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Serum concentrations of per- and polyfluoroalkyl substances (PFAS) in Danish pregnant women—temporal trends during pregnancy, correlations with partners, associations with physical activity, and blood lipid concentrations

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Abstract

Background Per- and polyflouralkyl substances (PFAS) are a group of persistent chemicals used extensively in industries and consumer products due to their water-repellent properties. Studies have linked PFAS exposure to adverse health effects, and human exposure to PFAS, especially during pregnancy, is of great concern. In this study, we report how serum PFAS concentrations during pregnancy correlated with serum PFAS of partners from the same household. Further, we report how serum PFAS concentrations change during the course of pregnancy and associations between PFAS and blood lipid concentrations as well as exploratory analyses of associations between physical activity and PFAS concentrations.

Methods In this secondary analysis of data from the FitMum study conducted from 2018 to 2021, 216 healthy, pregnant women, and 110 of their partners were included. Non-fasting venous blood samples were collected from the mothers at three test visits during pregnancy and at delivery, where blood from partners were also collected. Serum samples from all timepoints were analyzed for 15 short- and long-chained PFAS using liquid chromatography triple quadrupole linear ion trap mass spectrometry. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride blood concentrations were measured at three test visits during pregnancy and at delivery. Physical activity was measured with a wrist-worn activity tracker 24/7 from inclusion before gestational age week 15 + 0 and throughout pregnancy.

Results In serum samples we detected the following PFAS: PFOS, PFOA, PFHxS, PFNA, PFDA, and PFUnDA. The maternal median concentrations at baseline were: PFOS: 4.09 ng/mL, PFOA: 0.81 ng/mL, PFHxS: 0.29 ng/mL, PFNA: 0.42 ng/mL, PFDA: 0.25 ng/mL, and PFUnDA: 0.19 ng/mL. Partner serum PFAS concentrations were 3–145% higher

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than maternal concentration (except for PFUnDA). PFAS concentrations correlated within couples. All PFAS decreased significantly during pregnancy (PFOS -23.1 percent 95%-CI [-31.9;-13.2] from baseline to delivery). All PFAS concentrations were associated with increased HDL-C concentrations. No associations between physical activity and maternal PFAS concentrations were found.

Conclusions Overall, serum PFAS concentrations decreased during pregnancy. PFAS concentrations within households were strongly correlated. PFAS and HDL-C concentrations were positively associated. We found no associations between physical activity and serum PFAS concentrations.

Trial registration The study was registered at ClinicalTrials.gov; NCT03679130; 20/09/2018.

Keywords Biomonitoring, Pregnancy, Perfluorinated substances, Polyflouroalkyl substances, Environmetal chemicals, Physical activity

Background

Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic chemicals with fluoride components that were first produced in the late 1940s, and were marketed as successful water-repellant substances with many uses in households and industry [1]. PFAS are chemically stable substances, and the extensive use has resulted in the fact, that PFAS are spread widely in the environment, and exposure is inevitable in the western society [1]. There are large geographical variations in PFAS exposures especially related to hotspots of production and use. Exposures at high levels have been reported in industrial productions sites [2] as well as sites with environmental water pollution e.g. from use of PFAS containing fire extinction foams [3]. Many different PFAS have been produced, but some are more commonly used than others. Previous studies have focused mainly on perfluorooctanoic acid (PFOA) and perfluorooctanelufonic acid (PFOS), while regulations of these substances have led to increased production of other PFAS such as PFHxS, PFNA, PFDA, as well as small-chain PFAS [4]. Epidemiological studies have consistently found associations between PFAS exposure and reproductive and developmental toxicity, neurotoxicity, hormone disruption, liver, renal, and cardiovascular toxicity as well as immunotoxicity [5–8]. Human exposure to PFOA has been classified as carcinogenic (group 1) by the International Agency for Research on Cancer (IARC) based on strong mechanistic evidence in exposed humans [9]. These negative health consequences of PFAS exposure were recognised in the early 2000's and since then, regulations of the use of PFAS have been introduced continuously in Denmark and the rest of Europe [10]. As a consequence, PFOS and PFOA are no longer allowed for production and use in almost all sectors of European societies. However, they are still present in the environment in significant amounts due to the slow degradation and long half-lifes of up to several years [8, 11].

Sources of PFAS exposure are mainly food related while specific sources from industry and household are also reported. In a Danish study of school children and mothers in 2011 Mørck et al. found a significant correlation between the PFAS concentrations in children and their mothers, indicating a family-related exposure pattern [12]. Mørck et al. also found a positive association between plasma PFAS levels and the age of the mothers as well as lower levels of plasma PFAS in mothers with high parity. This is in line with studies reporting human transplacental transfer [13, 14] as well as transfer by breastfeeding [15, 16]. Pregnancy is a vulnerable state of life and PFAS exposure during pregnancy is a major public health concern as specified by the general DoHAD Developmental Origins of Health and Diseases theory as recently found related to overweight in children prenatally exposed to PFAS [17]. This is in line with adverse effects of maternal exposure to PFAS on growth and development of the fetus and child reported in several international studies [18–22] and no safe levels have been established.

A decline in exposure levels of selected PFAS of PFOA and PFOS measured in human serum over past decades has been reported in Denmark [23, 24] reflecting the impact of regulations on selectively these compounds. Other PFAS persist and newer smaller chained PFAS are introduced in pesticide formulations however not all analytically detectable yet [25]. Little is known about natural excretion, especially during pregnancy, where physiological and metabolic changes possibly plays a large role on the excretion rates. To our knowledge, only few studies have examined the changes in PFAS concentrations during pregnancy and the results are not consistent [26, 27].

In both epidemiological and mechanistic studies, PFAS exposures have been reported to be associated with increased cholesterol levels [28, 29]. The mechanism behind the linkage of PFAS exposures and lipid concentrations are not fully understood, but several plausible mechanisms have been suggested such as changes in the hepatic and endocrine metabolisms as refered to by Hasegawa et al. [29]. Adverse outcome pathways (AOP) based on human data have been described for PFAS

exposures and metabolic health outcomes, including dyslipidemia, hypertension, insulin resistance, and obesity [30]. AOP based on animal data have found thyroid-damaging effects of PFAS affecting thyroid hormones, thyroid hormone gene expression, and histology that are associated in animal studies with decreased body and organ weight [31]. During pregnancy, the lipid metabolism changes in order to ensure the fetal growth and development [32]. Interruptions in the lipid metabolism during pregnancy may pose significant risks to the fetus, highlighting the importance of exploring the connection between PFAS and lipids in pregnancy. Our study enables to test for associations between PFAS exposure and blood lipid concentrations in commonly exposed Danish pregnant women.

