at six months postpartum the mothers had lower median concentrations of sum PFHxS (−24%, $p < 0.001$), sum PFHpS (−33%, $p < 0.001$), and sum PFOS (−19%, $p < 0.001$), while the infants had higher median concentrations of sum PFHxS (15%, $p = 0.009$) and sum PFHpS (33%, $p < 0.001$), and equal sum PFOS (8%, $p = 0.52$). At six months postpartum, sum PFHxS was 51% higher, sum PFHpS 100% higher and sum PFOS was 33% higher in the infants compared to their mothers. The ratio between infant serum PFSA at six months and maternal concentrations in pregnancy week 18 was median1.5 (IQR 1.2, 1.7, range: 0.6–3.5) for sum PFHxS, median 1.5 (IQR 1.2, 2.0, range: 0.5–5.9) for sum PFHpS and for sum PFOS median 1.1 (IQR 0.9, 1.3, range: 0.4–2.1).

Table 1
Baseline characteristics of healthy, fertile, never-pregnant women and pregnant women in pregnancy week 18.

Parameters	Never pregnant women N = 158	Pregnant women (week 18) N = 114
Age, years, mean (SD)	25.3 (4.8)	31.5 (4.3)
Pre-pregnancy Body Mass Index, kg/m^2, mean (SD)	22.5 (3.0)	22.8 (3.1)
Education ≥ 12 years, n (%)	93 (60)	67 (59)
Para 0, n (%)	158 (100)	63 (55)
Regular smoking, n (%)	4 (3)	2 (2)
Alcohol units per week, median (IQR)[a]	2 (0.5, 3.8)	0 (0)
Regular use of micronutrient supplements (≥3 days/week), n (%)	35 (22)	47 (41)

[a] Number of drinks containing about 8 g of pure alcohol; Mann-Whitney test.

Fig. 2 shows a significant linear relationship between the individual maternal branched, linear and sum PFSA (except for branched PFHxS) concentrations in pregnancy week 18 and infant concentrations at age six months adjusted for birth weight and months of exclusive breastfeeding.

Maternal concentrations of PFSA in pregnancy week 18 were strong positive determinants of both linear and branched PFSA concentrations in infants at six months, after adjustment in multiple linear regression models for months of exclusive breastfeeding, gender, gestational age, birth weight and weight at six months (Table 3). In infants, months of exclusive breastfeeding was a positive determinant of both linear and branched PFOS and only of linear forms of PFHxS and PFHpS. Infant weight at six months had a slight negative impact on branched PFOS, apart from this no significant effects on infant serum PFSA concentrations were seen for gender, gestational age, infant birth weight and weight at six months.

The relations between branched and linear isomers of PFHxS, PFHpS and PFOS differed between never-pregnant, pregnant and postpartum women and infants (Table 2, Fig. 3).

3.2.1. PFHxS

Maternal sum PFHxS decreased by mean 17% from pregnancy week 18 to six months postpartum. Both branched and linear PFHxS changed significantly during pregnancy and postpartum ($p < 0.001$), but contrary to the other PFSAs, branched PFHxS increased in pregnancy week 36, while linear PFHxS decreased from week 18 to six months postpartum. As a result, the branched/linear PFHxS ratio increased from pregnancy week 36 and remained significantly higher compared to never-pregnant women up to six months postpartum ($p < 0.03$).

Infants had the lowest median branched and the highest median linear PFHxS concentrations compared to mothers at six months ($p < 0.001$ for both branched and linear) and never-pregnant women ($p < 0.001$ for both branched and linear). Their branched/linear PFHxS ratio was also lower than in mothers at six months ($p < 0.001$) and in never-pregnant women ($p < 0.001$) (Table 2, Fig. 3).

3.2.2. PFHpS

Maternal sum PFHpS decreased by mean 25% from pregnancy week 18 to six months postpartum. Branched and linear PFHpS both decreased ($p < 0.001$ for branched and linear), but as linear decreased more than branched, their ratio increased slightly from pregnancy week 18 to six months postpartum ($p = 0.05$).

Infants had the highest branched and linear PFHpS concentrations compared to both mothers at six months postpartum and never-pregnant women and ($p < 0.001$ for both groups). At six months, both median branched and linear PFHpS concentrations were twice as high in the infants compared to their mothers. The infant branched/linear ratio was however equal to their mother at six months postpartum ($p = 0.27$), but was significantly higher than in never-pregnant women ($p = 0.001$) (Table 2, Fig. 3).

3.2.3. PFOS

Maternal sum PFOS decreased by mean 18% from pregnancy week 18 to six months postpartum. Both branched and linear PFOS and the branched/linear PFOS ratio decreased significantly from pregnancy week 18 to six months postpartum ($p < 0.001$ for branched, linear and ratio). The ratios during this period were at all time points significantly lower than in never-pregnant women ($p < 0.001$).

Infants had significantly higher branched PFOS concentrations and branched/linear PFOS ratio than the mothers already from pregnancy week 18 throughout six months postpartum ($p < 0.009$). Infant branched and linear PFOS concentrations and the ratio were equal between infants and never-pregnant women ($p > 0.28$) (Table 2, Fig. 3).

Table 2
Linear and branched isomers and percent branched PFHxS, PFHpS and PFOS in never-pregnant women, pregnant and postpartum women and their infants at age 6 months.

