18 months of age. In addition, we did not observe any significant association between PFOS concentrations and neurodevelopmental outcomes in early infancy. In conclusion, our results suggest that prenatal PFOA exposure may affect female mental scales of neurodevelopment at 6 months of age. Further studies with larger sample sizes and longer observation periods are required to clarify sex difference of the neurodevelopmental effects.

1. Introduction

Perfluorinated chemicals (PFCs) are persistent and ubiquitous chemicals widely used in several industrial applications and consumer products (Lau et al., 2007). The most common route of exposure to PFCs is dietary via the consumption of contaminated food and drinking water; indoor air and dust are other potential source of exposure to PFCs (Fromme et al., 2009; Kato et al., 2009; Beesoon et al., 2011). The most well-detected PFCs in humans and biota are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). While PFOS and PFOA are being voluntarily phased out by several industries, they are still present in older products. Additionally, PFCs are resistant to metabolism, and PFOS and PFOA are slowly eliminated from the human body with mean half-lives of 5.4 and 3.8 years, respectively (Olsen et al., 2007).

In rats, neonatal exposure to PFCs can inhibit synaptogenesis in the developing brain (Liao et al. 2008; Johansson et al., 2009, Johansson et al., 2008), and exposure to PFOS and PFOA in neonatal mice results in spontaneous deranged behavior, including irreversibly reduced habituation or hyperactivity in adult mice (Johansson et al., 2008). In vitro and in vivo studies have suggested that PFCs exert toxic effects on developing brains via the disruption of thyroid hormone balances (Lau et al., 2003; Wang et al., 2011).

Fetuses are exposed to PFCs during the maternofetal passage (Inoue et al., 2004; Monroy et al., 2008). Infants and children may experience higher exposure to PFCs than adults due to crawling, inhalation and ingestion of indoor air dust. PFCs are detectable in brain tissue following oral exposure (Bogdanska et al., 2011), and the blood brain barrier is not completely formed until early infancy (Adinolfi, 1985), leaving infants more susceptible to the potential adverse effects of chemicals including PFCs.

Animals and humans are vulnerable to exposure of chemicals at the period of extensive growth and development of the brain called the brain growth sprout (BGS). In humans, BGS begins during the last trimester of pregnancy and extends throughout the first two years of life (Mariussen, 2012). A Taiwanese study showed a negative association between PFOS levels in cord blood and neurodevelopmental scales, particularly the gross-motor subdomain at 2 years of age (Chen et al., 2013). In contrast, no convincing associations were reported between prenatal PFOS/PFOA exposure and neurodevelopmental milestones at 6 and 18 months of age in the prospective Danish birth cohort study (Fei et al., 2008). In addition, no association was found between prenatal PFC levels and childhood behavioral or coordination problems at 7 years of age in the same cohort (Fei and Olsen, 2011).

There are a limited number of studies focusing on the potential neurobehavioral effects of PFCs in humans, and these studies have non-conclusive results; the potential effects of prenatal exposure to PFCs on the neurodevelopment of humans and especially in early infancy are not well understood. Therefore, in the present analysis, we explored the relationship between prenatal exposure to PFOS/PFOA and the neurodevelopment of infants at 6 and 18 months of age assessed by the Bayley Scales of Infant Development.

2. Material and methods

2.1. Study population

This study was a part of the Hokkaido Study on Environment and Children's Health performed between July 2002 and October 2005, and the details have been previously described (Kishi et al., 2011 and 2013). In this prospective birth cohort study, pregnant women were recruited between 23–35 weeks of gestation and delivered their children in one hospital in Sapporo, Japan. Of 1796 potentially eligible women, the following subjects were excluded: the women who decided to participate in the Japanese cord blood bank (22% of those approached), and the women who decided to deliver at another hospital (3% of those approached). Ultimately, 514 (28.6%) pregnant women agreed to participate in this study. All participants were natives of Japan and residents of Sapporo or the surrounding areas. For the analysis of the associations between maternal PFCs and BSID II, the following subjects were excluded: women with pregnancy-induced hypertension ($n = 11$), women with diabetes mellitus ($n = 1$), mother–infant pairs with fetal heart failure ($n = 1$), and twins ($n = 7$). After the exclusion of these subjects, 428 mother–infant pairs had available PFOS and PFOA concentrations. In addition, the eligibility criteria for the analysis of subjects included the term newborns of 37–42 weeks of gestation, Apgar score of >7 at 1 min, infants without congenital anomalies or diseases, and having completed BSID-II. To assess the association between PFCs and neurodevelopment at 6 months of age, we included infants who were examined between 5.5 and 6.5 months of age, and we excluded 4 subjects because the neurodevelopment examination was performed after 6.5 months of age.

