Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth

Irina Gyllenhammar,⁎ Barbro Diderholm, Jan Gustafsson, Urs Berger, Peter Ridefelt, Jonathan P. Benskin, Sanna Lignell, Erik Lamp, Anders Glynn

⁎ Corresponding author.
E-mail address: irina.gyllenhammar@slv.se (I. Gyllenhammar).

Abstract

We investigated if maternal body burdens of perfluoroalkyl acids (PFAAs) at the time of delivery are associated with birth outcome and if early life exposure (in utero/nursing) is associated with early childhood growth and weight gain. Maternal PFAA body burdens were estimated by analysis of serum samples from mothers living in Uppsala County, Sweden (POPUP), sampled three weeks after delivery between 1996 and 2011. Data on child length and weight were collected from medical records and converted into standard deviation scores (SDS). Multiple linear regression models with appropriate covariates were used to analyze associations between maternal PFAA levels and birth outcomes (n = 381). After birth Generalized Least Squares models were used to analyze associations between maternal PFAS and child growth (n = 200). Inverse associations were found between maternal levels of perfluoromonoalkyl acids (PFMAs) and birth weight SDS but positive associations with birth length SDS. Inverse associations were also found between maternal body burdens of perfluoroundecanoic acid (PFUnDA), and birth weight SDS with a change of −0.10 to −0.18 weight SDS for an inter-quartile range (IQR) increase in ng/g PFAA. After birth, weight and length SDS were not significantly associated with maternal PFAA. However, BMI SDS was significantly associated with PFOA, PFNA, and PFHxS at 3 and 4 years of age, and with PFOS at 4 and 5 years of age. If causal, these associations suggest that PFAA affects fetal and childhood body development in different directions.

1. Introduction

Owing to their stability and combined water and oil repelling properties, perfluoroalkyl moieties have been incorporated into numerous commercial products for over 50 years. Over 3000 per- and polyfluoroalkyl substances (PFASs) are known to exist on the global market. Among these, the perfluoroalkyl acids (PFAAs) have garnered considerable international attention due to their environmental persistence and global occurrence in humans and wildlife. Pathways of human exposure are numerous and include food, drinking water, dust and air. In addition to direct exposure to PFAAs via any one of the aforementioned routes, exposure can also occur via precursors which may transform to PFAAs after exposure (D’Hollander et al., 2010; Vestergren et al., 2012; Gebbink et al., 2015; Gyllenhammar et al., 2015).

Studies investigating associations between maternal PFAS concentrations in serum/plasma and birth weight in humans have reported conflicting results (Johnson et al., 2014; Bach et al., 2015). Most studies have focused on perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) although humans are exposed to numerous other PFASs, only a fraction of which are known.

Studies of associations between maternal PFAA levels and childhood weight gain are scarce. Previous work has pointed out negative associations between prenatal PFOA and PFOS exposure and child weight at 5 and 12 months of age (Andersen et al., 2010) and positive associations between maternal PFOS levels and weight at 20 months (Maisonet et al., 2012), and PFOA and risk of overweight at 20 years of age (Halldorsson et al., 2012). Increased prenatal PFAA exposure has also been associated with increased adiposity in 3–20-year-olds (Halldorsson et al., 2012; Braun et al., 2016; Mora et al., 2017). However, results from studies of prenatal PFAS exposure and childhood weight gain and adiposity are not consistent as some of them show no significant associations (Andersen et al., 2013; Barry et al., 2014).

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Intra uterine growth restriction (IUGR) and/or being born small for gestational age (SGA) have been related to increased risk of impaired glucose tolerance, increased adiposity, and increased blood pressure later in life, especially among individuals with an early and rapid catch-up in weight after birth (Ibanez et al., 2006; Kerkhof et al., 2012; Gishti et al., 2014). Therefore, it is of interest to investigate fetal/neonatal PFAA exposure in relation to fetal, infant and child weight development and growth.

The aim of the present study was to investigate if maternal body burdens of PFAA at delivery are associated with birth outcomes and offspring weight gain and growth. Based on the present knowledge, we hypothesize that fetal PFAA exposure could lead to a decreased birth weight and thereby possibly also influence offspring weight development.