Serum PFSA, ng/mL Median (IQR) Total range	Never pregnant women N = 158	Pregnant women			Postpartum women			Infants 6 months N = 94
		Week 18 N = 107	Week 28 N = 114	Week 36 N = 114	6 weeks N = 114	4 months N = 114	6 months N = 114	
Sum PFHxS[a]	0.59 (0.44, 0.78) 0.20, 17.19	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	0.68 (0.53, 0.92) 0.20, 2.26
Linear PFHxS	0.44 (0.29, 0.62) 0.13, 16.98	0.42 (0.28, 0.53) 0.13, 1.26	0.37 (0.26, 0.49) 0.07, 1.16	0.36 (0.24, 0.48) 0.14, 2.15	0.36 (0.26, 0.47) 0.09, 0.97	0.31 (0.24, 0.43) 0.11, 0.86	0.30 (0.24, 0.40) 0.09, 0.83	0.61 (0.47, 0.85) 0.14, 2.15
Branched PFHxS	0.12 (0.08, 0.19) 0.00, 1.01	0.11 (0.07, 0.14) 0.00, 0.46	0.10 (0.07, 0.16) 0.00, 0.45	0.16 (0.10, 0.24) 0.00, 0.81	0.12 (0.08, 0.16) 0.00, 1.0	0.11 (0.08, 0.18) 0.00, 1.56	0.11 (0.07, 0.21) 0.00, 1.30	0.08 (0.06, 0.10) 0.03, 0.22
% Branched of Sum PFHxS	20	20	20	30	23	25	24	12
Sum PFHpS[a]	0.09 (0.07, 0.12) 0.001, 0.41	0.08 (0.05, 0.10) 0, 0.25	0.07 (0.05, 0.10) 0, 0.25	0.07 (0.05, 0.09) 0.00, 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	0.12 (0.08, 0.16) 0.03, 0.46
Linear PFHpS	0.07 (0.05, 0.10) 0, 0.37	0.07 (0.04, 0.09) 0, 0.22	0.06 (0.04, 0.08) 0, 0.22	0.05 (0.04, 0.08) 0, 0.19	0.05 (0.04, 0.08) 0, 0.22	0.05 (0.03, 0.07) 0, 0.20	0.05 (0.03, 0.07) 0, 0.16	0.10 (0.07, 0.13) 0.02, 0.41
Branched PFHpS	0.014 (0.011, 0.020) 0,0.00, 0.040	0.013 (0.007, 0.019) 0.000, 0.04	0.012 (0.007, 0.016) 0.00, 0.05	0.012 (0.008, 0.017) 0.00, 0.03	0.011 (0.007, 0.017) 0.00, 0.03	0.010 (0.007, 0.013) 0.00, 0.04	0.010 (0.006, 0.015) 0.00, 0.03	0.021(0.015, 0.026) 0.01, 0.06
% Branched of Sum PFHpS	16	16	17	17	18	17	17	18
Sum PFOS[a]	4.38 (3.03, 5.94) 1.14, 21.30	4.30 (3.23, 5.94) 0.70, 11.64	4.00 (3.03, 5.58) 0.68, 9.98	3.86 (3.00, 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	4.71 (3.53, 6.23) 0.94, 10.99
Linear PFOS	2.41 (1.70, 3.42) 0.62, 10.32	2.61 (1.91 3.60) 0.42, 5.82	2.38 (1.80 3.34) 0.40, 5.39	2.32 (1.71, 3.13) 0.41, 5.07	2.31 (1.67, 3.07) 0.52, 4.75	2.18 (1.60, 2.99) 0.45, 4.44	2.14 (1.49, 2.82) 0.42, 4.29	2.48 (1.87, 3.41) 0.46, 6.13
Branched PFOS	1.89 (1.40, 2.66) 0.51, 10.98	1.71 (1,21, 2.37) 0.28, 6.23	1.61 (1.20, 2.13) 0.29, 5.60	1.53 (1,11, 1.99) 0.27, 5.87	1.48 (1.01, 1.84) 0.32, 4.30	1.30 (0.94, 1.76) 0.35, 4.16	1.22 (0.91, 1.65) 0.29, 4.31	2.13 (1,48, 2.67) 0.48, 5.55
% Branched of Sum PFOS	43	40	40	45	39	37	35	45
Sum PFSA	5.20 (3.84, 6.91) 1.82, 22.70	5.18 (3.67, 6.69) 0.87, 12.84	4.68 (3.55, 6.32) 0.80, 11.55	4.43 (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	5.48 (4.15, 7.30) 1.16, 13.34

4. Discussion

There were near linear relations between maternal concentrations of PFHxS, PFHpS and PFOS in pregnancy week 18 and infant 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.

Both the concentrations and the ratio between branched and linear isoforms of PFHxS, PFHpS and PFOS differed substantially between never-pregnant, pregnant and postpartum women and infants. Infants had high concentrations of both branched and linear PFHpS and PFOS isoforms, the linear isoform was the dominating PFHxS, while the branched isoform was low.

4.1. PFSAs in pregnant and postpartum women and in infants

All people in both industrialised and less industrialised countries have measurable amounts of PFSA in their blood (Eriksson et al., 2017; Hanssen et al., 2013; Sunderland et al., 2019). While the concentrations of PFOS in humans have declined over the past decade in most countries, the concentrations of PFHxS initially increased, but then declined from 2000 (Glynn et al., 2012; Lin et al., 2021).

When comparing PFSA concentrations in different populations one needs to take into account year of sampling. When comparing pregnant and postpartum populations one also needs to consider parity, in which pregnancy week the blood sampling was done, as well as months of breastfeeding, as all these factors may influence PFSA status in pregnant and lactating women.

