Original Contribution

Perfluorinated Compounds in Relation to Birth Weight in the Norwegian Mother and Child Cohort Study

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Perfluorooctane sulfonate and perfluorooctanoic acid are perfluorinated compounds (PFCs) widely distributed in the environment. Previous studies of PFCs and birth weight are equivocal. The authors examined this association in the Norwegian Mother and Child Cohort Study (MoBa), using data from 901 women enrolled from 2003 to 2004 and selected for a prior case-based study of PFCs and subfecundity. Maternal plasma samples were obtained around 17 weeks of gestation. Outcomes included birth weight z scores, preterm birth, small for gestational age, and large for gestational age. The adjusted birth weight z scores were slightly lower among infants born to mothers in the highest quartiles of PFCs compared with infants born to mothers in the lowest quartiles: for perfluorooctane sulfonate, $\beta = -0.18$ (95% confidence interval: $-0.41, 0.05$) and, for perfluorooctanoic acid, $\beta = -0.21$ (95% confidence interval: $-0.45, 0.04$). No clear evidence of an association with small for gestational age or large for gestational age was observed. Perfluorooctane sulfonate and perfluorooctanoic acid were each associated with decreased adjusted odds of preterm birth, although the cell counts were small. Whether some of the associations suggested by these findings may be due to a noncausal pharmacokinetic mechanism remains unclear.

birth weight; MoBa; Norwegian Mother and Child Cohort Study; perfluorinated compounds; perfluorooctane sulfonate; perfluorooctanoic acid

Abbreviations: CI, confidence interval; LGA, large for gestational age; MoBa, Norwegian Mother and Child Cohort Study; OR, odds ratio; PFC, perfluorinated compound; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; SGA, small for gestational age.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are perfluorinated compounds (PFCs), a class of man-made chemicals used in commercial and industrial applications. The potential human health effects of exposure to PFOS and PFOA have drawn recent attention despite the fact that their use is being phased out in favor of shorter chained PFCs (1). PFOS and PFOA have relatively long half-lives in humans: The median serum PFOS half-life was estimated at between 2.3 (2) and 4.6 (3) years, and the median serum PFOA half-life was estimated at 3.4 years (3). These compounds exhibit widespread distribution in the environment, and they are not subject to further degradation. Dietary exposure, particularly through seafood consumption, may be a major exposure route in humans (4, 5). PFOS and PFOA are stored primarily in the blood, liver, and kidneys, and both compounds have high binding affinity for albumin, the major protein component of plasma (6–8). Further, both compounds have the ability to cross the placental barrier (9, 10).

Reproductive toxicity of PFOS and PFOA has been demonstrated in animal studies that have shown exposure-associated increases in neonatal mortality and decreases in fetal and postnatal growth (11–13). The dose in animal studies, however, was often several orders of magnitude greater than that in the general population. Epidemiologic investigations have explored associations between plasma levels of PFOS and PFOA during pregnancy and birth weight in background-exposed populations; the results are equivocal (Table 1). None reported positive associations with either
PFC; 3 reported inverse associations with PFOS (9, 14, 15), and 4 reported inverse associations with PFOA (9, 14–16). A study of past pregnancies among background-exposed women found an association between maternal PFOS levels and decreased birth weight (17). A study among occupationally exposed women found no association between PFOS and birth weight (18). Further, in 2 separate studies, among women exposed to levels of PFOA higher than background levels, no association was found between PFOA and birth weight (17, 19). In the present study, we examined the relation between maternal plasma levels of PFOS and PFOA and birth weight z scores in a background-exposed population of Norwegian women.

MATERIALS AND METHODS

Study population

This study was based on the Norwegian Mother and Child Cohort Study (MoBa) conducted by the Norwegian Institute of Public Health. Enrollment for MoBa was from 1999 to 2008 (20). Pregnant women in Norway were invited to participate during a routine ultrasound examination offered to all women around 17 weeks of gestation. Overall, 38.5% of invited women participated, although participation was greater during the earlier years (21). MoBa was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate, and informed consent was obtained from each participant. The present study was based on MoBa data file version 4.301.