Some studies have shown that physical activity can possibly mitigate the negative health effects of PFAS [33–35]. One study found that maternal PFOA concentration during pregnancy was associated with a worse cardiometabolic risk score in the offspring at 12 years of age but found that physical activity weakened this association [33]. The mechanism is not completely understood, but a plausible explanation could be that physical activity induce increased excretion of PFAS. Our study opened the possibility of investigating if physical activity – at relatively low intensity – was associated with PFAS concentrations during pregnancy.

With access to repeated sampling from pregnant women in the FitMum study, we aimed to explore temporal changes in serum PFAS concentrations during pregnancy in a healthy commonly exposed population. Further, we aimed to examine correlations between serum PFAS concentrations of mothers and partners from the same household. Last but not least, we aimed to investigate associations between physical activity level and PFAS concentrations, and associations between maternal PFAS serum concentrations and maternal blood lipid concentrations during pregnancy.

We hypothesized that physical activity can increase the excretion rate of PFAS and this way lower PFAS serum concentrations. Further, we hypothesized that increased PFAS concentrations to be associated with increased blood lipid concentrations.

Methods

Participants and study design

This study was a secondary, exploratory analysis of data from the FitMum study. The FitMum study was a randomized controlled trial conducted from October 2018 to June 2021 at the Department of Gynecology and Obstetrics at Copenhagen University Hospital – North Zealand, Hilleroed, Denmark.

The FitMum study included 220 healthy, inactive pregnant women. The participants were included before gestational week 15+0 and randomized 2:2:1, to 1) structured supervised exercise sessions, 2) motivational counselling on physical activity or 3) a control group receiving standard care. The intervention continued until delivery. A detailed description of the study and primary outcomes are published elsewhere [36, 37]. This paper includes all participants (from now on referred to as mothers) and partners (biological fathers) with accessible blood samples. Demographic information was obtained at inclusion from both mothers and partners. Body mass index (BMI) (pre-pregnancy, for mothers) was calculated based on self-reported weight and height.

Biochemical analyses

Blood sample collection

Non-fasting venous blood samples were obtained from mothers at Visit 1 at 11.7 ± 2.5 weeks of gestation ranging from week 6+1 to 15+0, Visit 2 at 28.4 ± 0.3 weeks of gestation ranging from week28+0 to 28+6), Visit 3 at 34.4 ± 0.3 weeks of gestation ranging from week week 34+0 to 34+0 and at Visit 4, shortly after delivery at 40.1 ± 1.6 weeks of gestation ranging from week 32+1 to 42+0. Non-fasting venous blood samples were obtained from the partners at Visit 4. Blood used for analyses of PFAS concentrations was collected in serum tubes, and blood used for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride analyses was collected in lithium-heparin-plasma tubes at Visit 1, 2, and 3, and in serum tubes at Visit 4. The plasma samples were kept at room temperature and lipid concentrations were analyzed within a few hours after collection. For the serum samples, the blood were allowed to coagulate at room temperature for at least 30 min before being centrifuged. Serum was subsequently collected and stored at −80°C until analyses of PFAS and lipid concentrations.

Analyses of per-and polyfluoroalkyl substances

The serum samples were analyzed for PFAS using liquid chromatography-tandem mass spectrometry (LC–MS/MS; QTRAP 6500+; AB Sciex, Framingham, MA, USA) at the Division of Occupational and Environmental Medicine at Lund University according to Norén et al. [38]. Briefly, 100 µl serum samples were added with isotopically labelled internal standards for most compounds, acetonitrile, and shaken vigorously for 30 min to precipitate proteins and thereafter centrifuged. All samples were analyzed in 9 batches of 96-well plates with glass inserts. Each batch, numbered 1–9, included four wells with chemical blanks and four wells with homemade pooled serum samples as quality controls (QC).

In the analytical method 15 PFAS compounds were included: perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) were quantified and reported in the samples, the others were below the limit of detection. The limit of detection (LOD) was defined as three times the standard deviation of the concentrations in chemical blank samples. The value of the PFAS below the the LOD were included in the statistical analyses. We estimated between-run precision using the quality controls (QC), as shown in Table S1 in supplement. The laboratory participated successfully in the HBM4EU QA/QC program for PFAS analysis, and participates bi-annually in the German External Quality Assessment Scheme (G-EQUAS) coordinated by the University of Erlangen-Nuremberg, and currently participates in ICI/EQUAS for PARC.

Analyses of lipids

The plasma and serum samples were analyzed at the Department of Clinical Biochemistry, Copenhagen University Hospital, Hilleroed, Denmark. TC, HDL-C, and triglyceride concentrations were measured by photometry (Dimension Vista 1500, Siemens Medical Solutions USA, Inc., Malvern, PA, US). LDL-C concentration was calculated using the Friedewald formula [39]. A detailed description of lipid analyses and effects of physical activity on maternal blood lipids is published elsewhere [40].

Measurement of physical activity

Physical activity was measured 24/7 from randomization to delivery by an activity tracker (Garmin Vívosport), worn on the wrist of the non-dominant hand. The activity tracker had a built-in heart rate monitor and accelerometer. Days with more than 6 h of non-wear time and weeks with less than 4 days of valid measurements were considered invalid and removed from the data set. In total the participants wore the activity tracker for a 77% of the potential days, as previously described [41]. The average wear time was 192 ± 20 days for each participant, after removing invalid days. Measures from the tracker included in the present study were minutes per day of moderate-to-vigorous-intensity physical activity (MVPA), active kilocalories (kcal) per day, number of steps per day, and hours per day of awake sedentary behaviour. MVPA was registered by the tracker when it recorded physical activity with a metabolic equivalent of task value of ≥ 3 in bouts of at least 10 consecutive minutes. Active kcal is assumably the calories burned through movement during a day, and these were calculated by the tracker based on age, weight, height, sex, and accelerometer and heart rate measures. Steps were registered by the tracker based on accelerometry [42]. Sedentary behaviour was defined as awake time spent on little or no activity like sitting and resting.