2. Materials and methods

2.1. Participants

Serum samples were collected 1996–2011 from first-time mothers from Uppsala County, Sweden, within the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas). The study was restricted to singleton births with no birth defects. All women were Swedish born and almost all women had a full-term pregnancy (37–42 weeks), except in a few cases with the shortest gestational length being 35 weeks and the longest 43 weeks. The women in the POPUP cohort are homogenous regarding ethnic background, as indicated by the fact that only about 4% of the Swedish population had one or two foreign-born parents in 2006 (Socialstyrelsen, 2009). Samples were taken three weeks after delivery and thereafter biobanked at the Swedish National Food Agency. For details about recruitment, blood sampling, and collection of personal characteristics data see Glynn et al. (2007) and Lignell et al. (2009). In total 381 mothers who delivered their first child were analyzed for PFAAs (Table 1). During the first years of the study (1996–1999) women were also sampled in the 3rd trimester of pregnancy. The study was approved by the local ethics committee in Uppsala, Sweden, and the participating women gave informed consent for themselves and for their children.

2.2. Chemical analyses

Serum levels of 7 PFAAs, including perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS), were determined in mothers three weeks after delivery. In a subset of the participating women (n = 20), the aforementioned PFAAs were also analyzed in 3rd trimester serum in order to investigate correlations in levels between late pregnancy and 3 weeks after delivery. The analytical procedure has been described previously (Glynn, 2012; Vestergren et al., 2012). Briefly, serum was spiked with isotopically-labeled internal standards, extracted with acetonitrile, and then subjected to clean-up with graphitized carbon, prior to instrumental analysis by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Method detection limits (MDLs) were defined based on the quantified background contamination signals. In the absence of procedural blank contamination, MDLs were defined as the lowest concentration in a serum sample giving a signal-to-noise ratio of 3. All batches included a procedural blank (water) and a control sample (replicate pooled human serum, n = 21). In addition, NIST SRM 1578 was analyzed to evaluate the accuracy of the method. CVs for control samples were typically between 15 and 22% with the exception of PFBS which showed higher variability, owing to concentrations closer to MDLs (Supplementary data, Table A1). Analysis of NIST reference material revealed that measured concentrations were consistent with reference values for all targets (Supplementary data, Table A2).

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Mean (range)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mothers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>381</td>
<td>29 (20–41)</td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>380</td>
<td>23 (16–40)</td>
<td></td>
</tr>
<tr>
<td>Weight gain during pregnancy (%)</td>
<td>379</td>
<td>22 (–4.1–54)</td>
<td></td>
</tr>
<tr>
<td>Weight loss from delivery to sampling (%)</td>
<td>343</td>
<td>14 (4.3–28)</td>
<td></td>
</tr>
<tr>
<td>Fish consumption (g/day)</td>
<td>360</td>
<td>30 (0–220)</td>
<td></td>
</tr>
<tr>
<td>Years of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4 years of high school</td>
<td>123</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>1–3 years of higher education</td>
<td>81</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>&gt; 3 years of higher education</td>
<td>168</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>380</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>44</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>234</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>102</td>
<td>27</td>
<td></td>
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<tr>
<td><strong>Children</strong></td>
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<td></td>
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<tr>
<td>Boys</td>
<td>217</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>164</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Gestational length (days)</td>
<td>381</td>
<td>280 (244–302)</td>
<td></td>
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<tr>
<td>Birth weight (g)</td>
<td>381</td>
<td>3575 (2159–5420)</td>
<td></td>
</tr>
<tr>
<td>Birth weight (SDS)</td>
<td>381</td>
<td>–0.15 (–3.1–3.1)</td>
<td></td>
</tr>
<tr>
<td>Birth length (SDS)</td>
<td>381</td>
<td>–0.05 (–3.9–3.0)</td>
<td></td>
</tr>
<tr>
<td>Head circumference</td>
<td>376</td>
<td>–0.37 (–3.6–3.3)</td>
<td></td>
</tr>
<tr>
<td>SGAₗ</td>
<td>14</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>SGAₘ</td>
<td>21</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>GFRₗ (ml/min/1.73 m²)</td>
<td>305</td>
<td>103 (48–176)</td>
<td></td>
</tr>
<tr>
<td>GFRₗₑₘₖ (ml/min/1.73 m²)</td>
<td>305</td>
<td>92 (60–166)</td>
<td></td>
</tr>
<tr>
<td>Total breastfeeding (months)</td>
<td>192</td>
<td>6.5 (0–12)</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding at 3 months</td>
<td>184</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding at 6 months</td>
<td>161</td>
<td>84</td>
<td></td>
</tr>
</tbody>
</table>

a Body mass index.
b Weight gain was calculated as weight of the mother just before delivery (Wₐ) compared to weight before pregnancy (W₀): [(Wₑ – W₀) / W₀] × 100.
c Weight of the mother just before delivery (Wₐ), weight of the mother at the time of blood sampling (Wₜ), and the birth weight of the child (Wₛ) was used to calculate this weight loss: [(Wₛ – Wₜ – W₀) / Wₛ] × 100.
d Small for gestational age, weight or length < –2 SDS.
e Estimated glomerular filtration rate, calculated using cystatin C-levels.