The PFSA concentrations in the present study are comparable, but somewhat lower compared to Swedish women in early pregnancy, recruited during 2007 to 2010 (median PFOS 5.38 ng/mL, PFHxS 1.23 ng/mL) (Wikström et al., 2020). In Norwegian women recruited in pregnancy week 20 during 2007 to 2009, the median concentration of PFOS (8.03 ng/mL) was higher, but the concentrations of sum PFHxS and sum PFHpS were equal to our pregnant population in week 18 (Berg et al., 2014).

Our findings indicate that there has been a decline in PFOS exposure after the phase-out in 2002, though the substance is still present in women and infants. On the other hand, according to our results, the exposure of other types of PFSA, introduced as substitutes for the C_8-PFOS analogues, have increased.

During pregnancy PFAS, including PFSA, pass the placenta and accumulate in fetal blood and tissues (Mamsen et al., 2019). Previous published studies have detected several PFASs in fetal tissues already from the first trimester (Gützkow et al., 2012; Mamsen et al., 2019), a fact which gives rise to concern for fetal development (Lau et al., 2004). Mamsen et al. were able to find PFASs in the fetus already from week seven of gestation (Mamsen et al., 2019).

The prevalence of breastfeeding in Norway is high (Häggkvist et al., 2010), and in this study 89% of the infants were still breastfed at six months, either exclusively or in addition to other food. After birth, PFASs are transferred to the child through breastmilk (Kärrman et al.,

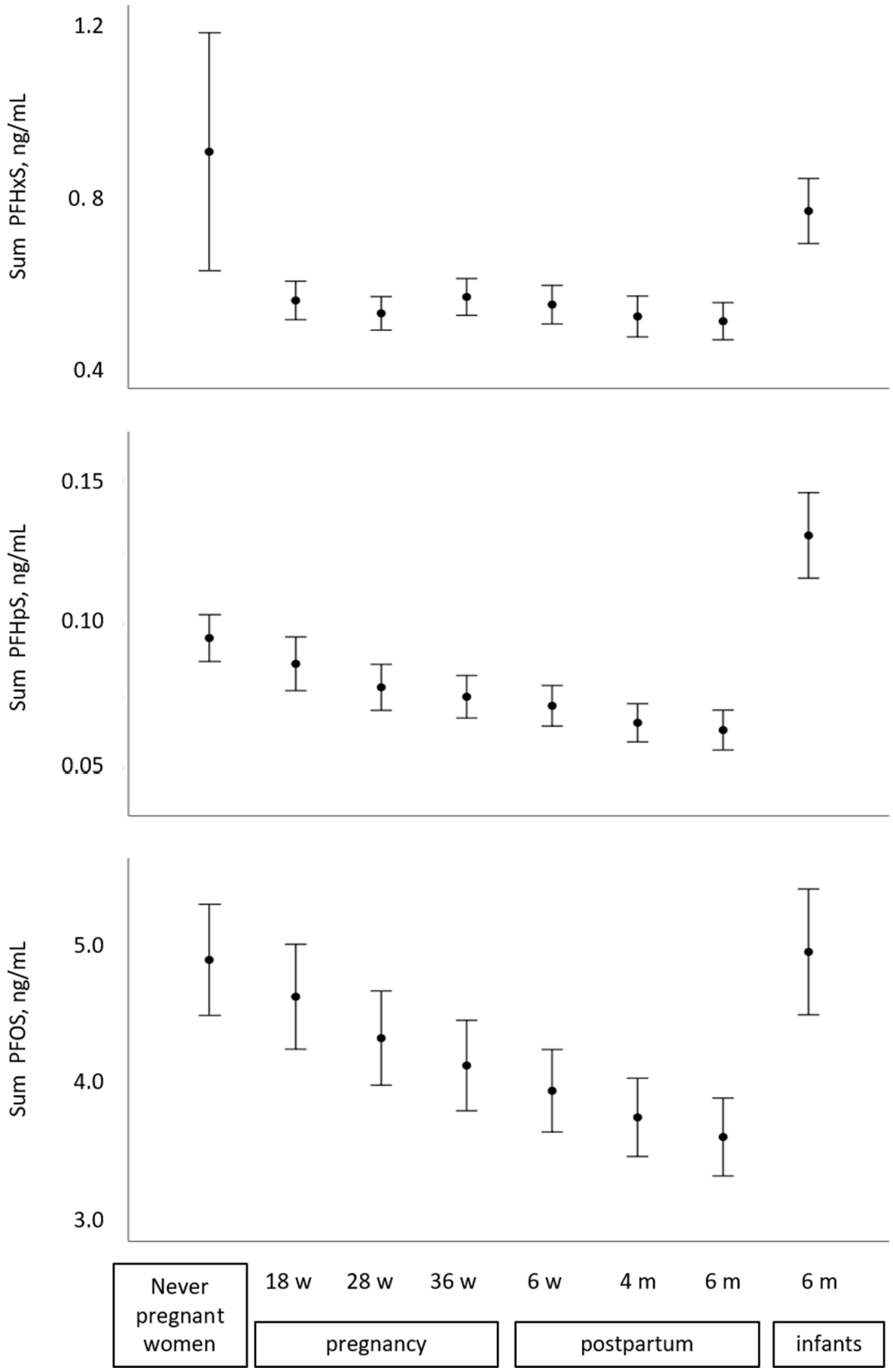

Fig. 1. Serum sum PFHxS, PFHpS and PFOS concentrations in never-pregnant (n = 158), pregnant and postpartum women (n = 114) and their infants (n = 94).

2007), and increasing serum concentrations of PFOA, PFOS and PFHxS with extended breastfeeding periods have been reported in infants (Fromme et al., 2010).