2.2. Questionnaires and medical records

A self-administered questionnaire survey was completed after the second trimester of pregnancy (Washino et al., 2009) containing information related to previous medical history, smoking, economic status, educational levels, alcohol and caffeine intake during pregnancy, and dietary intake during pregnancy including daily fish intake. For estimation of alcohol and caffeine intake during pregnancy, a self-administered questionnaire was used as described by Nagata et al. (1998). Medical information including maternal age, maternal body mass index (BMI) before pregnancy, parity, gestational age, pregnancy complications, type of delivery, infant's sex, and birth size (weight, length, and head circumferences) was obtained from participant medical records. This study was conducted with the written informed consent of all participants, and the study protocol was approved by the institutional ethical board for epidemiological studies at the Graduate School of Medicine and Center for Environmental and Health Sciences, Hokkaido University.

2.3. Blood sampling and exposure assessment

A 40-mL blood sample was taken from the maternal peripheral vein during the perinatal period after the second trimester of pregnancy. All samples were stored at −80 °C until analysis. Detailed methods for the measurement of PFOS and PFOA have been described in our previous report (Nakata et al., 2009). In brief, serum samples (0.1 mL) were mixed with 0.2 mL internal standard ($^{13}C_4$–PFOS–Na^+ and $^{13}C_2$–PFOA) solution containing acetonitrile, centrifuged at 1450 ×g for 10 min, and the supernatant was transferred to a polypropylene tube. An aliquot of the filtered sample solution was subjected to column-switching liquid chromatography–tandem mass spectrometry (LC/MS/MS). The detection limit for both PFOS and PFOA was 0.5 ng/mL. PFOS levels were detected in

all samples, and for samples with PFOA levels below detection limit, we used a value of half the detection limit (0.25 ng/mL). Detection rate for PFOA was 91.3% and 93.2% in maternal serum whose children examined at 6 and 18 months, respectively.

2.4. Neurodevelopmental assessment

Details of the developmental measures have been previously described by our group (Nakajima et al., 2006). Briefly, infants' neurodevelopment was assessed at 6 and 18 months of age using the Bayley Scales of Infant Development, second edition (BSID-II, 1993). This test measures the mental developmental index (MDI) and psychomotor developmental index (PDI) from 0 to 3 years of age. The BSID-II mental scale assesses the age-appropriate level of cognitive, language, and personal/social development. The motor scale examines fine and gross motor development. Mental and motor scores are based on the calibration scale from a raw score and are represented as index scores. The mean scores of MDI and PDI (±SD) are 100 (±15). All children were examined by one examiner in a quiet, private room in the presence of the parent(s). The indicators of neurodevelopment were evaluated by three occupational therapists with clinical experience in the field of developmental disabilities. The examiners were unaware of the participants' PFC levels. First, the scoring was performed by the examiner who conducted the assessment of all of the children, and two other examiners then verified and confirmed the scores by reviewing videotaped evaluations. In addition, we assessed the index of the childcare environment of the subjects using the questionnaire (Anme et al., 1997).

2.5. Data analysis

We analyzed the correlations between PFOS and PFOA concentrations and the characteristics of the mothers and infants using the Spearman rank correlation coefficient (rs), the Mann–Whitney *U*-test, and the Kruskal–Wallis test. The same statistical analyses were performed to find correlations between infants' BSID II scores and the participants' characteristics. We performed multiple-regression analysis to examine the association between BSID-II scores (MDI, PDI) and the levels of PFCs in maternal serum samples. The levels of PFCs in maternal blood were $\log_{10}$ transformed, and potential confounders were selected according to the previous literature and current results in this paper. The analysis was adjusted for maternal age (year), parity (0/≥1), maternal educational levels (categorical), alcohol consumption and smoking during pregnancy (yes/no), caffeine intake during pregnancy (milligrams/day), blood sampling period (before and after delivery), breastfeeding (less or more than 3 months). Previously, our group reported that prenatal exposure to some dioxin congeners is negatively associated with MDI and PDI scores at 6 months of age (Nakajima et al., 2006). Therefore, prenatal dioxin levels were added to the aforementioned confounders in an additional adjustment model (fully adjusted model). Moreover, the associations between the PFCs and BSID II scores were assessed after stratifying by the infants' sex. For the assessment of dose–response, the prenatal PFC concentrations in the maternal blood samples were divided into quartiles, and least square means (LSMs) and 95% confidence interval (CI) were calculated. For calculation of *p* for trend, we used the linear contrast coefficient −3, −1, +1, +3 assigned to quartile 1, 2, 3, and 4, respectively. The LSM of the BSID II for each quartile was compared using the Hsu–Dunnet method to accommodate for multiple comparisons. We performed all of the statistical analyses using JMP pro 11 (SAS Institute Inc., NC, USA). Results were considered significant if $p < 0.05$.