2.3. Infants/children

Weight, length and head circumference of the newborn infants (n = 381) were collected from the Swedish Medical Birth Register. During infancy and childhood, measurements of weight, length/height and head circumference at 3, 6, 12 and 18 months and 3, 4 and 5 years of age (n = 200) were collected from child health records in Uppsala County. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared at ages 3, 4, and 5 years. Standard deviation scores (SDS) were calculated from growth standards according to Karlberg et al. (2001), Wikland et al. (2002), and Niklasson and Albertsson-Wikland (2008).

2.4. Glomerular filtration rate (GFR)

In order to calculate estimated maternal GFR (eGFR), levels of creatinine and cystatin C were analyzed in serum samples from the mothers three weeks after delivery. In 20 women creatinine and cystatin C were also measured in late pregnancy, in order to investigate if the eGFR at this time point corresponded to that estimated after delivery.

Serum samples were analyzed on Abbott Architect ci8200 and ci16200 instruments (Abbott Park, IL, USA). Reagents (8L24-02) for the enzymatic IDMS-traceable creatinine method were from Abbott. Reagents for the immunoturbidimetric cystatin C method (Cystatin C reagent kit, no 1101) were from Gentian (Moss, Norway), and the recently described IFCC-certified international calibration procedure (Grubb et al., 2014) was used. The total analytical imprecision of
cystatin C measurements were 3.1% and 2.0% at 12 and 38 μmol/L respectively. Corresponding figures for creatinine were 3.4% and 2.3% at 1.7 and 3.2 g/L. eGFR was calculated using either cystatin C (GFR<sub>crea</sub>) or creatinine level (GFR<sub>crea</sub>). GFR<sub>c</sub> was calculated according to Grubb et al. (2014) and GFR<sub>crea</sub> according to Nyman et al. (2014) in the unit mL/min/1.73 m<sup>2</sup> BSA (body surface area).

2.5. Statistical analyses

Statistical analyses were performed using R version 3.3.1 (R Core Team, 2016) with the rms (Harrell, 2017) and nime (Pinheiro et al., 2016) packages. PFBS, PFDA, and PFUnDA were the only targets investigated in this work with concentrations below MDLs. Due to the reduced number of detects for PFBS (67%), samples were grouped based on mothers with PFBS concentrations < MDL and mothers with PFBS concentrations ≥ MDL. In contrast, PFDA and PFUnDA displayed a much higher frequency of detects (96 and 90% of samples, respectively). For these targets, concentrations below the MDL were imputed by log<sub>e</sub>-transforming the data ≥ MDL for a given year and then fitting these data to a cumulative normal distribution using a Generalized Reduced Gradient algorithm available through the Solver add-in for Microsoft Excel (John, 1998). Prior studies have demonstrated that imputation-based approaches such as this offer a better estimate of non-detectable concentrations with smaller bias and fewer errors compared to replacement techniques (e.g. 0.5 × MDL or MDL/2<sup>1/2</sup>) (Croghan and Egeghy, 2003).

The correlations between PFAA serum levels in 3rd trimester and 3 weeks after delivery were tested with Spearman’s rank correlation test. Multiple linear regression models were used to analyze associations between maternal PFAA levels and birth outcomes. Generalized Least Squares models (Pinheiro and Bates, 2000) were used to estimate associations between PFAA levels and child weight gain and growth. For birth outcomes the covariates sampling year, maternal age, pre-pregnancy BMI, years of education, smoking during pregnancy, maternal weight gain during pregnancy, maternal weight loss after delivery, and total fish consumption were included as covariates in the regression models. We have registered medical data on 278 of the women in the study and 2 women had gestational diabetes and 9 women had gestational hypertension. It was not considered as confounders in this size of population. For child weight gain and growth the covariates sampling year, maternal age, pre-pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, whether or not the child was breastfeeding at three and/or six months and duration of breastfeeding and years of education were included. These variables have been shown to be significantly associated with serum PFAA levels (Bjermo et al., 2013; Brantsaeter et al., 2013; Gyllenhammar et al., 2015) or with birth weight and child growth (Preedy, 2012). To investigate possible confounders, a DAG was performed and the covariates that are used in the models have been evaluated.