Statistical analyses

Demographic characteristics of the study population are presented as mean and standard deviation for approximately symmetric distributions, median and interquartile range for asymmetric distributions, and frequency and proportion for categorical data.

PFAS concentrations in serum from mother and partner respectively are presented as median with 5 and 95% percentiles and range from minumum to maximum concentrations. Maternal and partner concentrations were compared by calculating the relative difference (mother vs partner in %) and the difference was tested in paired t-tests for log-transformed concentrations. Within-household correlations were analyzed using Pearson correlation coefficients with 95% confidence intervals between log-transformed PFAS concentrations in mothers and partners. Sensitivity analyses excluding mothers and partners who smoked were performed, but did not change the results significantly. The results of the sensitivity analyses are presented in the supplementary material.

Concentrations of PFAS at Visit 1, 2, 3, and 4 are presented as geometric mean with 95% confidence intervals. Spaghetti plots illustrating the raw maternal concentrations at the different time points during pregnancy are reported in the supplementary material. Changes in PFAS concentrations during pregnancy were analyzed using linear mixed model regression analyses with log-transformed PFAS concentrations as the outcome variable and visit, parity, maternal age, pre-pregancy body mass index and batch number as covariates. To account for repeated measurements, we allowed residuals in the same participant at different visits to be correlated. The mixed model allows for inclusion of mothers with incomplete PFAS data (missing concentrations at some visits) using a maximum likehood approach.

In a similar mixed model, associations between PFAS serum concentrations and blood lipid concentrations were analyzed using a linear mixed model regression analyses with the log-transformed blood lipid concentrations at different visits as the outcome. This model assumed log-transformed PFAS serum concentrations at baseline to affect log-transformed baseline lipid concentration and in these analyses, we reported the percentage change in the lipid concentration at baseline (Visit 1) associated with of a doubling in PFAS concentration at baseline, presented as change with 95% confidence intervals. The analyses were adjusted for pre-pregancy BMI, maternal age, parity, and MVPA. Similarly, the difference

between the (log-transformed) lipid concentration at Visit 1 and Visit 4 was assumed to depend on the PFAS concentration at baseline. In these analyses, we reported (in Supplementary material) the percentage change in the ratio of lipid concentrations at Visit 4 and 1 (Visit 4/Visit 1) associated with a doubling in PFAS concentration at baseline. The analyses were adjusted for pre-pregancy BMI, maternal age, parity, and MVPA.

Finally, associations between physical activity measures and PFAS concentrations were analyzed. Here, PFAS concentrations at baseline were assumed to depend on physical activity measures in the baseline period, while the difference between the PFAS concentrations at Visit 1 and Visit 4 was assumed to depend on effects of physical activity level during pregnancy. In these analyses, we report the percentage change in the PFAS concentration at baseline (Visit 1) associated with a one unit increase in the physical activity measures at baseline. Further, we reported the percentage change in the ratio of PFAS concentrations at Visit 4 and 1 (Visit 4/Visit 1) associated with a one unit increase in the mean physical activity measures from Visit 1 to Visit 4. Results for theses analyses are presented as percentage change with 95% confidence intervals. Analyses were adjusted for pre-pregnancy BMI, maternal age, and parity. Physical activity measures were daily mean values in the baseline period, and from randomization to Visit 4, respectively. Days with missing observations in activity tracker data were imputed by multiple imputations in 25 data sets using the random forest imputation model from the mice R package as previously described [37].

For all linear mixed models we performed sensitivity analyses, adjusting for maternal educational level, but only found minor changes. Thus, we did not include educational level in the final results, but the results of the sensitivity analyses are presented in the supplementary material.

All statistical analyses were performed in R, except for linear mixed models which were analyzed using SAS version 9.4 and Mplus version 8.8. Statistical significance was defined as a p-value below 0.05.

Results

The FitMum study included 220 pregnant women and 198 of their partners who were the self reported biological fathers and at least one blood sample was accessible from 216 mothers and 110 partners (Fig. 1).

Descriptive characteristics of the study population are presented in Table 1. Generally, the study population was normal-weight, as the mothers' median pre-pregnancy BMI was 24.1 kg/m^2 (21.9–28.7) and partners' median BMI was 25.4 kg/m^2 (23.6–27.7). Further, they were well-educated with 91% of mothers and 75% of their partners having completed ≥ 12 years of school. Only 1% of