The present study used data from 950 MoBa subjects included in a prior case-based study of PFCs and subfecundity (22). In the previous study, 2 groups were selected from all MoBa subjects who were enrolled in 2003–2004, delivered a liveborn child, provided a plasma sample at enrollment, and reported time to pregnancy. The first group (the base group) consisted of a random sample (n = 550), while the second group (the case group) was a random sample of subfecund (time to pregnancy, >12 months) women (n = 400). For the present study, we excluded subjects missing prepregnancy body mass index (n = 15), gestational age at birth (n = 7), and twin (n = 27) pregnancies. There were 35 preterm deliveries, which were excluded from the analysis of birth weight z scores, for a total of 866 subjects available for the z score analysis and 901 subjects available for analyses of preterm birth, small for gestational age (SGA), and large for gestational age (LGA).

Ascertainment of birth outcomes

MoBa pregnancies were linked with the Medical Birth Registry of Norway, which includes pregnancy and birth records reported by the physician or midwife attending the childbirth (23). Birth weight and gestational age (estimated from both the woman’s last menstrual period and ultrasound measurements) are reported to this registry. We used gestational age based on the last menstrual period unless it differed from the ultrasound estimate by more than 14 days, in which case the ultrasound estimate was used (24); z scores for birth weight were calculated by standardizing birth weights for gender and gestational age, based on Norwegian births from 1987 to 1998 (25). Preterm birth was defined as gestational age less than 37 weeks (n = 35). SGA was defined as gender- and gestational age-specific birth weight less than the 10th percentile (n = 60), and LGA was defined as gender- and gestational age-specific birth weight greater than the 90th percentile (n = 125).

Measurement of exposure

At the time of enrollment, women provided plasma samples that were shipped from the collection site to Oslo at ambient temperature. Because of the chemical stability of PFCs (26), alterations in the plasma concentrations during shipping were unlikely. PFC concentrations were measured in 150 μL of plasma by using high-performance liquid chromatography/tandem mass spectrometry at the Norwegian Institute of Public Health. Details of the analytical method have been published elsewhere (27). For quality assurance purposes, 50 aliquots of a single plasma pool were analyzed...
alongside the specimens in 17 batches; the lab technicians were blind to their identity. The median concentrations of PFOS and PFOA in the 50 pooled specimens were 19.3 ng/mL and 3.7 ng/mL, respectively. No values of PFOS and PFOA were below the limit of quantification (0.05 ng/mL). Based on the 50 quality assurance specimens, the within-batch correlation of variation for PFOS was 4.5%, and the between-batch correlation of variation was 11.3%. For PFOA, the within-batch correlation of variation was 3.5%, and the between-batch correlation of variation was 6.7%.

**Covariates**

At enrollment, women completed a questionnaire covering sociodemographic and lifestyle factors and medical and reproductive history. Around 22 weeks of gestation, women completed a food frequency questionnaire designed to capture dietary habits during the first part of pregnancy (28). From this questionnaire, we obtained women’s consumption (g/day) of lean fish (composed of 15 types of fish and shellfish) and oily fish (composed of 4 types of fish). All questionnaires can be found online (http://www.fhi.no/eway/default.aspx?pid=238&trg=MainArea_5811&MainArea_5811=5903:0:15,3138:1:0:::0:0). Birth dates for older children were also obtained from the Medical Birth Registry of Norway. The interpregnancy interval, defined as the number of months from the birth of the previous child to the conception of the index child, was calculated for each woman. Additionally, plasma specimens were analyzed for albumin concentration.

**Statistical analyses**

We used weighted methods for the analysis of secondary outcomes in data sets collected by using case-control designs (29). The sampling weights for the present analysis were the inverse of the sampling probability for either the case or base group in the previous study. We used PROC SURVEYREG in SAS software (SAS Institute, Inc., Cary, North Carolina) to estimate beta coefficients and 95% confidence intervals for the association between weight z scores and each PFC. Beta coefficients were estimated for both continuous exposures as well as for quartiles of PFOS and PFOA. Trend tests (1 df) were constructed by using a score comprising the median PFC level for each quartile (30).