Generalized Least Squares is an extension of ordinary least squares to handle repeated measurements within an individual but circumvents the problem mixed models with random effects have of requiring complex approximations of distributions of test statistics (Harrell, 2001). What is required is the specification of the within individual correlation structure. The correlation structure used in this study was an autoregressive-moving average lag 1, which assumes that the correlation between two time points depends on the distance in time between them.

Initially, all continuous variables were modeled using restricted cubic splines with four interior knots. Knowing that these models may overfit the data, degrees of freedom were re-allocated based on each variable’s importance in each model as judged by each variable’s χ<sup>2</sup> statistic minus the degrees of freedom. The rationale behind this strategy is that it is probably more important to model the important variables correctly, i.e. allow for non-linear/non-monotonic dependencies, than it is for less important variables. Associations were allowed to differ between boys and girls by including interaction terms between sex and the PFAs. The interaction terms were then all kept or removed based on their importance in the models. Sensitivity analyses were done with eGFR as an additional adjustment factor.

Estimates in tables and figures are presented as the predicted change in the outcome variable for an inter-quartile range (IQR) increase in each PFAA, i.e. when the PFAA is increased from the 25th to the 75th percentile.

3. Results

3.1. PFAA levels in maternal serum

Maternal PFAA levels are shown in Table 2. In the Uppsala mothers, PFOS was present at the highest concentrations, followed by PFHxS and PFOA. Levels of PFBS above the MDL were found in 57% of the mothers. Serum levels of each homologue (PFOA, PFNA, PFDA, PFUnDA, PFHxS, and PFOS) were significantly correlated between late pregnancy and three weeks after delivery (Spearman’s correlation coefficient: 0.64–0.94, n = 20, p < 0.001–0.002) in a subset of the participating women (Table 3).

3.2. eGFR

eGFR differed slightly between calculations based on creatinine and cystatin C (Table 1). Simple regression analyses showed that maternal

### Table 2. PFAA levels (ng/g) in mothers 3 weeks after delivery.

<table>
<thead>
<tr>
<th>PFAA</th>
<th>n</th>
<th>Mean (SE)</th>
<th>Min</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>Max</th>
<th>&lt; MDL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>381</td>
<td>2.4 (0.06)</td>
<td>0.20</td>
<td>1.6</td>
<td>2.3</td>
<td>3.0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>PFNA</td>
<td>381</td>
<td>0.46 (0.01)</td>
<td>0.062</td>
<td>0.31</td>
<td>0.41</td>
<td>0.57</td>
<td>2.4</td>
<td>0</td>
</tr>
<tr>
<td>PFDA</td>
<td>381</td>
<td>0.23 (0.007)</td>
<td>&lt; MDL</td>
<td>0.14</td>
<td>0.20</td>
<td>0.28</td>
<td>1.1</td>
<td>3</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>381</td>
<td>0.19 (0.006)</td>
<td>&lt; MDL</td>
<td>0.11</td>
<td>0.17</td>
<td>0.26</td>
<td>0.91</td>
<td>15</td>
</tr>
<tr>
<td>PFBS</td>
<td>351</td>
<td>0.03 (0.003)</td>
<td>&lt; MDL</td>
<td>0.012</td>
<td>0.037</td>
<td>0.80</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>PFHxS</td>
<td>377</td>
<td>3.6 (0.20)</td>
<td>0.32</td>
<td>1.4</td>
<td>2.4</td>
<td>4.0</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>PFOS</td>
<td>377</td>
<td>14 (0.44)</td>
<td>2.1</td>
<td>7.4</td>
<td>13</td>
<td>19</td>
<td>46</td>
<td>0</td>
</tr>
</tbody>
</table>

* 30 samples were not quantifiable due to elevated blank levels.
* The internal standard signal was too low for quantification in 2–4 samples.