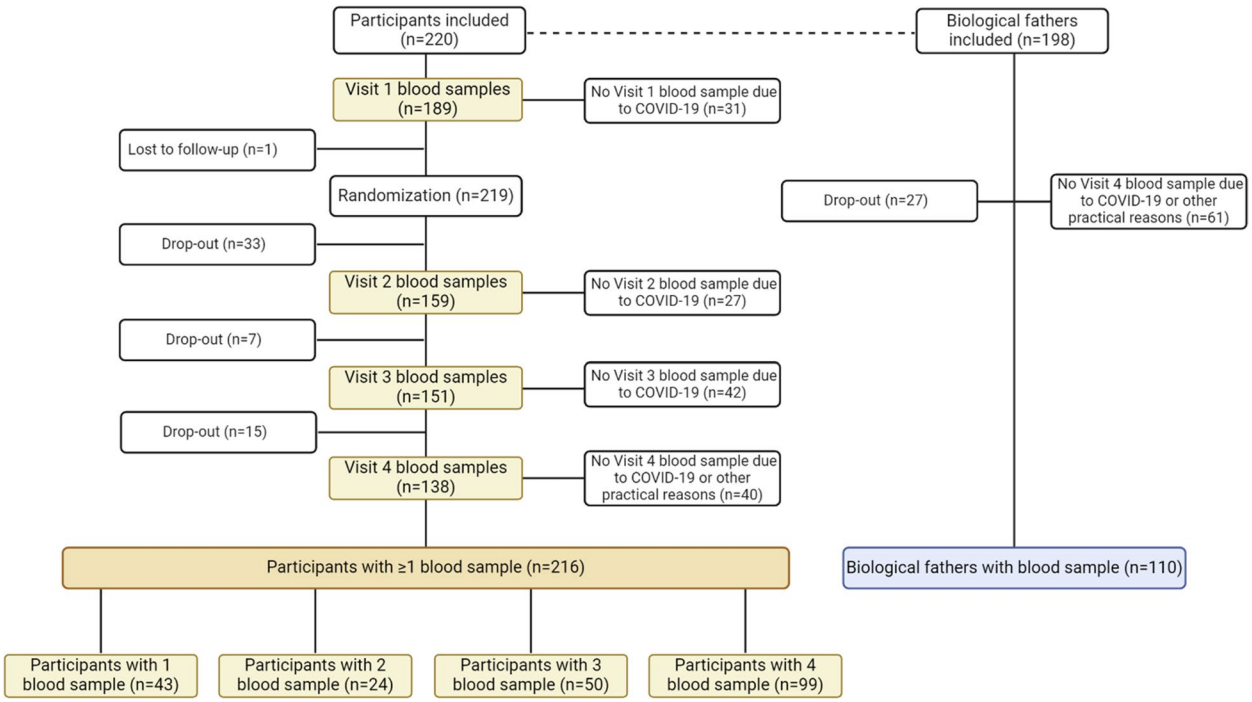

Fig. 1 Flowchart of inclusion. Visit 1: Gestational age week 6 + 1 to 15 + 0. Visit 2: Gestational age week 28 + 0–6. Visit 3: Gestational age week 34 + 0–6. Visit 4: Delivery, gestational age week 32 + 1 to 42 + 0. *Figure created with BioRender.com*

Table 1 Demographic characteristics of the study population

Number of participants	**Mothers** ***n = 216***	**Partners** ***n = 110***
Age (years)	31.5 ± 4.3	33.5 ± 5.7
BMI (kg/m²)[a]	24.1 (21.9–28.7)	25.4 (23.6–27.7)
GA at inclusion (weeks)	12.6 (9.4–13.8)	*NA*
GA at delivery (weeks)[b]	40.4 (39.4–41.1)	*NA*
Parity (n (%))		
0	82 (38)	*NA*
1	98 (45)	*NA*
2	31 (14)	*NA*
3	5 (2)	*NA*
Educational level (n (%))[c]		
School ≥ 12 years	189 (88)	83 (75)
Further education ≥ 3 years	196 (91)	67 (60)
Employed/studying (n (%))	196 (90)	106 (96)
Smoking (n (%))		
Currently smoking	2 (1)	16 (15)
Stopped recently	6 (7)	2 (2)

Data are presented as mean ± standard deviation, median (interquartile range) and n (%). No statistical comparisons have been performed

BMI Body mass index; GA: gestational age

[a] Maternal BMI is self-reported pre-pregnancy BMI

[b] GA at delivery, $n = 178$

[c] School ≥ 12 years corresponds to high school, and Further education ≥ 3 years corresponds to a bachelor's or master's level

mothers were smoking during pregnancy, while 15% of their partners were smoking. 38% of the mothers were nulliparous.

Maternal and partner PFAS concentrations

In serum samples we detected PFOS, PFOA, PFHxS (2 samples below limit of detection), PFNA, PFDA, PFUnDA (98 samples below limit of detection). Maternal median concentrations at baseline were PFOS: 4.09 ng/mL, PFOA: 0.81 ng/mL, PFHxS: 0.29 ng/mL, PFNA: 0.42 ng/mL, PFDA: 0.25 ng/mL, PFUnDA: 0.19 ng/mL. Overall, partner PFOS, PFOA, PFHxS, and PFNA concentrations were 3–145% higher than maternal PFAS concentrations (Table 2). Correlations between mothers' and partners' concentrations within households were 0.41 [95% CI 0.22;0.56] for PFOS, 0.20 [95% CI 0.00;0.39] for PFOA, 0.14 [95% CI −0.06;0.34] for PFHxS, 0.33 [95% CI 0.14;0.50] for PFNA, 0.51 [95% CI 0.33;0.64] for PFDA, and 0.39 [95% CI 0.20;0.55] for PFUnDA.

Changes in PFAS concentrations during pregnancy

Overall, PFAS concentrations decreased during the course of pregnancy (Fig. 2 and Table 3). PFOS, PFOA, PFNA, and PFDA decreased between 18 and 35 percent during the course of pregnancy (overall p-values < 0.005), while PFHxS only decreased significantly from Visit 1 to Visit 2 (change −18.4 percent [95% CI −31.1;−3,4]). No statistically significant association was detected between visit number and PFUnDA concentrations. Being pregnant for the 3rd time was associated with a 18.84 percent lower PFOS concentration [95% CI −31.58;−3.73] compared to being nulliparous. However, no significant overall effect of parity on PFOS concentration was detected (p-value 0.078). Being pregnant with the 4th child was associated with a 40.37 percent lower PFOA concentration [95% CI −58.26;−14.81] and a 39.49 percent lower PFHxS concentration [95% CI −61.48;−4.94] compared to being nulliparous. Meanwhile, parity was not associated with a decline in PFNA, PFDA or PFUnDA concentrations.

Table 2 Percentage of all samples above limit of detection as well as concentrations of PFHxS, PFOA, PFNA, total-PFOS, PFDA, PFUnDA (ng/mL) in maternal serum from visit 1 and partner serum presented as median (5–95% percentiles), and range [minimum and maximum concentrations]. Percentage difference between (geometric means of) paternal and maternal PFAS concentrations, and correlations of (log-transformed) PFAS concentrations within households, both presented with 95% confidence intervals

	Detection (%) ***n = 774***	**Maternal, visit 1** ***n = 187***	**Partner** ***n = 110***	**Difference [95% CI]** ***n = 92***	**Correlation [95% CI]**
PFOS	100%	4.09 (1.92–7.26) [1.22–11,77]	6.94 (3.31–12.49) [1.12–22.03]	70.41 [52.89;89.93]*	0.41 [0.22;0.56]*
PFOA	100%	0.81 (0.36–1.61) [0.16–2.43]	1.30 (0.54–2.04) [0.05–2.47]	47.38 [29.41;67.85]*	0.20 [0.00;0.39]
PFHxS	99.7%	0.29 (0.12–0.65) [0.04–5.65]	0.76 (0.38–1.32) [0.08–9.83]	145.02 [110.34;185.42]*	0.14 [−0.06;0.34]
PFNA	100%	0.42 (0.23–0.89) [0.12–1.34]	0.64 (0.32–1.11) [0.09–1.37]	38.11 [24.20;53.57]*	0.33 [0.14;0.50]*
PFDA	100%	0.25 (0.13–0.49) [0.08–0.92]	0.30 (0.14–0.54) [0.06–0.67]	3.03 [−5.56;12.4]	0.51 [0.33;0.64]*
PFUnDA	87.3%	0.19 (0.08–0.40) [0.03–0.64]	0.21 (0.08–0.37) [0.04–0.47]	−10.2 [−19.81;0.58]	0.39 [0.20;0.55]*