Pregnancy and breastfeeding obviously represent a cleaning process for the mother, while the infant is the unfortunate recipient (Grandjean 2010; Grandjean and Landrigan 2006). We observed a gradual decline of serum PFSA concentrations in mothers during pregnancy and the postpartum period, as documented by others (Brantsæter et al., 2013). In

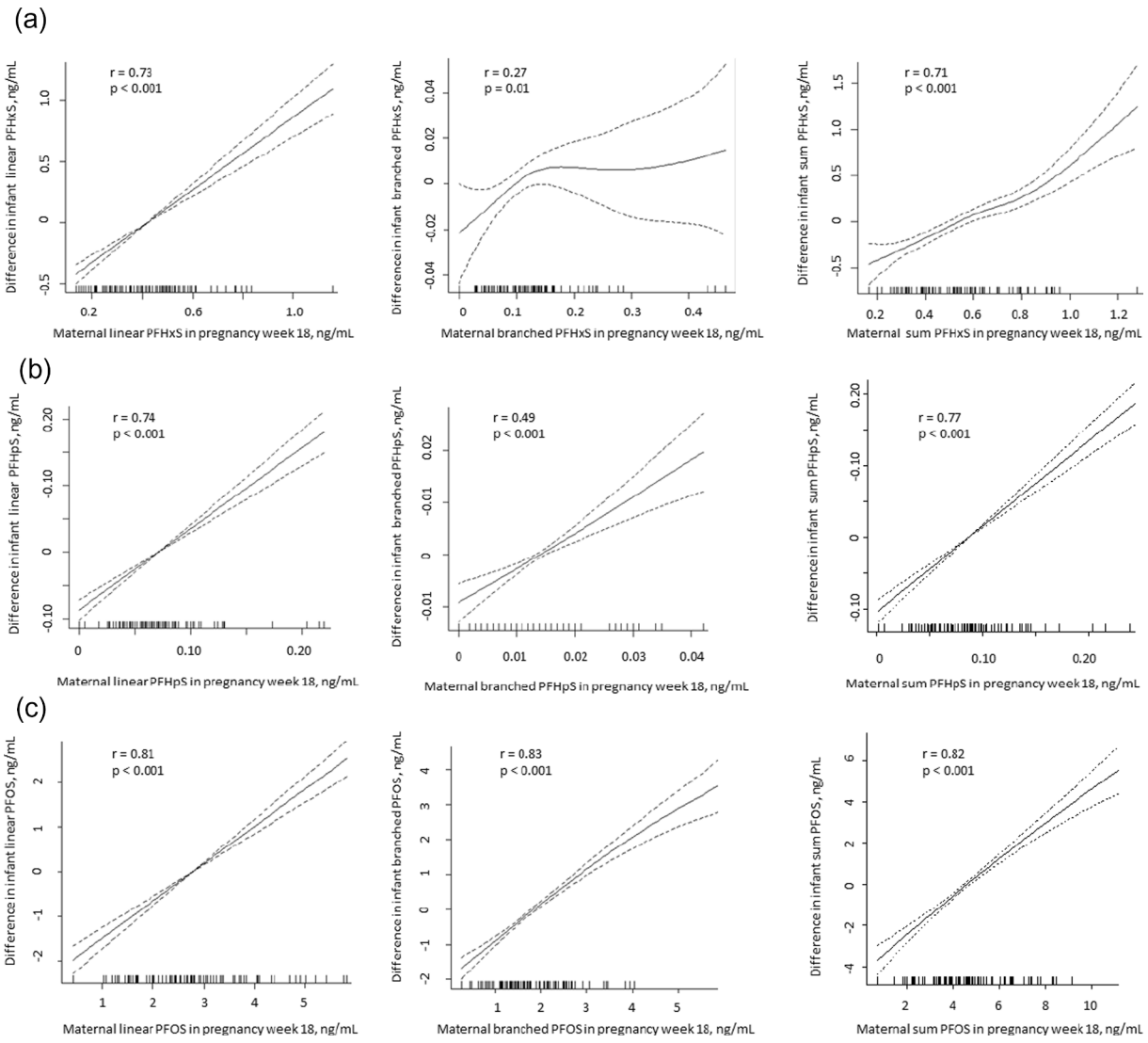

Fig. 2. Infant branched, linear and sum PFHxS, PFHpS and PFOS serum concentrations at age six months in relation to the respective maternal serum concentration in pregnancy week 18 by generalized additive models (GAM) adjusted for birth weight and months of exclusive breastfeeding (n = 94). The values on the y-axes represents the difference from the respective mean infant PFSA values. Spearman correlation coefficients (r = rho, p = p value).

Table 3
Determinants of serum PFSA concentrations in infants aged six months by multiple linear regression, n = 94.

Variables included in the model	Infant PFSA status at age 6 months											
	PFHxS				PFHpS				PFOS			
	Linear		Branched		Linear		Branched		Linear		Branched	
	B[a]	95% CI for B[a]	B[a]	95% CI for B[a]	B[a]	95% CI for B[a]	B[a]	95% CI for B[a]	B[a]	95% CI for B[a]	B[a]	95% CI for B[a]
Maternal serum PFSA in pregnancy week 18[b]	1.493	1.208, 1.778	0.088	0.000, 0.169	1.253	1.037, 1.470	0.727	0.476, 0.977	0.844	0.711, 0.978	1.014	0.879, 1.149
Exclusive breastfeeding, months	0.058	0.023, 0.093	−0.002	−0.007, 0.002	0.008	0.003, 0.014	0.001	0.000, 0.002	0.216	0.115, 0.317	0.167	0.087, 0.247
Gender, boy- girl	−0.067	−0.175, 0.040	0.009	−0.005, 0.024	−0.010	−0.028, 0.008	−0.002	−0.006, 0.002	0.016	−0.301, 0.333	−0.118	−4.340, 7.416
Gestational age, months	−0.014	−0.079, 0.050	0.002	−0.007, 0.011	0.002	−0.009, 0.013	−0.001	−0.004, 0.001	0.045	−0.145, 0.236	−0.005	−0.154, 0.145
Infant birth weight, kg	0.032	−0.110, 0.174	−0.004	−0.023, 0.015	−0.003	−0.027, 0.021	−0.001	−0.007, 0.004	−0.183	−0.605, 0.240	−0.046	−0.382, 0.289
Infant weight at six months, kg	−0.040	−0.107, 0.026	0.007	−0.002, 0.016	−0.007	−0.018, 0.005	−0.001	−0.003, 0.002	−0.170	−0.365, 0.025	−0.161	−0.318, −0.003