![Image](193)
GFRcc was significantly associated with maternal levels of PFOA ($\beta = 0.004 \pm 0.002, p = 0.022$). GFRcc was also inversely associated with gestational length ($\beta = -0.07 \pm 0.03, p = 0.025$) but not with birth SDS weight or SDS length. GFRcc was slightly lower in late pregnancy than three weeks after delivery, estimated to $95 \pm 20$ and $107 \pm 14$ mL/min/1.73 m$^2$, respectively (Mann Whitney test, $p = 0.066$). GFRcreat was higher in late pregnancy than three weeks after delivery, $117 \pm 10$ and $97 \pm 11$ mL/min/1.73 m$^2$, respectively (Mann Whitney test, $p < 0.0001$). No statistically significant correlations were seen between late pregnancy and post-delivery GFRcc or GFRcreat (data not shown).

3.3. Birth outcomes

At birth there were inverse associations between birth weight SDS and maternal levels of long-chained PFCAs (Fig. 1), being significant for PFDA and PFUnDA and borderline so for PFNA ($p = 0.054$). The results for associations between PFAAs and birth length SDS followed the same pattern as for birth weight SDS but were not significant. Maternal PFOS concentrations had a borderline significant association with gestational length ($p = 0.056$) (Fig. 1). All variables were modeled linearly in all models with the exception of maternal BMI and weight gain during pregnancy which were modeled using restricted cubic splines with three knots. When the associations were adjusted for eGFR the statistical significance for birth weight remained for PFDA and PFUnDA (Supplementary data, Figs. A1 and A2). Since a high maternal weight gain during pregnancy could be a consequence of a fetus gaining a lot of weight, adjusting for it may bias the association between PFAA and birthweight. The statistical analyses for birth outcomes were thus performed without maternal weight gain during pregnancy as sensitivity analyses but the results showed no significant differences from those with adjustment for maternal weight gain (Fig. 1, Supplementary data, Fig. A3).

No associations were found between PFAA levels and head circumference (SDS) (Fig. 1) or between PFBS and birth outcomes (data not shown).

3.4. Growth and weight development in early childhood

We observed no significant associations between maternal PFAA levels and childhood weight SDS during the period from 3 months to 5 years of age, although there was a tendency of positive associations for PFOA, PFNA, PFHxS, and PFOS already at 3 months of age (Fig. 2). No associations were observed between length SDS and maternal PFAA levels (Fig. 3). For BMI SDS, only analyzed at 3, 4, and 5 years of age, PFOA, PFNA and PFHxS had a similar pattern with positive associations between maternal levels and BMI SDS in children at age 3 and 4 although becoming less strong and not significant at age 5 (Fig. 4).
Maternal PFOS levels were also positively associated with BMI SDS but on the contrary the association became stronger with increasing age and was significant at 4 and 5 years of age (Fig. 4).

Maternal levels of PFOA and PFHxS were positively associated with head circumference SDS and significant at 18 months for PFOA and 12 and 18 months for PFHxS (Fig. 5). No other significant associations were found. No evidence of different associations for boys or girls were found as p-values for the interaction terms between sex and the PFAAs in the models varied between 0.20 and 0.79 for birth outcomes and between 0.19 and 0.99 for the longitudinal outcomes.

All variables were modeled linearly in the models for growth and weight development with the exception of attained age which was modeled using a restricted cubic spline with four knots. Attained age was modeled with a second degree polynomial for the outcomes BMI and Head circumference since there were not enough time points to generate the spline basis functions.

4. Discussion

In the present study increased maternal levels of PFNA, PFDA and PFUnDA were associated with reduced birth weight. We observed no consistently significant associations for PFBS, PFHxS, PFOS, and PFOA. Associations between birth weight and exposure to PFOA and PFOS have been examined in several previous studies. However, investigations of other PFAAs and birth outcomes are scarce. In Taiwanese girls, similar to our results, prenatal levels of PFNA, PFDA, PFUnDA, and perfluorododecanoic acid (PFDoDA) were inversely associated with birth weight (Wang et al., 2016). In contrast to our observations, in a study of Danish mothers and children 2008–2013, estimated mean birth weight was reduced in groups with PFHxS exposure above the lowest exposure quartile. This was also observed for perfluorohexane sulfonate (PFHxS), which was not studied by us (Bach et al., 2016). In another study, from South Korea, on cord serum levels of nine PFAAs (including PFOA and PFOS) and birth weight no significant associations were found (Lee et al., 2016). Our results are consistent with previous studies showing associations between increased PFAA exposure and reduced birth weight, however the relation of different homologues remains unclear.