PFOS perfluorooctane sulfonic acid, *PFOA* perfluorooctanoic acid, *PFHxS* perfluorohexane sulfonate, *PFNA* perfluorononanoic acid, *PFDA* perfluorodecanoic acid, *PFUnDA* perfluoroundecanoic acid

* p-value < 0.05

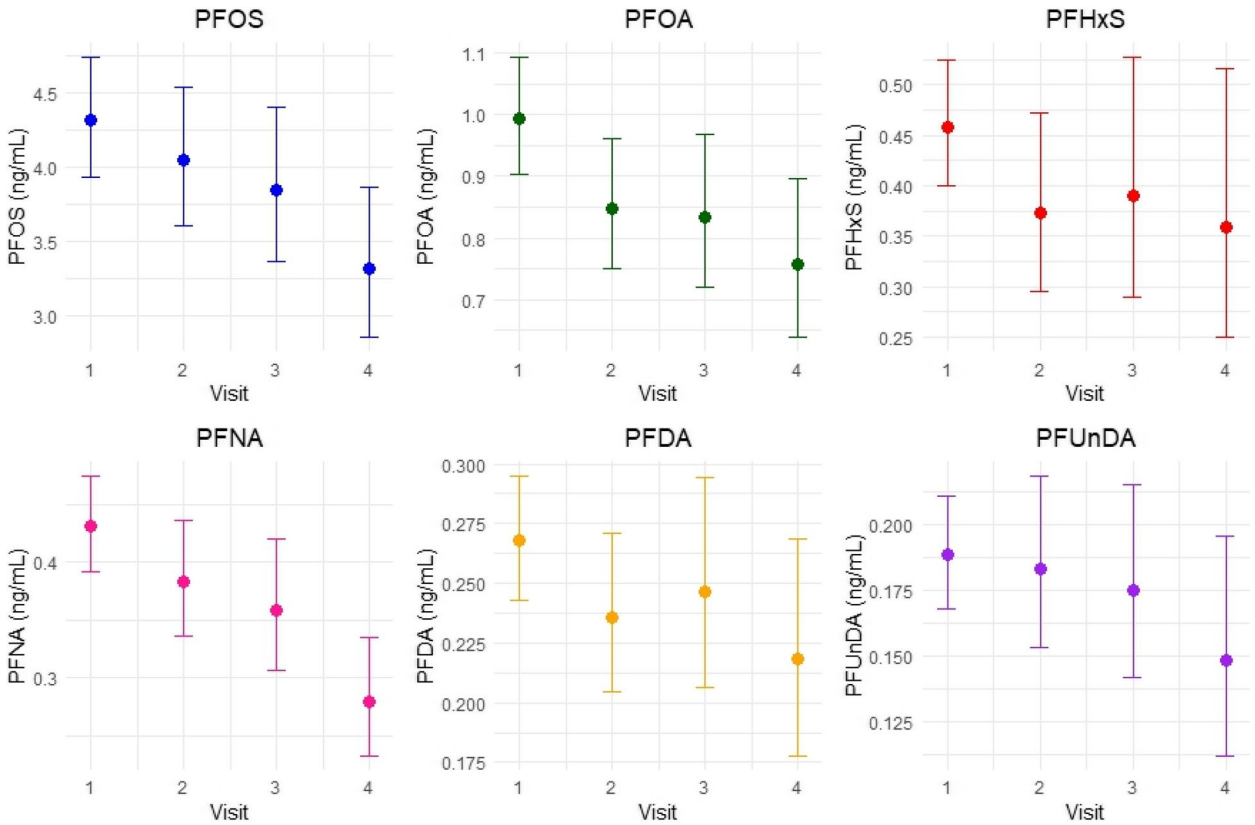

Fig. 2 Maternal serum PFAS concentrations at Visit 1, 2, 3, and 4 presented as geometric mean with 95% confidence intervals. Visit 1: Gestational age week 6 + 1 to 15 + 0. Visit 2: Gestational age week 28 + 0–6. Visit 3: Gestational age week 34 + 0–6. Visit 4: Delivery, gestational age week 32 + 1 to 42 + 0. PFAS; Per- and polyfluoroalkyl substances, PFOS; perfluorooctane sulfonic acid, PFOA; perfluorooctanoic acid, PFHxS; perfluorohexane sulfonate, PFNA; perfluorononanoic acid, PFDA; perfluorodecanoic acid, PFUnDA; perfluoroundecanoic acid. *Figure created with BioRender*

PFAS and blood lipid concentrations

At baseline, a doubling of serum concentrations of all PFAS were associated with increased concentrations of HDL-C at Visit 1 (Table 4). Further, a doubling of serum concentrations of all PFAS were positively associated with TC/HDL-C ratio at Visit 1 (Table 4). Results from analyses on changes from Visit 1 to Visit 4 can be found in Supplementary material (Table S1). A doubling of PFOA was associated with less increase of HDL-C from Visit 1 to Visit 4 (Table S1). No significant effect of any PFAS on TC, LDL-C or triglyceride concentrations were detected.

Physical activity and PFAS concentrations

We did not detect significant associations between any physical activity measure and PFOS, PFNA, PFDA or PFUnDA serum concentrations (Table 5). At baseline, one hour more spent sedentary seemed to be negatively associated with PFOA (change: −3.88 percent, p-value 0.046) (Table 5). More minutes per day of MVPA were associated with a smaller decrease in PFHxS from Visit 1 to Visit 4 (change: −1.23 percent per daily increase of one minute of MVPA, *p*-value 0.024). Likewise, higher burned active kcals were associated with a smaller decrease in PFHxS from Visit 1 to Visit 4 (change: −5.30 percent per 100 burned active kcal per day, *p*-value 0.010) and more sedentary time were associated with a larger decrease in PFHxS from Visit 1 to Visit 4 (change: −11.08 percent per daily hour of sedentary time, *p*-value 0.027) as well as a larger decrease in PFUnDA (−8.99 percent per daily hour of sedentary time, *p*-value 0.036).