[a] Unstandardized coefficient.
[b] Corresponding maternal serum PFSA in pregnancy week 18.

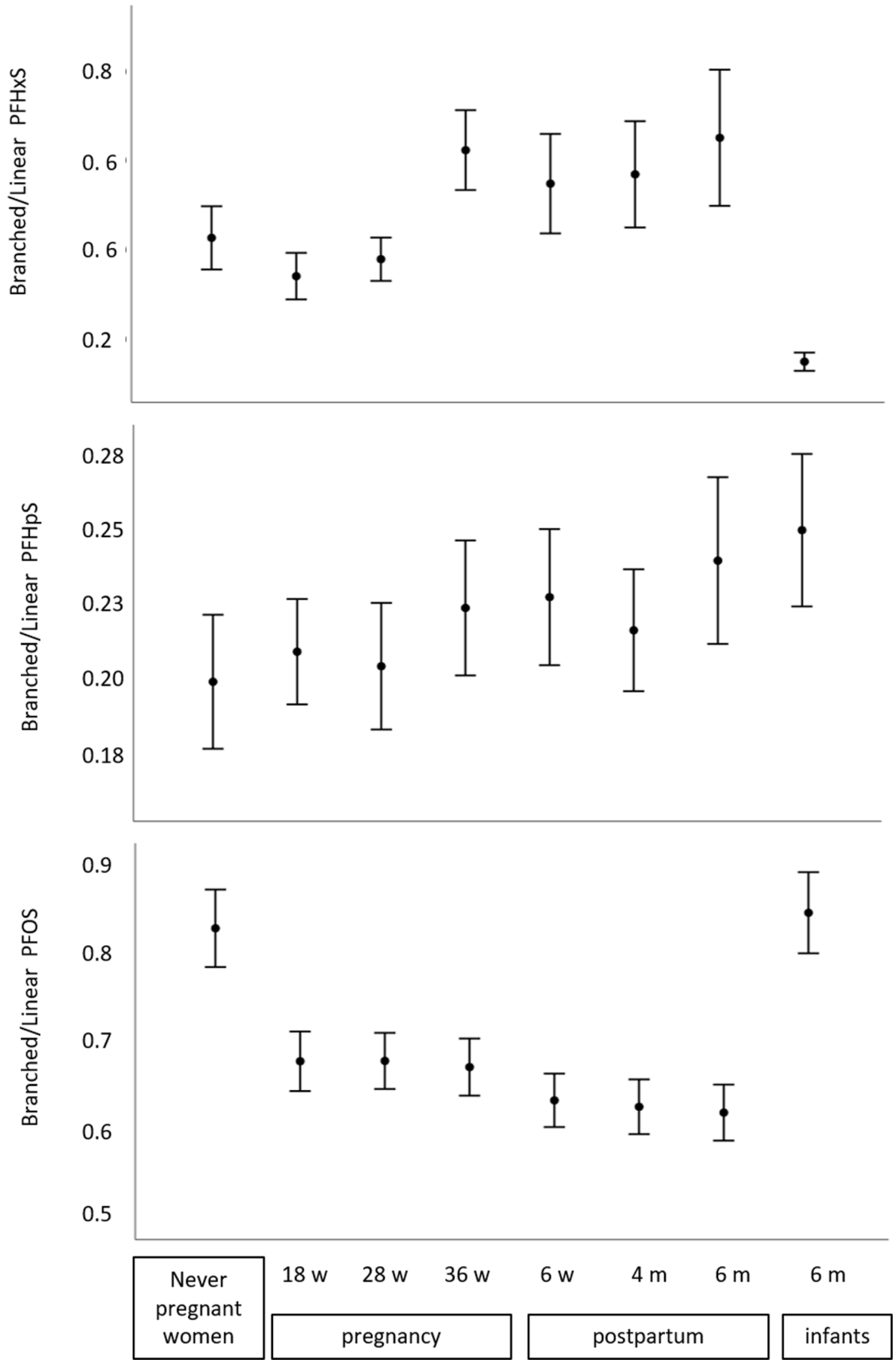

Fig. 3. Branched/linear ratio of serum PFHxS, PFHpS and PFOS (mean ± 2SD) in never pregnant women (n = 158), in pregnant and postpartum women (n = 114) and their infants (n = 94).

uncomplicated pregnancies, the maternal blood volume increases up to 40–50%, starting in the first trimester with a peak in week 34–36 (Costantine 2014; Hytten 1985). There is additionally a progressive increase up to 50% in glomerular filtration rate (GFR) from pregnancy week 14 with peak at term, and the hyperfiltration continues at levels 20% above normal at postpartum week two and normalizes by one month postpartum (Davison and Dunlop 1980; Krutzén et al., 1992). The pregnancy induced hemodilution and increased GFR may of course affect the blood levels of several analytes in pregnancy. However, as our data show there was a gradual decrease in PFSA concentrations both during pregnancy and in the postpartum period to six months, when pregnancy related changes in blood volume and GFR were normalized.