3. Results

The basic characteristics of the study population are presented in Table 1. The mean (SD) scores were 90.5 (5.7) for MDI and 90.1 (10.1) for PDI in infants at 6 months of age, whereas the mean (SD) for MDI and PDI at 18 months of age was 84.2 (12.0) and 86.3 (10.9), respectively. The median value of prenatal PFOS and PFOA levels in mothers whose infants were examined at 6 months of age was 5.7 ng/mL (25–75 percentile: 4.4–7.4) and 1.2 ng/mL (25–75 percentile: 0.8–1.7), respectively (Table 2). Tables 2 and 3 show maternal PFOA and PFOS levels according to characteristics of the mothers and the infants at 6 and 18 months of age, respectively. Prenatal PFOS levels were significantly correlated with maternal age, parity, blood sampling period in mother–infant pairs at 6 and 18 months of age. PFOA levels were negatively associated with parity (Tables 2 and 3).

Birth weight was associated with the infants' MDI at 6 months of age ($rs = 0.182$; $p = 0.016$). At 6 months of age, PDI scores were negatively correlated with caffeine intake during pregnancy and positively correlated with gestational age, birth weight and length (Table 4). At 18 months, MDI was correlated positively with higher annual income during pregnancy, female gender, and longer gestational age, whereas PDI was correlated positively with higher annual income during pregnancy and female gender and negatively correlated with pre-pregnancy BMI (Table 5).

After adjusting for appropriate confounders, PFOS and PFOA did not show any significant association with MDI nor PDI at 6 months of age in total infants (Table 6). After sex stratification, we found a significant negative association between prenatal PFOA exposure and MDI scores only in female infants ($\beta = -0.296$; 95% confidence interval: −11.96, −0.682) in the model 2. We also examined the association between the serum PFOA quartiles and MDI at 6 months of age. In the adjusted model, female infants whose mothers were in the highest quartile of PFOA concentration had MDI scores −5.05 [95% CI: −10.66 to 0.55]

Table 1
Characteristics of mother–infant pairs.

Characteristics	6-month postpartum assessment (n = 173)	18-month postpartum assessment (n = 133)
	No. (%)/mean ± SD	No. (%)/mean ± SD
Maternal characteristics		
Age (years)[a]	30.8 ± 4.6	31.0 ± 4.4
Prepregnancy BMI (kg/m^2)[a]	21.1 ± 2.9	21.2 ± 2.8
Primipara	83 (48.0)	65 (48.9)
Educational ≤ 12 years	66 (38.1)	48 (36.1)
Annual income during pregnancy < 5 million yen	112 (64.7)	85 (64.0)
Smoking during pregnancy (yes)	20 (11.6)	11 (8.3)
Alcohol intake during pregnancy (yes)	51 (29.5)	40 (30.1)
Child characteristics		
Male infant	83 (48.0)	66 (49.6)
Gestational age (days)[a]	276.3 ± 8.3	276.3 ± 8.5
Birth weight (g)[a]	3111.2 ± 358.5	3096.8 ± 323.8
Birth length (cm)[a]	48.2 ± 1.7	48.2 ± 1.6
Breast-feeding < 3 months	71 (41.0)	50 (37.6)
BSID II mental index score (MDI)[a]	90.5 ± 5.7	84.0 ± 12.0
BSID II psychomotor index score (PDI)[a]	90.1 ± 10.1	86.3 ± 10.9
Index of child care environment[b]	22.2 ± 3.9	28.2 ± 3.5

[a] Mean ± SD.
[b] Perfect score is 30 points for 6 months and 38 for 18 months of age, respectively.

lower than female infants born to mothers with PFOA concentrations in the first quartile. The p-value between quartile 1 and 4 did not meet significance ($p = 0.086$), but the p for trend was significant ($p = 0.045$) (Fig. 1, and Supplementary Table 1). However, at 18 months of age, we observed no significant association of PFCs with MDI and PDI (Table 7). Moreover, we did not find any sex differences in the

Table 2
Maternal blood PFC levels (ng/mL) in relation to characteristics of mothers and 6-month old infants (n = 173).

