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Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003–2011☆,☆☆

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ABSTRACT

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that are used in a wide range of consumer products. Recent epidemiological studies have shown that prenatal exposure to toxic levels of PFAAs in the environment may adversely affect fetal growth and humoral immune response in infants and children. Here we have characterized levels of prenatal exposure to PFAA between 2003 and 2011 in Hokkaido, Japan, by measuring PFAA concentrations in plasma samples from pregnant women. The study population comprised 150 women who enrolled in a prospective birth cohort study conducted in Hokkaido. Eleven PFAAs were measured in maternal plasma samples using simultaneous analysis by ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. At the end of the study, in 2011, age- and parity-adjusted mean concentrations of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were 1.35 ng/mL, 1.26 ng/mL, 0.66 ng/mL, 1.29 ng/mL, 0.25 ng/mL, 0.33 ng/mL, 0.28 ng/mL, and 3.86 ng/mL, respectively. Whereas PFOS and PFOA concentrations declined 8.4%/y and 3.1%/y, respectively, PFNA and PFDA levels increased 4.7%/y and 2.4%/y, respectively, between 2003 and 2011. PFUnDA, PFDoDA, and PFTrDA were detected in the vast majority of maternal samples, but no significant temporal trend was apparent. Future studies must involve a larger population of pregnant women and their children to determine the effects of prenatal exposure to PFAA on health outcomes in infants and children.

1. Introduction

Perfluoroalkyl acids (PFAAs) are used in a broad range of consumer products because of their surface properties, which include insulation and water resistance. These compounds are persistent and widespread organic pollutants within the environment, wildlife, and humans (Lau et al., 2007). Contamination of drinking water, foodstuffs such as seafood, leaching from food packaging and non-stick cookware, and household dust are known major routes of human exposure (Fromme et al., 2009). Potential health effects associated with PFAA exposure in humans are made worse by both bioaccumulation and persistence. In 2002, after 50 years of production, the 3M Company phased out the production and distribution of perfluorooctane sulfonate (PFOS) (Renner, 2001). PFOS has subsequently been regulated by the governments of the United States (Significant New Use Rules, United States Environmental Protection Agency, 2000), Canada (Schedule 1 of CEPA 1999 in Environment Canada, 2006), and the European Union (Directive 76/769/EEC, European Commission, 2006). PFOS was also included in

Abbreviations: PFAAs, perfluoroalkyl acids; PFCAs, perfluorinated carboxylic acids; PFSAs, perfluoroalkane sulfonates; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFDoDA, perfluorododecanoic acid; PFTrDA, perfluorotridecanoic acid; PFTeDA, perfluorotetradecanoic acid; PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; BEH, ethylene-bridged hybrid; UPLC-MS/MS, ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry; MDL, method detection limit; CI, confidence interval; GM, geometric mean.

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☆☆ Ethics approval: This study was conducted with written informed consent from all patients and was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

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Annex B of the 2009 Stockholm Convention on Persistent Organic Pollutants (UNEP, 2007; Wang et al., 2009). United States Environmental Protection Agency (2006) launched a 2010/2015 PFOA Stewardship Program to voluntarily reduce perfluorooctanoic acid (PFOA) emissions. Recent studies have indicated that concentrations of PFOS and PFOA are declining in the general human population (Kato et al., 2011; Olsen et al., 2012; Sundström et al., 2011; Wang et al., 2011). In contrast, concentrations of long-chain perfluorinated carboxylic acids (PFCAs) in the general human population are increasing (Wang et al., 2011).

PFOS and PFOA pass the placental barrier and are transferred to the fetus in humans (Midasch et al., 2007; Monroy et al., 2008). Previous epidemiological studies have reported a negative association between prenatal PFOS or PFOA exposure and birth weight (Andersen et al., 2010; Chen et al., 2012; Fei et al., 2007; Washino et al., 2009). Moreover, maternal PFOS levels correlate negatively with antibody concentrations in children aged 5 years (Grandjean et al., 2012). However, the effects of prenatal exposure to other PFAAs [e.g., long-chain perfluorinated carboxylic acids (PFCAs), such as perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA)] remain unclear. PFCAs with chains longer than those in PFOA have high bioconcentration factors, suggesting their environmental persistence (Martin et al., 2003). It is necessary to measure, therefore, levels of exposure of pregnant women to PFOS, PFOA, and other PFAAs. It is also critical to determine whether environmental levels of these compounds are changing over time.

Here we have measured the concentration of 11 PFAAs in blood samples taken from pregnant women in Hokkaido, Japan. Analysis of samples from 2003 to 2011 allowed us to assess temporal trends associated with changes in the levels of these compounds.