Discussion

PFAS concentrations

Overall, the PFAS concentrations in this study were comparable to concentrations in recent Danish studies and with PFOS found at higher concentrations than PFOA [23, 24]. In our dyads of mothers and partners, maternal PFOS, PFOA, PFHxS, and PFNA concentrations at baseline were lower than partner concentrations. This could be partly explained by different occupational exposure patterns and by different excretion rates due to hormonal and physiological differences [43]. Another possible explanation is that women excrete some PFAS every

Table 3 Percentage changes in (geometric mean) PFAS concentrations as a function of visit and parity, estimated in a linear mixed model with adjustment for pre-pregnancy body mass index, maternal age, and batch number. The last column shows the p-value in test of the hypothesis for no differences between groups

PFAS	Covariate		Change [95% CI]	*p*-value	Overall *p*-value
PFOS					
	Visit	1[a]	0		< 0.001
	Visit	2	−6.28 [−11.74;−0.49]	0.034	
	Visit	3	−10.82 [−18.15;−2.84]	0.009	
	Visit	4	−23.14 [−31.92;−13.22]	< 0.001	
	Parity	0[a]	0		0.078
	Parity	1	−10.78 [−20.71;0.40]	0.058	
	Parity	2	−18.84 [−31.58;−3.73]	0.017	
	Parity	3	−12.53 [−39.04;25.53]	0.466	
PFOA					
	Visit	1[a]	0		< 0.001
	Visit	2	−14.60 [−20.28;−8.51]	< 0.001	
	Visit	3	−16.06 [−24.29;−6.95]	< 0.001	
	Visit	4	−23.89 [−34.04;−12.19]	< 0.001	
	Parity	0[a]			< 0.001
	Parity	1	−36.69 [−43.64;−28.88]	< 0.001	
	Parity	2	−35.65 [−45.65;−23.82]	< 0.001	
	Parity	3	−40.37 [−58.26;−14.81]	0.005	
PFHxS					
	Visit	1[a]	0		0.065
	Visit	2	−18.38 [−31.08;−3.35]	0.019	
	Visit	3	−14.69 [−33.23;8.99]	0.203	
	Visit	4	−21.56 [−43.88;9.63]	0.154	
	Parity	0[a]	0		< 0.001
	Parity	1	−34.69 [−43.73;−24.20]	< 0.001	
	Parity	2	−48.35 [−58.43;−35.82]	< 0.001	
	Parity	3	−39.49 [−61.48;−4.94]	0.030	
PFNA					
	Visit	1[a]	0		< 0.001
	Visit	2	−11.23 [−17.69;−4.26]	0.002	
	Visit	3	−16.80 [−25.59;−6.97]	0.001	
	Visit	4	−35.22 [−44.58;−24.27]	< 0.001	
	Parity	0[a]	0		0.251
	Parity	1	−10.50 [−20.48;0.72]	0.066	
	Parity	2	−12.25 [−26.03;4.11]	0.133	
	Parity	3	−8.85 [−36.42;30.68]	0.613	
PFDA					
	Visit	1[a]	0		0.005
	Visit	2	−12.04 [−19.66;−3.70]	0.006	
	Visit	3	−8.04 [−19.24;4.73]	0.206	
	Visit	4	−18.42 [−31.91;−2.26]	0.027	
	Parity	0[a]	0		0.423
	Parity	1	2.09 [−8.99;14.53]	0.723	
	Parity	2	9.85 [−7.00;29.76]	0.267	
	Parity	3	27.60 [−9.83;80.58]	0.168	
PFUnDA					
	Visit	1[a]			0.253
	Visit	2	−2.78 [−13.72;9.54]	0.642	
	Visit	3	−7.10 [−20.76;8.93]	0.363	
	Visit	4	−21.26 [−38.75;1.22]	0.062	
	Parity	0[a]			0.829
	Parity	1	1.14 [−10.25;13.97]	0.852	
	Parity	2	7.79 [−10.03;29.14]	0.414	
	Parity	3	10.41[−23.16;58.64]	0.591	

Visit 1: Gestational age week 6 + 1 to 15 + 0

Visit 2: Gestational age week 28 + 0–6

Visit 3: Gestational age week 34 + 0–6

Visit 4: Delivery, gestational age week 32 + 1 to 42 + 0

PFAS per- and polyfluoroalkyl substances, *PFOS* perfluorooctane sulfonic acid, *PFOA* perfluorooctanoic acid, *PFHxS* perfluorohexane sulfonate, *PFNA* perfluorononanoic acid, *PFDA* perfluorodecanoic acid, *PFUnDA* perfluoroundecanoic acid

[a] Reference group

month during the menstural period [44]. However, we found that maternal and partner PFAS concentrations within households were correlated, especially for PFOS, PFNA, PFDA and PFUnDA. This suggests that a large proportion of the exposure to these PFAS is coming from within the household, such as food and drinking water. This is not surprising, and in line with comparable positive correlations found between mothers and children in a Danish study from 2011 [12].

We found that PFAS concentrations decreased during pregnancy, and being multiparous was associated with lower PFAS concentrations. We did not detect any significant changes in PFUnDA concentrations. However, due to the relatively low concentrations of PFUnDA, these results should be interpreted with caution.