German infants aged six months and recruited during 2007 and 2009, had lower PFOS (3.0 ng/mL) concentrations, while PFHxS (0.6 ng/mL) concentration was similar to our Norwegian infants (Fromme et al., 2010).

4.2. Variations in PFSA transfer from mother to child

The exact mechanisms for placental transfer of different PFASs, including linear and branched PFSA isomers, are largely unknown. With passive diffusion across the placenta, hydrophobic compounds are expected to exhibit higher placental transfer than more hydrophilic compounds (van der Aa et al., 1998). Branched PFSA isomers are more hydrophilic than the linear isomers, but have also a more "rounded" molecular shape, which further inhibits placental transfer (van der Aa et al., 1998). Protein binding may also influence transport of chemicals across the placenta and into the milk ducts of the breast (Beesoon et al., 2011; Garcia-Lino et al., 2019; Hill and Abramson 1988).

We observed a linear relation between PFSAs in the mother from pregnancy week 18 and infant levels at six months in the present study, indicating that maternal PFSAs status in early pregnancy is an important determinant of infant PFSAs concentration during the first months of life.

Based on the calculated median ratios between infant serum PFSA concentrations at six months and maternal concentrations in pregnancy week 18, one can expect to find infant PFSA concentrations to be 1.1 to 1.5 times higher than maternal serum PFSA concentrations obtained in pregnancy week 18. Infant PFSA concentrations were higher than maternal concentrations at six months postpartum, and also higher than in never-pregnant women. The exception was branched PFHxS, which was lower in the infants compared to their mothers, indicating that this compound is not easily transferred over the placenta or in the breastmilk. We did not find any significant association between months of exclusive breastfeeding and infant branched PFHxS concentrations at six months. Chen et al. has reported reduced transfer across the placenta for branched PFHxS, with retention in the placenta as a possible mechanism (Chen et al., 2017).

Unlike the other PFSAs investigated in our study, branched PFHxS concentration initially decreased in pregnancy week 18 and 28, but then increased to maximal level in pregnancy week 36, and was reduced to concentrations observed in never-pregnant women postpartum. The branched/linear PFHxS ratio was significantly higher in mothers at pregnancy week 36 and postpartum compared to never-pregnant women, and conversely very low in infants, indicating a more selective transfer of the linear isomer to the infant. By calculating branched/linear ratio from Gyllenhammar et al, the infant branched/linear PFHxS ratio was 0.08 at age 13 weeks vs 0.13 in our study at six months age. The calculated maternal branched/linear PFHxS ratio of 0.06 three weeks postpartum was much lower compared to the median ratio of 0.55 we found in mothers six weeks postpartum. The dissimilar results in the mothers might be due to different mixtures of branched isomers in the two studies and/or sampling time after birth.

We observed no difference in branched/linear PFHpS ratios between infant, pregnant and postpartum women, indicating that both linear and branched isomers are equally transferred from mother to infant, however, we did not find any significant association between months of exclusive breastfeeding and infant branched PFHpS concentrations at six months.

The branched/linear PFOS ratio in infants was substantially higher than in both pregnant and postpartum women, indicating a higher transfer of the branched isoforms during both periods In our study, the branched/linear PFOS ratio was 0.85 in the infants versus 0.58 in the infants from the Gyllenhammar study, and 0.64 in our mothers versus 0.50 in the mothers from the Gyllenhammar study (Gyllenhammar et al., 2018) These findings are in line with Chen et al, who reported lower branched PFOS in maternal sera than in cord sera, indicating a more efficiently transfer of branched than linear PFOS across the placenta (Chen et al., 2017).

Months of exclusive breastfeeding was a significant positive determinant of both infant linear and branched serum PFOS concentrations, indicating that also breastmilk is an important source of both PFOS isoforms.

The observed gradual decline in maternal PFSA concentrations during both pregnancy and postpartum period, as well as the strong association between maternal PFSA concentrations in early pregnancy and months of breastfeeding with infant PFSA concentrations, and the very high PFSA concentrations in the infants at six months, confirm that pregnancy and lactation are major excretion routes for PFSAs, that unfortunately result in accumulation in the infant (Brantsæter et al., 2013). There are only a few studies specifically targeting infants and young children, but they suggest that peak concentration of PFOS occur before age 20 months (Winkens et al., 2017). During fetal life and early childhood the organism respond to adverse environmental situations by adaptations at the cellular, molecular and biochemical levels. These adaptations are found to have an effect on development and may cause later disease (Olsen and Liew 2018; Sookoian et al., 2013). The observed wide concentration ranges and the right skewed distribution of PFSAs, as well as the obvious wide variation in transfer of PFSA from the mother to the baby (the infant/maternal ratio for sum PFHpS varied from 0.5 to 5.9), indicate that there is a great variability in PFSA burden among people. In our study, some women and infants had a PFSA concentration, which was 10–20 times higher than the lowest concentration measured in the corresponding population, something which may influence the adverse health effects associated with PFSA exposure.

4.3. Strengths and limitations

To our knowledge, longitudinal measurement of this subgroup of PFAS; branched and linear PFSAs during pregnancy and postpartum period and in infants at six months age have not been published earlier. Comparison with never-pregnant women is a strength to the study, as this totally exclude parity interference on PFSA concentrations. However, as the never-pregnant women were younger than the pregnant women, this may influence the difference of PFSA concentrations between the groups, as age is a known positive determinant of PFSA concentrations. We did not have any information about menstrual cycle characteristics, something which may be a limitation to the study, as menstrual bleeding has been suggested to be a potential elimination pathway in fertile women (Singer et al., 2018).