The models of birth weight z scores at term were adjusted for maternal age (years), prepregnancy body mass index (weight (kg)/height (m)^2), and parity (0, 1, ≥2) a priori. We used a backwards deletion strategy (31), with a 10% change in estimate rule, to determine the magnitude of other potential confounding for PFOS and PFOA, separately. Potential confounders included the following: pregnancy weight gain at 17 weeks (kg), maternal oily fish consumption (g/day), maternal lean fish consumption (g/day), interpregnancy interval (represented with a linear and quadratic term), gestational age at the time of the blood draw (weeks), maternal plasma albumin concentration (g/dL), maternal smoking at 17 weeks (nonsmoker, quit during pregnancy, smoker), maternal alcohol intake at 17 weeks (yes/no), maternal education (less than high school, high school, some college, college or greater), household income (high, medium, low), any maternal diabetes (yes/no), and child’s gender. Based on this procedure, in addition to the a priori variables, the following variables were included in the PFOS model: maternal albumin concentration, consumption of lean fish, maternal education, and interpregnancy interval. In the PFOA model, weight gain at 17 weeks was the only additional covariate included. Because of missing covariate information, a total of 838 subjects were included in the PFOS analysis, and 849 subjects were included in the PFOA analysis.

We performed secondary weighted analyses using PROC SURVEYLOGISTIC in SAS software to estimate odds ratios and 95% confidence intervals for the association between the outcomes (preterm birth, SGA, and LGA) and the exposures (PFOS and PFOA). For these analyses, we used the same a priori variables as in the birth weight z-score analysis and assessed the same covariates previously listed as potential confounders. The deletion of none of the covariates changed the odds ratio between any outcome and either PFC by more than 10%; therefore, the preterm birth, SGA, and LGA analyses were adjusted for only the a priori adjustment variables. These analyses included 901 subjects.

Because these data came from a prior study of subfecundity, we performed sensitivity analyses for birth weight z scores, restricting the analyses to subjects previously selected in the “base” group. We adjusted for the same a priori variables and used the same procedure for evaluating confounding as in the primary analyses. Among the base group, only smoking and income were identified as confounders of the PFOS–z-score association, and the following covariates were identified as confounders of the PFOA–z-score association: intake of lean fish, intake of oily fish, pregnancy weight gain, albumin concentration, smoking, interpregnancy interval, and income. To further examine whether selection status in the original study affected our results, we tested for interaction between selection status (case vs. base) and each PFC level (ng/mL). We also tested for interaction between PFCs and child’s gender. Finally, because of missing covariate information, we used PROC MI and PROC MIANALYZE in SAS software to impute missing values, and we reran the z-score analyses.

**RESULTS**

The mean birth weight among the infants in this study was 3.640 g; the mean gestational age was 40 weeks (Table 2). Nearly half (48%) of the infants were born to nulliparous women, and the majority were born to women who reported no smoking in early pregnancy (76%) and who had at least a college education (59%). On average, women were 31 years of age at enrollment and had a prepregnancy body mass index of 24.9. The median PFOS level was 13.0 ng/mL (interquartile range: 10.3–16.6); the median PFOA level was 2.2 ng/mL (interquartile range: 1.6–3.0). A total of 7% of the infants were classified as SGA, 14% were classified as LGA, and 4% were preterm.

Our crude analyses indicated reduced birth weight z scores associated with both PFOS and PFOA, as well as
among infants born to mothers in the highest quartile of each compound (Table 3). Compared with infants born to mothers in the lowest quartile, infants born to mothers in the highest quartile had slightly lower adjusted \(z\) scores for both PFOS (\(\beta = -0.18, 95\% \text{ confidence interval (CI): } -0.41, 0.05\)) and PFOA (\(\beta = -0.21, 95\% \text{ CI: } -0.45, 0.04\)). Analyses of birth weight adjusted for gestational age revealed similar results. Infants born to mothers with the highest PFOS levels weighed 85 g less (95% CI: \(-194.1, 23.6\)) than those in the lowest quartile; similarly, those born to mothers with the highest PFOA levels weighed 106 g less (95% CI: \(-219.6, 7.2\)) (refer to Web Table 1, which is posted on the Journal’s website (http://www.aje.oxfordjournals.org/)).

After imputing missing values, we found similar results (data not shown). In the event that adjusting the PFOS model for fish consumption may have resulted in overadjustment, we dropped this variable from the model and obtained similar results (data not shown).