In line with a study by Li et al. [45] reporting that PFOA decreased by 26% per year compared with 20% for PFOS and 13% for PFHxS in a non-pregnant population, we also found that PFOA decreased more during pregnancy than any of the other measured PFAS. In a recent study by Møller et al. [46], the natural excretion of PFOA in a non-pregnant population of highly exposed men and women was 3.3% over a period of 12 weeks, corresponding to 0.27% decrease per week. Furthermore, in a study by Oh et al. [47] the serum concentration of PFOS, PFOA, PFNA, PFHxS decreased by 10–12% per year in a population of 2–5 year old children. Thus, our results suggest that the excretion of PFAS is larger during pregnancy compared to outside pregnancy, as expected. This is in line with results from the Chen et al. [26] where all detected PFAS

Table 4 Percentage change in maternal PFAS concentration at baseline for a one unit increase in physical activity measure and percentage change in the relative change in PFAS concentration for a one unit increase in physical activity measure. Associations were estimated in a linear mixed model with adjustment for pre-pregnancy BMI, maternal age, and parity

	PFOS Change [95% CI]	PFOA Change [95% CI]	PFHxS Change [95% CI]	PFNA Change [95% CI]	PFDA Change [95% CI]	PFUnDA Change [95% CI]
Visit 1						
MVPA	−0.01 [−3.46;3.56]	0.21 [−3.64;4.21]	−0.17 [−10.16;10.93]	−0.10 [−5.11;5.17]	0.08 [−6.18;6.75]	−0.80 [−7.10;5.93]
Steps	−0.22 [−3.68;3.36]	2.76 [−1.22;6.90]	1.14 [−9.18;12.64]	−0.03 [−5.05;5.25]	0.47[−5.82;7.17]	−1.59 [−7.87;5.12]
Active kcal	−1.70 [−5.10;1.82]	3.40 [−0.61;7.57]	−0.87 [−10.74;10.10]	0.24 [−4.79;5.53]	0.08 [−6.18;6.76]	−2.04 [−8.26;4.60]
Sedentary time	−1.13 [−4.53;2.38]	−3.88 [−7.54;−0.07]*	−0.51 [−9.66;11.81]	−0.82 [−5.79;4.42]	−1.88 [−8.00;4.66]	1.22 [−5.21;8.08]
Change Visit 1 to Visit 4						
MVPA	0.02 [−0.34;0.38]	−0.05 [−0.53;0.43]	1.23 [0.16;2.30]*	−0.30 [−0.79;0.19]	0.06 [−0.46;0.59]	−0.15 [−1.07;0.78]
Steps	0.18 [−1.59;1.98]	1.40 [−1.02;3.87]	−0.16 [−5.35;5.30]	−0.40 [−2.85;2.11]	0.26 [−2.34;2.93]	0.20 [−4.20;4.79]
Active kcal	0.44 [−0.85;1.75]	−0.04 [−1.83;1.78]	5.30 [1.26;9.50]*	−1.23 [−3.01;0.59]	0.27 [−1.64;2.22]	2.52 [−0.88;6.04]
Sedentary time	−2.53 [−5.90;0.97]	−4.59 [−9.02;0.05]	−11.08 [−19.86;1.35]*	−0.63 [−5.46;4.45]	−2.61 [−7.65;2.71]	−8.99 [−16.66;−0.62]*

Units for physical activity measures are MVPA: minutes, Steps: per 1000, Actkcal: per 100, Sedentary time: hours

Visit 1: Gestational age week 6 + 1 to 15 + 0

Visit 4: Delivery, gestational age week 32 + 1 to 42 + 0

PFOS perfluorooctane sulfonic acid, *PFOA* perfluorooctanoic acid, *PFHxS* perfluorohexane sulfonate, *PFNA* perfluorononanoic acid, *PFDA* perfluorodecanoic acid, *PFUnDA* perfluoroundecanoic acid, *MVPA* moderate-to-vigourous intensity physical activity, *Kcal* kilocalories

* *p*-value < 0.05

Table 5 Maternal PFAS concentrations at Visit 1 presented as mean ± standard deviation. Percentage change in plasma lipid concentration at Visit 1 for a doubling of serum PFAS concentration presented as relative change with 95% confidence intervals. Associations were estimated in a linear mixed model and adjusted for pre-pregnancy BMI, maternal age, parity, and daily minutes of moderate-to-vigorous-intensity physical actitivty

	TC	HDL-C	TC/HDL-C	LDL-C	Triglyceride
Visit 1 (mmol/L)	**4.52 ± 0.79**	**1.74 ± 0.35**	**2.68 ± 0.61**	**2.17 ± 0.64**	**1.33 ± 0.61**
	Change [95% CI]	**Change [95% CI]**	**Change [95% CI]**	**Change [95% CI]**	**Change [95% CI]**
PFOS	−0.49 [−4.64;3.84]	5.83 [1.23;10.63]*	−5.95 [−10.35;1.35]*	−3.62 [−10.46;3.73]	−6.12 [−15.45;4.25]
PFOA	−0.82 [−4.44;2.95]	5.64 [1.63;9.80]*	−6.10 [−9.93;2.10]*	−5.92 [−11.75;0.29]	−1.93 [−10.55;7.51]
PFHxS	−0.67 [−3.60;2.34]	5.16 [1.97;8.45]*	−5.53 [−8.62;−2.34]*	−4.51 [−9.29;0.52]	−5.00 [−11.72;2.24]
PFNA	0.22 [−4.05;4.67]	8.01 [3.29;12.95]*	−7.20 [−11.60;−2.58]*	−6.30 [−13.05;0.97]	0.22 [−9.98;11.57]
PDFA	−0.40 [−4.58;3.96]	6.13 [1.50;10.98]*	−6.13 [−10.54;−1.49]*	−4.84 [−11.61;2.46]	−4.77 [−14.31;5.83]
PFUnDA	0.63 [−2.94;4.34]	7.06 [3.16;11.10]*	−5.98 [−9.70;−2.10]*	−3.16 [−9.02;3.08]	−3.60 [−11.82;5.38]

Visit 1: Gestational age week 6 + 1 to 15 + 0

PFAS per- and polyfluoroalkyl substances, *PFOS* perfluorooctane sulfonic acid, *PFOA* perfluorooctanoic acid, *PFHxS* perfluorohexane sulfonate, PFNA; perfluorononanoic acid, PFDA; perfluorodecanoic acid, PFUnDA; perfluoroundecanoic acid, *TC* Total cholesterol, *HDL-C* High-density lipoprotein cholesterol, *LDL-C* Low-density lipoprotein cholesterol

* *p*-value < 0.05

concentrations, except PFHxS, decreased during the course of pregnancy. However, a recent study by Tan et al. [27] found the opposite, that maternal PFAS concentrations, except PFOA, increased during pregnancy. Thus, it remains unclear what the natural excretion rate of PFAS is during pregnancy, and more investigations are needed in order to determine this. It is well-known that PFAS pass the placenta, and it seems logical that the PFAS concentration during pregnancy decrease, partly explained by the transplacental transfer of PFAS [14, 48]. Further, it is worth noticing that the blood volume increases with up to 50% during pregnancy [49], and part of the explanation for the decrease in PFAS concentrations may be the larger distribution volume. However, not much is known about the compartments of which the PFAS are stored, and future studies should

investigate this in order to more precisely estimate the excretion rates during pregnancy.