This study included a rather low number of pregnant women, which is a limitation, but there was no lost to follow up. As PFSAs were not measured in breastmilk, the concentrations measured in the infants reflects the total PFSA burden resulting from placental transfer, breastfeeding and intake of drinking water, formula and solid food in the first six months of life. Clinical data were collected by questionnaires, prone to recall bias, but all interviews were done by the same two doctors throughout the study period.

5. Conclusion

Longitudinal measurements of PFSAs, a subgroup of PFAS, during

pregnancy and postpartum confirm the cleaning effect of pregnancy and lactation at the expense of the infant. There were linear relations between maternal PFSA concentrations in pregnancy week 18 and in the infant at age six months, with the highest PFSA concentrations seen in the infants. Wide concentration ranges and right skewed distribution of PFSAs, indicate a great variability in PFSA burden among mothers and infants.

The transfer of branched and linear isoforms of PFHxS, PFHpS and PFOS differ in pregnancy and the postpartum period, indicating reduced transfer from the mother to the infant of the branched PFHxS isoforms and an increased transfer of the branched PFOS isoforms as compared to the respective linear isoforms.

The observed great variability in PFSA burden among mothers and infants, as well as the variability in maternal transfer of branched versus linear isoforms of PFSAs, might have an impact on adverse health effects associated with PFSA exposure, something which needs to be further studied.

Financial Support

This study was financially supported by Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital and from Department of Laboratory Medicine, University Hospital of North Norway.

Funding

This study was financially supported by Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital and from Department of Laboratory Medicine, University Hospital of North Norway.

CRediT authorship contribution statement

Kristin Varsi: Conceptualization, Methodology, Investigation, Writing – original draft. **Sandra Huber:** Methodology, Investigation, Writing – review & editing. **Maria Averina:** Writing – review & editing. **Jan Brox:** Methodology, Writing – review & editing. **Anne-Lise Bjørke-Monsen:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision.

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.

Acknowledgements

The authors would like to express their gratitude to all the women and infants who participated in the study. The authors will also thank Sten-Kristian Odden and Christina R. Hansen, Department of Laboratory Medicine, University Hospital of North Norway, Norway, for performing the sample preparation and instrumental analysis for the PFSAs. The financial support of the Department of Laboratory Medicine, University Hospital of North Norway, Norway and the North Norway Regional Health Authority, Norway, is highly appreciated.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.107065.

References

Allen, L.H., 2012. B vitamins in breast milk: relative importance of maternal status and intake, and effects on infant status and function. Adv. Nutrit. 3, 362–369.
Beesoon, S., Webster, G.M., Shoeib, M., Harner, T., Benskin, J.P., Martin, J.W., 2011. Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. Environ. Health Perspect. 119 (11), 1659–1664.
Berg, V., Nøst, T.H., Huber, S., Rylander, C., Hansen, S., Veyhe, A.S., Fuskevåg, O.M., Odland, J.Ø., Sandanger, T.M., 2014. Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use. Environ. Int. 69, 58–66.
Bjorke Monsen, A.L., Ueland, P.M., Vollset, S.E., Guttormsen, A.B., Markestad, T., Solheim, E., Refsum, H., 2001. Determinants of cobalamin status in newborns. Pediatrics 108, 624–630.
Brantsæter, A.L., Whitworth, K.W., Ydersbond, T.A., Haug, L.S., Haugen, M., Knutsen, H. K., Thomsen, C., Meltzer, H.M., Becher, G., Sabaredzovic, A., Hoppin, J.A., Eggesbø, M., Longnecker, M.P., 2013. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. Environ. Int. 54, 74–84.
Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.PJ., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr. Environ. Assess Manage. 7 (4), 513–541.
Chen, F., Yin, S., Kelly, B.C., Liu, W., 2017. Isomer-specific transplacental transfer of perfluoroalkyl acids: results from a survey of paired maternal, cord sera, and placentas. Environ. Sci. Technol. 51 (10), 5756–5763.
Conder, J.M., Hoke, R.A., Wolf, W.d., Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ. Sci. Technol. 42 (4), 995–1003.
Costantine, M.M., 2014. Physiologic and pharmacokinetic changes in pregnancy. Front. Pharmacol. 5, 65.
Davison, J.M., Dunlop, W., 1980. Renal hemodynamics and tubular function normal human pregnancy. Kidney Int. 18, 152–161.
Eriksson, U., Mueller, J.F., Toms, L.-M., Hobson, P., Kärrman, A., 2017. Temporal trends of PFSAs, PFCAs and selected precursors in Australian serum from 2002 to 2013. Environ. Pollut. 220, 168–177.
European Chemicals Agency (ECHA), 2018. T.E.U. Information on Chemicals.
Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Boroviczény, O., Koletzko, B., Völkel, W., 2010. Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environ. Sci. Technol. 44 (18), 7123–7129.
Garcia-Lino, A.M., Alvarez-Fernandez, I., Blanco-Paniagua, E., Merino, G., Alvarez, A.I., 2019. Transporters in the mammary gland-contribution to presence of nutrients and drugs into milk. Nutrients 11.
Glynn, A., Berger, U., Bignert, A., Ullah, S., Aune, M., Lignell, S., Darnerud, P.O., 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. Environ. Sci. Technol. 46 (16), 9071–9079.
Grandjean, P., 2010. Even low-dose lead exposure is hazardous. Lancet 376 (9744), 855–856.
Grandjean, P., Landrigan, P.J., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368 (9553), 2167–2178.
Gützkow, K.B., Haug, L.S., Thomsen, C., Sabaredzovic, A., Becher, G., Brunborg, G., 2012. Placental transfer of perfluorinated compounds is selective–a Norwegian Mother and Child sub-cohort study. Int. J. Hyg. Environ. Health 215 (2), 216–219.
Gyllenhammar, I., Benskin, J.P., Sandblom, O., Berger, U., Ahrens, L., Lignell, S., Wiberg, K., Glynn, A., 2018. Perfluoroalkyl Acids (PFAAs) in serum from 2-4-month-old infants: influence of maternal serum concentration, gestational age, breast-feeding, and contaminated drinking water. Environ. Sci. Technol. 52 (12), 7101–7110.
Häggkvist, A.-P., Brantsæter, A.L., Grjibovski, A.M., Helsing, E., Meltzer, H.M., Haugen, M., 2010. Prevalence of breast-feeding in the Norwegian Mother and Child Cohort Study and health service-related correlates of cessation of full breast-feeding. Public Health Nutr. 13 (12), 2076–2086.
Hanssen, L., Dudarev, A.A., Huber, S., Odland, J.Ø., Nieboer, E., Sandanger, T.M., 2013. Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan. Sci. Total Environ. 447, 430–437.
Hill, M.D., Abramson, F.P., 1988. The significance of plasma protein binding on the fetal/maternal distribution of drugs at steady-state. Clin. Pharmacokinet. 14 (3), 156–170.
Huber, S., Brox, J., 2015. An automated high-throughput SPE micro-elution method for perfluoroalkyl substances in human serum. Anal. Bioanal. Chem. 407 (13), 3751–3761.
Hytten, F., 1985. Blood volume changes in normal pregnancy. Clin. Haematol. 14 (3), 601–612.
Kärrman, A., Ericson, I., van Bavel, B., Darnerud, P.O., Aune, M., Glynn, A., Lignell, S., Lindström, G., 2007. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. Environ. Health Perspect. 115 (2), 226–230.
Krutzén, E., Olofsson, P., Bäck, S.-E., Nilsson-Ehle, P., 1992. Glomerular filtration rate in pregnancy: a study in normal subjects and in patients with hypertension, preeclampsia and diabetes. Scand. J. Clin. Lab. Invest. 52 (5), 387–392.
Lau, C., Butenhoff, J.L., Rogers, J.M., 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicol. Appl. Pharmacol. 198 (2), 231–241.