Three recent reviews of PFOA and PFOS associations with birth weight have concluded that there is sufficient human and animal evidence that developmental exposure to PFOA and PFOS reduces fetal growth/birth weight (Johnson et al., 2014; Lam et al., 2014; Bach et al., 2015). Although PFOA and PFOS concentrations were in the same range in the present study we did not observe associations between increased maternal PFOS and PFOA and decreased birth weight in the statistical analyses. This could for instance be due to difference in study design, covariates included in the regression models, and the relatively limited sample size in our study.

At least three potential pathways have been suggested by which in utero exposure to the long-chain perfluorinated carboxylic acids (PFCAs) might affect offspring weight (Halldorsson et al., 2012): Firstly, they may interfere with ovary development in utero, leading to impaired estrogen synthesis; secondly, PFAAs may interact with the peroxisome proliferator-activated receptors (PPAR) PPARα and PPARγ, which are involved in lipid metabolism in adipocytes; and thirdly, PFAA may interact with thyroid hormones (Halldorsson et al., 2012).

It has been suggested that fetal size, maternal GFR, and maternal PFAA concentrations are all correlated, and that assessment of the fetal size (or birth weight) associations with maternal PFAA should be adjusted for maternal GFR, especially if maternal PFAA is determined around delivery (Verner et al., 2015). Urinary excretion of PFCAs decreases with increased chain-length of the homologues up to C9-C10, and among the PFAAs studied by us PFOA appears to be the homologue with the highest degree of urinary excretion (Zhang et al., 2013). In our
study, PFOA was the only PFAA for which maternal serum levels were significantly associated with maternal eGFR measured 3 weeks after birth, in this case a positive association. This is in contrast to data indicating that higher GFR would lead to lower PFOA levels (Verner et al., 2015). Other studies have shown associations between low GFR and reduced birth weight (Morken et al., 2014), however that was not shown in the present study when eGFR was used. Maternal eGFR adjustment of the associations between birth weight and maternal PFAAs had no marked influence on the results in the present study (Supplementary data, Figs. A1 and A2). All women in this study were healthy with normal renal function, and it may be possible that the inter-individual variation in GFR was not large enough to confound associations between long-chain PFCAs and birth weight due to low urinary excretion of these PFAA homologues. GFR during the 3rd trimester did not correlate with that analyzed 3 weeks after delivery demonstrating that the intra-individual variation also was large in this cohort with healthy pregnant women. This could be due to the changes in renal function that take place during normal pregnancy and the first weeks postpartum (Hussein and Lafayette, 2014). Blood samples for eGFR estimations taken at other times before or during pregnancy, or in the first weeks after delivery may give other results. There are also uncertainties using eGFR, since all equations estimating GFR from creatinine or cystatin C are approximations. However, the gold standard for measuring GFR is using plasma or urinary clearance of an exogenous injected filtration marker, e.g. iohexol clearance (Levey and Inker, 2016). Such methods are invasive and not feasible in the present setting.

There is an association between intrauterine growth restriction and risk of impaired glucose tolerance and increased blood pressure later in life possibly leading to metabolic syndrome in adulthood (Ibanez et al., 2006). Especially individuals with an early and rapid catch-up weight gain and growth are at risk (Kerkhof et al., 2012). Animal models have shown possible mechanisms via reduced number of nephrons in the kidney and pancreatic beta-cell hypoplasia underlying these conditions (Woods et al., 2004; Green et al., 2010). Early catch-up weight gain is also associated with obesity later in life (Monteiro and Victora, 2005). Since we found that maternal PFDA and PFUnDA, and to some extent also PFNA, were negatively associated with birth weight SDS, the question is whether higher maternal levels of these PFAAs at delivery are associated with faster weight gain and growth during childhood.

We observed a tendency of positive associations between maternal PFOA, PFNA, PFHxS and PFOS and child weight already at 3 months of age. This result did however not give strong support for the hypothesis that reduced fetal growth due to in utero exposure to PFAA results in early-life catch-up growth, since we did only observe borderline significant decreased birth weight SDS with increased maternal PFNA, and no significant associations for PFOA, PFHxS, and PFOS. Higher maternal PFOA, PFNA, and PFHxS levels were associated with increased BMI SDS at 3 and 4 years of age, and higher maternal PFOS with higher BMI SDS at 4 and 5 years of age. Rapid growth during early childhood and high childhood BMI has been associated with obesity later in life (Brisbois et al., 2012).