Characteristics	N	PFOS (mean or correlation)	p-Value	PFOA (mean or correlation)	p-Value
Median (25–75 percentile)		5.7 (4.4–7.4)		1.2 (0.8–1.7)	
Maternal characteristics					
Age (years)		rs = −0.186	**0.014**	rs = −0.093	0.220
Prepregnancy BMI (kg/m²)		rs = 0.017	0.822	rs = −0.088	0.247
Parity (times)					
0	83	6.95 ± 0.29	**0.003**	1.65 ± 0.08	**<0.001**
≥1	90	5.54 ± 0.28		1.04 ± 0.07	
Educational level (years)					
≤12	66	5.79 ± 0.33	0.056	1.30 ± 0.09	0.695
≥13	107	6.48 ± 0.26		1.35 ± 0.07	
Annual income (million yen)					
<5	112	6.27 ± 0.25	0.757	1.34 ± 0.07	0.856
≥5	61	6.12 ± 0.35		1.32 ± 0.10	
Smoking during pregnancy					
Yes	20	5.25 ± 0.61	0.092	1.21 ± 0.18	0.519
No	153	6.34 ± 0.22		1.35 ± 0.06	
Alcohol intake during pregnancy					
Yes	51	6.38 ± 0.38	0.208	1.34 ± 0.11	0.731
No	122	6.15 ± 0.24		1.33 ± 0.07	
Alcohol intake during pregnancy (g/day)		rs = 0.078	0.306	rs = 0.020	0.793
Caffeine intake during pregnancy (mg/day)		rs = −0.136	0.073	rs = −0.048	0.526
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	95	6.02 ± 0.28	0.218	1.31 ± 0.08	0.482
More than 1–2 times/week	78	6.46 ± 0.31		1.36 ± 0.09	
Deep-sea fish					
Less than 1–2 times/month	81	6.02 ± 0.30	0.135	1.36 ± 0.08	0.727
More than 1–2 times/week	92	6.39 ± 0.28		1.31 ± 0.08	
Blood sampling period					
During pregnancy	131	6.55 ± 0.23	**0.002**	1.40 ± 0.06	0.135
After delivery	42	5.19 ± 0.41		1.12 ± 0.12	
Child characteristics					
Sex					
Male	83	6.23 ± 0.30	0.805	1.45 ± 0.08	0.164
Female	90	6.21 ± 0.29		1.23 ± 0.08	
Type of delivery					
Vaginal	146	6.39 ± 0.22	0.061	1.36 ± 0.06	0.272
Cesarean section	28	5.26 ± 0.52		1.17 ± 0.15	
Gestational age (days)		rs = 0.071	0.352	rs = −0.018	0.812
Birth weight (g)		rs = −0.058	0.444	rs = −0.105	0.166
Birth length (cm)		rs = 0.151	0.046	rs = −0.004	0.949
Head circumference (cm)		rs = −0.013	0.855	rs = −0.020	0.791
Feeding					
Breast-feeding	72	6.30 ± 0.32	0.684	1.36 ± 0.09	0.929
Mix	79	6.02 ± 0.30		1.29 ± 0.08	
Bottle-feeding	6	6.66 ± 1.12		1.20 ± 0.32	
Breast-feeding (month)					
<3	71	6.26 ± 0.32	0.900	1.31 ± 0.09	0.603
≥3	102	6.19 ± 0.27		1.35 ± 0.07	

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.

Table 3
Maternal blood PFC levels (ng/mL) in relation to characteristics of mothers and 18-month old infants (n = 133).