In the analyses of birth weight \(z\) scores, we found no evidence of an interaction between either PFOS (\(P =\) ...)
when the z-score analysis was repeated among subjects selected for the base group in the original study, we observed results similar to those for the entire sample. For consistency, we also repeated the analysis in the base group while adjusting for those covariates that were found to be confounders in the total group of subjects (i.e., variables adjusted for in the primary analysis). The results of this analysis were also similar (data not shown).

The adjusted analyses of preterm birth revealed decreasing relative odds of preterm birth associated with increasing levels of both PFOS (\(P_{\text{trend}} = 0.03\) and PFOA (\(P_{\text{trend}} = 0.02\), with decreased odds in the highest quartile of both PFOS (odds ratio (OR) = 0.3, 95% CI: 0.1, 1.0) and PFOA (OR = 0.1, 95% CI: 0.03, 0.6). However, the number of cases of preterm birth was small.

There were increased adjusted relative odds of SGA among infants born to mothers in the third PFOS quartile (OR = 2.2, 95% CI: 1.0, 5.1) but not in the highest quartile (Table 4). We found little evidence of an association between PFOA and SGA. Although we found decreased adjusted odds of LGA among women in the highest PFOS quartile (OR = 0.7, 95% CI: 0.3, 1.4) and the highest PFOA quartile (OR = 0.6, 95% CI: 0.3, 1.4), the confidence intervals were wide. Additionally, no dose-response was detected for either PFC.

**DISCUSSION**

We found slightly lower adjusted birth weight z scores and absolute birth weight among infants born to women with the highest plasma levels of PFOA and PFOS, although

### Table 3. Estimated Change in Birth Weight z Scores Associated With PFOS and PFOA Among 849 Infants in the Norwegian Mother and Child Cohort Study, 2003–2004, Excluding Preterm and Multiple Births

<table>
<thead>
<tr>
<th></th>
<th>Crude (\beta) Estimate</th>
<th>95% CI</th>
<th>(P_{\text{trend}})</th>
<th>Adjusted(^a) (\beta) Estimate</th>
<th>95% CI</th>
<th>(P_{\text{trend}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOS (quartiles)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>&lt;10.27</td>
<td>0.00</td>
<td></td>
<td>0.00</td>
<td></td>
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<tr>
<td>10.27–12.97</td>
<td>−0.13</td>
<td>−0.35</td>
<td>0.10</td>
<td>−0.08</td>
<td>−0.29</td>
<td>0.13</td>
</tr>
<tr>
<td>12.98–16.58</td>
<td>−0.21</td>
<td>−0.44</td>
<td>0.01</td>
<td>−0.17</td>
<td>−0.39</td>
<td>0.05</td>
</tr>
<tr>
<td>≥16.59</td>
<td>−0.37</td>
<td>−0.60</td>
<td>0.15</td>
<td>&lt;0.001</td>
<td>−0.18</td>
<td>−0.41 0.05</td>
</tr>
<tr>
<td>PFOS, ng/mL</td>
<td>−0.02</td>
<td>−0.03</td>
<td>−0.01</td>
<td>−0.01</td>
<td>−0.02</td>
<td>0.01</td>
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<tr>
<td>PFOA (quartiles)</td>
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<tr>
<td>&lt;1.65</td>
<td>0.00</td>
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<td>0.00</td>
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<tr>
<td>1.65–2.24</td>
<td>−0.09</td>
<td>−0.31</td>
<td>0.13</td>
<td>−0.06</td>
<td>−0.28</td>
<td>0.16</td>
</tr>
<tr>
<td>2.25–3.03</td>
<td>−0.20</td>
<td>−0.43</td>
<td>0.03</td>
<td>−0.08</td>
<td>−0.32</td>
<td>0.16</td>
</tr>
<tr>
<td>≥3.04</td>
<td>−0.52</td>
<td>−0.73</td>
<td>−0.31</td>
<td>&lt;0.0001</td>
<td>−0.21</td>
<td>−0.45 0.04</td>
</tr>
<tr>
<td>PFOA, ng/mL</td>
<td>−0.15</td>
<td>−0.21</td>
<td>−0.09</td>
<td>−0.03</td>
<td>−0.10</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

\(^a\) PFOS analysis contains 838 subjects because of missing covariate information.