2. Materials and methods

2.1. Study population

Study participants included 150 pregnant women, between 28 and 32 weeks of gestation, who were enrolled in a prospective birth cohort study (the Hokkaido Study on Environment and Children's Health). This ongoing cohort study was initiated in February 2003, and details have been described (Kishi et al., 2011). Briefly, subjects were considered eligible if they were indigenous Japanese women who had antenatal care at one of 37 participating hospitals within Hokkaido during the first trimester of pregnancy. Healthcare personnel introduced the study and provided each potential participant with an invitation, which included a consent form and a baseline questionnaire. All participants provided written informed consent. Among the 20,737 women that were registered between February 2003 and December 2011, only patients associated with a consent form, a baseline questionnaire, medical records at birth, and a maternal blood sample were included in this study. This represented 1944 women selected during 2003, 2459 women selected during 2005, 1820 women selected during 2007, 1274 women selected during 2009, and 1103 women selected during 2011. From these populations, 30 women from each year were randomly selected for analysis. The protocol used in this study was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.2. Standards and reagents

Acetonitrile, methanol, ultrapure water, and an HPLC-grade ammonium acetate solution (1 mol/L) were purchased from Wako Pure Chemical Inc., Osaka, Japan. Bulk ENVI-Carb sorbent was purchased from Supelco, Bellefonte, PA, USA. Acetic acid (purity: 99.7%) was purchased from Kanto Chemicals, Tokyo, Japan. Perfluorohexane sulfonate (PFHxS; >98%), PFOS (>98%), and a mixture of native PFCAs [perfluorohexanoic acid (PFHxA; >98%), perfluoroheptanoic acid (PFHpA; >98%), PFOA (>98%), PFNA (>98%), PFDA (>98%), PFUnDA (>98%), PFDoDA (>98%), perfluorotridecanoic acid (PFTrDA; >98%), and perfluorotetradecanoic acid (PFTeDA; >98%)] were obtained from Wellington Laboratories, Inc., Guelph, Ontario, Canada. Wellington Laboratories also supplied $^{13}C_3$-labeled PFHxS (≥99%), $^{13}C_4$-labeled PFOS (≥99%), and a mixture of ^{13}C-labeled PFCAs [$^{13}C_2$-PFHxA (≥99%), $^{13}C_4$-PFOA (≥99%), $^{13}C_5$-PFNA (≥99%), $^{13}C_2$-PFDA (≥99%) and $^{13}C_2$-PFUnDA (≥99%)].

2.3. Sample preparation

A 10-mL blood sample was taken from the maternal peripheral vein between 28 and 32 weeks of pregnancy. All samples were stored at −80 °C before analysis. An internal standard, which consisted of $^{13}C_3$-labeled PFHxS, $^{13}C_4$-labeled PFOS, and $^{13}C_4$-labeled PFCAs (2.5 ng of each), was added to each human plasma sample (0.5 mL). Samples were extracted with 2 mL acetonitrile by vortexing for 30 s. After centrifugation (3000 ×*g* for 15 min), supernatants were transferred into new tubes containing 25 mg ENVI-Carb and 50 μL acetic acid. Solutions were mixed by vortexing for 30 s. After centrifugation (3000 ×*g* for 15 min), each supernatant taken from above the ENVI-Carb was concentrated to 0.25 mL under nitrogen, and 0.25 mL methanol was added with subsequent mixing.

2.4. Ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry (UPLC-MS/MS)

Extracted solutions were analyzed using UPLC-MS/MS instrumentation. The ACQUITY UPLC system (Waters, Tokyo, Japan) was used with ethylene-bridged (BEH) C18 columns (1.7 μm, 2.1 × 50 mm). The retention gap technique was used by installing retention gap columns [BEH C18 columns (1.7 μm, 2.1 × 100 mm)], which improved PFAA sensitivity by trapping mobile-phase PFAAs (contaminants) in the retention gap column. The column temperature was 55 °C, and the column oven was maintained at 57 °C. A Micromass Quattro Premier tandem quadruple mass spectrometer (Waters) was used for MS/MS. Conditions for MS/MS were as follows: desolvation and source temperatures were set at 350 °C and 120 °C, respectively. The capillary was held at a potential of 3.5 kV relative to the counterelectrode in the negative-ion mode for all compounds. Cone and desolvation gas flow rates were 50 and 800 L/h, respectively. Cone and collision voltages, and monitored transition ions are listed in Table 1. Analytes were eluted from the column with a linear gradient involving solvent A (2 mM ammonium acetate in water) and solvent B (2 mM ammonium acetate in acetonitrile) as follows: 10% B for the initial 0.2 min, then a gradient of 10–100% B from 0.2 min to 9 min. The effluent was maintained at 100% B from 9 min to 12 min. The total UPLC cycle time was 15 min including column re-equilibration. An eluent flow rate of 0.3 mL/min was employed for all analyses. The injection volume was 5 μL.

2.5. Quality control

Levels of PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFHxS, and PFOS were measured. Calibration curves were prepared using calibration standards that consisted of seven concentrations (between 0.1 and 10 ng/mL) prepared in 1:1 acetonitrile/methanol. Each calibration standard also contained the internal standard (5 ng/mL). Calibration curves were constructed to perform linear regressions (1/× weighting) that compared plots of peak area/internal standard area versus standard concentration/internal standard concentration. Plasma samples were quantified using calibration curves that showed good linearity and correlation coefficients (R^2) > 0.995 for all compounds. Quantification was performed using a relative-response ratio to an internal standard that most structurally matched the target analyte (Table 1).

Recoveries and relative standard deviations were evaluated using five replicate fortifications (fortified to 10 times the original