Liew, Z., Goudarzi, H., Oulhote, Y., 2018. Developmental Exposures to Perfluoroalkyl Substances (PFASs): an update of associated health outcomes. Curr. Environ. Health Rep. 5 (1), 1–19.

Lin, P.-I D., Cardenas, A., Hauser, R., Gold, D.R., Kleinman, K.P., Hivert, M.-F., Calafat, A. M., Webster, T.F., Horton, E.S., Oken, E., 2021. Temporal trends of concentrations of per- and polyfluoroalkyl substances among adults with overweight and obesity in the United States: Results from the Diabetes Prevention Program and NHANES. Environ. Int. 157, 106789. https://doi.org/10.1016/j.envint.2021.106789.

Mamsen, L.S., Björvang, R.D., Mucs, D., Vinnars, M.-T., Papadogiannakis, N., Lindh, C. H., Andersen, C.Y., Damdimopoulou, P., 2019. Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. Environ. Int. 124, 482–492.

Mogensen, U.B., Grandjean, P., Nielsen, F., Weihe, P., Budtz-Jørgensen, E., 2015. Breastfeeding as an exposure pathway for perfluorinated alkylates. Environ. Sci. Technol. 49 (17), 10466–10473.

Olsen, J., Liew, Z., 2018. Perfluoroalkyl substances and metabolic outcomes in pregnancy. J. Public Health Emerg. 2.

Schulz, K., Silva, M.R., Klaper, R., 2020. Distribution and effects of branched versus linear isomers of PFOA, PFOS, and PFHxS: A review of recent literature. Sci. Total Environ. 733, 139186. https://doi.org/10.1016/j.scitotenv.2020.139186.

Singer, A.B., Whitworth, K.W., Haug, L.S., Sabaredzovic, A., Impinen, A., Papadopoulou, E., Longnecker, M.P., 2018. Menstrual cycle characteristics as determinants of plasma concentrations of perfluoroalkyl substances (PFASs) in the Norwegian Mother and Child Cohort (MoBa study). Environ. Res. 166, 78–85.

Sookoian, S., Gianotti, T.F., Burgueño, A.L., Pirola, C.J., 2013. Fetal metabolic programming and epigenetic modifications: a systems biology approach. Pediatr. Res. 73 (2-4), 531–542.

Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo Sci. Environ. Epidemiol. 29 (2), 131–147.

van der Aa, E.M., Peereboom-Stegeman, J.H., Noordhoek, J., Gribnau, F.W., Russel, F.G., 1998. Mechanisms of drug transfer across the human placenta. Pharm. World Sci. 20, 139–148.

Wikström, S., Lin, P.-I., Lindh, C.H., Shu, H., Bornehag, C.-G., 2020. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr. Res. 87 (6), 1093–1099.

Winkens, K., Koponen, J., Schuster, J., Shoeib, M., Vestergren, R., Berger, U., Karvonen, A.M., Pekkanen, J., Kiviranta, H., Cousins, I.T., 2017. Perfluoroalkyl acids and their precursors in indoor air sampled in children's bedrooms. Environ. Pollut. 222, 423–432.