Studies on associations between PFAS exposure and childhood weight gain and growth are few and not consistent. In study on British girls high in utero exposure to PFOS was associated with a lower birth weight and a higher weight at 20 months, compared to those with lower exposure (Maisonet et al., 2012). Danish women had a higher risk of overweight at 20 years of age after prenatal exposure to PFOA (Halldorsson et al., 2012). In a Danish study maternal PFOS and PFOA levels during pregnancy were inversely related to child weight up to 7 years of age, although not statistically significant (Andersen et al., 2010, 2013). In this Danish cohort negative associations between PFOS and PFOA levels and birth weight have previously been reported (Fet et al., 2007, 2008). Increased prenatal PFAA exposure has also been
associated with increased adiposity in children and adolescents (Halldorsson et al., 2012; Braun et al., 2016; Mora et al., 2017). However, other studies of prenatal PFAA exposure and childhood weight and adiposity show no significant associations (Andersen et al., 2013; Barry et al., 2014; Timmermann et al., 2014). None of these studies investigated child weight gain or linear growth.

Some studies suggest different associations of PFAAs and birth outcomes by infant sex. Andersen et al. (2010) studied 1010 children and found that increased maternal levels of PFOS and PFOA were associated with lower weight and BMI at 5 and 12 months of age in boys. Washino et al. (2009) found negative associations between increased maternal levels of PFOS and birth weight in girls. Robledo et al. (2015) studied children to 234 couples and association between a several contaminants representing different classes and found an inverse association between maternal perfluorooctanesulfonamide (PFOSA) and birth weight in boys. Our data do not support different associations by sex for any of the studied outcomes. Tests for interaction in regression models require a larger sample size than tests for main effects and have limited power in small data sets. Our sample size is much smaller than in Andersen et al. (2010) but is on par with the sample size in Washino et al. (2009) and larger than the sample used by Robledo et al. (2015) so sample size alone cannot explain the differences. One explanation could be the different outcome measurements used. While the above mentioned studies studied birthweight in grams, we used sex and age standardized measures so the effects of sex on the outcomes are already accounted for. Differences in covariates included in the regression models and study design may also explain the lack of clear PFAA by sex interactions in our study.

4.1. Strengths and limitations

In the present study seven different PFAA were evaluated. Most other studies in this field have only presented results for PFOS and PFOA. The use of standard deviation scores have the advantage of directly correcting for gestational age as well as postnatal age, thus compensating for the variations in actual time for the individual measurements. In the present study maternal levels of PFAA were analyzed in serum sampled three weeks after delivery, which makes the results harder to interpret. The ideal study design would have included estimation of maternal PFAA before pregnancy or at least in early pregnancy, in order to avoid the suggested confounding with maternal GFR around delivery. Serum levels of each homologue (PFOA, PFNA, PFDA, PFUnDA, PFHxS, and PFOS) in POPUP women were strongly correlated between late pregnancy and three weeks after delivery and in an earlier study strong correlations have also been seen between PFAA concentrations after delivery and in both early and late pregnancy (Glynn et al., 2012). Method of delivery could be suggested to be a confounder in this type of study. We have not found any association with PFAA concentrations or birth outcome and method of delivery (data not shown). A study from Sweden found no difference in mean estimated blood loss between planned vaginal delivery and planned caesarean section studying 451 women (Larsson et al., 2011). This demonstrates that maternal PFAA levels measured 3 weeks after delivery provide good estimates of levels in early and late pregnancy, although residual confounding still cannot be excluded. We used birth outcomes data from the Swedish Medical Birth register and body weight and length from child health records, instead of self-reported data.

5. Conclusion

The present study (including PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS), on one hand indicates that early-life exposure to long-chained PFCAs is associated with decreased fetal growth, and on the other hand an increased childhood BMI with increasing maternal PFOA,
PFNA, PFHxS, and PFOS. Further follow-up of associations between early-life PFAA exposure and growth at later ages, including puberty, is warranted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2017.12.002.

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Fig. 5. Associations between maternal PFNA concentrations and head circumference SDS over time in children at 3 to 18 months (n = 152–177). Generalized Least Squares models were used with the covariates sampling year, maternal age, pre pregnancy BMI, maternal weight gain during pregnancy, maternal weight loss after delivery, years of education, and total time of breastfeeding. Results are shown as adjusted mean (95% confidence interval) change in SDS for an interquartile range increase in maternal PFNA.