We found strong associations between parity and PFOA and PFHxS concentrations and tendency towards an association between parity and PFOS was found. This might reflect that the different PFAS are transported differently across the placenta [50]. Our results are in line with previous results by Kang et al. [48] where PFOA and PFHxS had higher placental transfer rates than PFOS.

Blood lipid concentrations

Interestingly, we found that higher maternal PFAS concentrations were associated with higher HDL-C and lower TC/HDL ratio in early pregnancy. These results are partly in line with previous studies in non-pregnant populations where serum PFAS concentrations were positively associated with TC, HDL-C, and LDL-C concentrations [28]. Lipoprotein cholesterols, such as HDL-C and LDL-C, are mainly synthezised in the liver, and it is well-known that PFAS accumulate in the liver [51]. Mechanistic studies in both humans and rodents have shown that PFAS bind to proteins in the hepatocyte affecting the transport function of the hepathocytes [52]. The lipid metabolism changes drastically during pregnancy, and the placenta plays a key role in these changes [53]. Thus, the effect of PFAS on the lipid metabolism is not necessarily the same during pregnancy as in non-pregnant life periods. In a study of highly exposed, lean, pregnant women PFOS was positively associated with TC, HDL-C, and LDL-C concentrations in the first trimester, while both PFOA and PFHxS were inversely associated with TC and LDL-C concentrations in third trimester [54]. In the present study, of likewise lean, pregnant, but not highly exposed, pregnant women we found positive associations between all PFAS and HDL-C, suggesting PFAS are affecting mainly HDL-C metabolism during pregnancy. The role of placenta in the shift in the lipid metabolism during pregnancy and the interaction with PFAS should be further investigated.

Physical activity

We hypothesized that physical activity could increase the excretion of PFAS and this way lower the PFAS concentrations. Previous studies have shown that physical activity can mitigate the negative health effects of PFAS [33–35]. One study found that maternal PFOA concentrations were associated with lower cardiometabolic risk score and found that the children's physical activity level mitigated the effect while another study found that PFAS concentrations at 12 years of age was associated with lower bone mineral content and also found that physical activity mitigated the association. A possible explanation of these effects could be that physical activity induce an increased excretion rate. The excretion pathways of PFAS are not fully understood, but a recent study found that treatment with cholestyramine, a medication increasing fecal excretion of cholesterols, lowers PFAS concentrations [46], suggesting that PFAS excretion is closely linked to the cholesterol metabolism. Further, it is well documented that physical activity can lower lipid concentrations and it is also evident, that PFAS and lipid concentrations are associated, at least in non-pregnant individuals. Thus, it could be, that physical activity lowers both lipid and PFAS concentrations. However, in the present study, we did not detect any significant associations between physical activity and serum concentration of any PFAS except for PFHxS, where increased physical activity was associated with a smaller decline in PFHxS from Visit 1 to Visit 2. The negative findings might be due to the relatively low concentrations of PFAS combined with relatively small differences in physical activity level in the study sample. As previously described, the mothers in all intervention groups spent less than 1 h per week of MVPA recorded by the Garmin tracker [55], which is below the recommendations from the Danish Health Authorities [56]. Thus, studies in highly exposed populations with larger differences in physical activity level might be needed to provide the power to detect any possible effect of physical activity.

Strengths and limitations

It is a strength that both maternal and partner blood samples were obtained for a large number of pregnant women. It is a strength that we were able to measure physical activity objectively. However, the physical activity level did not differ much between the mothers, therefore the study might be under-powered to detect any association between physical activity and PFAS concentrations. Further, many analyses between physical activity and PFAS concentrations have been performed, leading to the risk of chance findings due to multiple testing. Further, this study is one of the first to describe associations between repeated serum PFAS concentrations and blood lipid concentrations measured concurrently in a pregnant study sample. However, volume of distribution changes during pregnancy on an individual level making it impossible to calculate exact excretion rates.

Conclusion

Serum PFAS concentrations decreased during pregnancy, as expected. Further, we found significant correlations between PFAS concentrations of pregnant women and their partners. Interestingly, high serum concentrations of PFAS were associated with higher HDL-C concentrations. No associations of physical activity and serum PFAS concentrations were found.

Abbreviations

AOP	Adverse outcome pathway
BMI	Body mass index
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
Kcal	Kilocalories
MVPA	Moderate-to-vigorous-intensity physical activity
PFAS	Per- and olyfluoroalkyl substances
PFOS	Perfluorooctane sulfonic acid
PFOA	Perfluorooctanoic acid
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
TC	Total cholesterol
QC	Quality control

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
B.S. initiated and directed the FitMum study and E.L. was principal investigator. E.L., I.K.B.J., and L.K. developed the study protocol for this secondary analysis of data from the FitMum study in collaboration with C.L. and O.H.M. I.K.B.J., C.B.R., and S.d.P.K., collected data in collaboration with research assistants and master students supported by J.B., S.M., and T.D.C.. C.L. analyzed serum samples for PFAS concentrations. L.K. supervised analysis of data and writing of the manuscript. E.B.J. performed statistical analyses. I.K.B.J. and L.K. drafted the manuscript. All authors read, contributed, and approved the final version of the manuscript.

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Data availability
The datasets generated and analyzed in the current study are not publicly available due to confidentiality but are available from the corresponding author upon reasonable request. Individual participant data will be transferred according to the Data Protection Act, when approval from the Danish Data Protection Agency is obtained, and a Standard Contractual Clause is completed, to ensure the legal basis of the transfer.

Declarations

Ethics approval and consent to participate
Written informed consent was obtained from all participants. The study was approved by the Regional Committee on Health Research Ethics (August 30, 2018, #H-18011067 with amendment for PFAS analyses #99743, approved on April 12, 2023) and the Danish Data Protection Agency (September 12, 2018, #P-2019–512). The study adheres to the principles of the Helsinki declaration.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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