\(^b\) PFOS analysis was adjusted for maternal age, prepregnancy body mass index, parity, and weight gain at 17 weeks.
Table 4. Adjusted\(^a\) Odds Ratios and 95% Confidence Intervals for the Association Between PFOS and PFOA and Preterm Birth, Small for Gestational Age, and Large for Gestational Age Among 901 Infants in the Norwegian Mother and Child Cohort Study, 2003–2004, Excluding Multiple Births

<table>
<thead>
<tr>
<th></th>
<th>Preterm Birth</th>
<th>SGA</th>
<th>LGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>PFOS (quartiles)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;10.27)</td>
<td>9</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>10.27–12.97</td>
<td>6</td>
<td>0.9</td>
<td>0.3, 2.8</td>
</tr>
<tr>
<td>12.98–16.58</td>
<td>10</td>
<td>0.9</td>
<td>0.3, 2.7</td>
</tr>
<tr>
<td>(\geq16.59)</td>
<td>10</td>
<td>0.3</td>
<td>0.1, 1.0, 0.03</td>
</tr>
<tr>
<td><strong>PFOA (quartiles)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;1.65)</td>
<td>7</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1.65–2.24</td>
<td>6</td>
<td>0.3</td>
<td>0.1, 1.3</td>
</tr>
<tr>
<td>2.25–3.03</td>
<td>13</td>
<td>0.7</td>
<td>0.2, 2.4</td>
</tr>
<tr>
<td>(\geq3.04)</td>
<td>9</td>
<td>0.1</td>
<td>0.03, 0.6, 0.02</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; LGA, large for gestational age; OR, odds ratio; PFOA, perfluoroctanoic acid; PFOS, perfluorooctane sulfonate; SGA, small for gestational age.

\(^a\) Analyses were adjusted for maternal age, prepregnancy body mass index, and parity.

filiation rate, in which case purported associations between PFC levels and birth weight may be due, in part, to reverse causality. However, at this time, it is not possible to disentangle how much of the suggested association may be due to the pharmacokinetics of PFCs in pregnancy versus causality.

The present study used data previously collected for a case-base study of subfecundity. We used weighted analysis methods (29) and included women who were selected for the base group in the original study as well as cases. To examine the potential effects of selection bias in our data, we restricted the \(z\)-score analyses to women randomly selected from the cohort (i.e., the base group), and we found similar results. Because of relatively few preterm, SGA, and LGA births, we were unable to repeat these analyses among the base group.

As clinically important outcomes, SGA, LGA, and preterm births may provide more useful information regarding the adverse effects of in utero exposure to PFCs. Although our data were suggestive of an inverse association between PFCs and preterm birth, a previous study found no such evidence (17). Additionally, the small numbers of SGA, LGA, and preterm infants in our study limited our ability to draw meaningful conclusions.

We had the advantage of including women who were part of a large national prospective pregnancy cohort in Norway. Although overall participation in the cohort is low, estimates of exposure–disease associations from this large study should be internally valid and generalizable (21). Birth weight and gestational age were obtained from the Medical Birth Registry of Norway, which is completed by the physician or midwife attending the birth using a standardized form (23). We used the ultrasound-based estimation of gestational age only if it differed from the last menstrual period-based estimate by more than 14 days. We did not rely solely on ultrasound-based estimates, because these estimates could be biased if exposure to PFCs affects very early growth. Our analysis also included the evaluation of 2 potential confounders not examined in previous studies—plasma albumin concentration and interpregnancy interval. PFOS and PFOA are bound primarily to albumin in serum (6–8), and maternal albumin may be related to adverse pregnancy outcomes (39–41). Although it did not make a large contribution to the PFOA–birth weight association, the plasma albumin concentration was found to be a confounder of the PFOS–birth weight association, moving the association toward the null. The interpregnancy interval was also found important for the PFOS–birth weight association. Both long and short interpregnancy intervals have been associated with decreased birth weight (42). Additionally, because plasma PFC concentrations decrease during pregnancy and lactation (10, 43, 44), the timing since previous pregnancies may also affect women’s current plasma PFC concentrations. We had extensive data regarding fish consumption during pregnancy, a major source of human exposure to PFCs, and included this dietary predictor of PFC levels to capture potential confounding effects (45).

Although not definitive, our analysis suggests an inverse association between maternal plasma levels of PFCs and birth weight among Norwegian women. Whether the associations suggested by these findings may be due to a noncausal pharmacokinetic mechanism remains unclear.

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