Characteristics	N	PFOS (mean or correlation)	p-Value	PFOA (mean or correlation)	p-Value
Median (25–75 percentile)		5.8 (4.5–7.4)		1.2 (0.8–1.7)	
Maternal characteristics					
Age (years)		rs = −0.251	**0.003**	rs = −0.137	0.130
Prepregnancy BMI (kg/m²)		rs = 0.049	0.569	rs = −0.056	0.518
Parity (times)					
0	65	6.87 ± 0.31	**0.019**	1.69 ± 0.09	**<0.001**
≥1	68	5.64 ± 0.30		1.09 ± 0.09	
Educational level (years)					
≤12	48	5.81 ± 0.37	0.083	1.45 ± 0.12	0.480
≥13	85	6.49 ± 0.28		1.34 ± 0.09	
Annual income (million yen)					
<5	85	6.08 ± 0.28	0.408	1.38 ± 0.09	0.990
≥5	48	6.53 ± 0.37		1.38 ± 0.12	
Smoking during pregnancy					
Yes	11	5.20 ± 0.78	0.164	1.23 ± 0.25	0.700
No	122	6.33 ± 0.23		1.40 ± 0.07	
Alcohol intake during pregnancy					
Yes	40	6.41 ± 0.41	0.231	1.40 ± 0.13	0.609
No	93	6.17 ± 0.27		1.37 ± 0.08	
Alcohol intake during pregnancy (g/day)		rs = 0.088	0.313	rs = 0.022	0.800
Caffeine intake during pregnancy (mg/day)		rs = −0.215	**0.012**	rs = −0.081	0.349
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	73	6.10 ± 0.30	0.487	1.34 ± 0.09	0.291
More than 1–2 times/week	60	6.41 ± 0.33		1.44 ± 0.10	
Deep-sea fish					
Less than 1–2 times/month	64	5.98 ± 0.32	0.105	1.40 ± 0.10	0.872
More than 1–2 times/week	69	6.48 ± 0.31		1.37 ± 0.10	
Blood sampling period					
During pregnancy	106	6.56 ± 0.24	**0.006**	1.45 ± 0.08	**0.083**
After delivery	27	5.00 ± 0.49		1.12 ± 0.16	
Child characteristics					
Sex					
Male	66	6.17 ± 0.32	0.388	1.52 ± 0.10	0.053
Female	67	6.31 ± 0.32		1.25 ± 0.10	
Type of delivery					
Vaginal	110	6.46 ± 0.24	**0.036**	1.42 ± 0.08	0.328
Cesarean section	23	5.18 ± 0.53		1.21 ± 0.17	
Gestational age (days)		rs = 0.138	0.112	rs = −0.039	0.654
Birth weight (g)		rs = −0.062	0.474	rs = −0.067	0.440
Birth length (cm)		rs = 0.169	0.051	rs = 0.066	0.447
Head circumference (cm)		rs = −0.099	0.256	rs = 0.013	0.874
Feeding					
Breast-feeding	59	6.40 ± 0.34	0.759	1.43 ± 0.11	0.808
Mix	66	6.03 ± 0.32		1.35 ± 0.10	
Bottle-feeding	5	6.66 ± 1.10		1.16 ± 0.37	
Breast-feeding (month)					
<3	50	6.04 ± 0.37	0.403	1.40 ± 0.11	0.935
≥3	83	6.36 ± 0.28		1.37 ± 0.09	

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.

Table 4
Characteristics of mother–infant pairs in relation to 6-month old MDI and PDI (n = 173).

Characteristics	No.	MDI		PDI	
		Mean ± SD	p-Value	Mean ± SD	p-Value
Maternal characteristics					
Age (years)		rs = −0.046	0.539	rs = −0.001	0.987
Prepregnancy BMI (kg/m^2)		rs = −0.008	0.911	rs = 0.023	0.755
Parity (times)					
0	83	91.1 ± 0.6	0.484	89.9 ± 1.1	0.681
≥1	90	90.0 ± 0.6		90.2 ± 1.0	
Educational level (years)					
≤12	66	91.0 ± 0.7	0.346	91.0 ± 1.2	0.448
≥13	107	90.2 ± 0.5		89.5 ± 0.9	
Annual income (million yen)					
<5	112	90.4 ± 0.5	0.532	89.5 ± 0.9	0.344
≥5	61	90.7 ± 0.7		91.2 ± 1.3	
Smoking during pregnancy					
Yes	20	90.1 ± 1.2	0.441	88.7 ± 2.2	0.630
No	153	90.6 ± 0.4		90.2 ± 0.8	
Alcohol intake during pregnancy					
Yes	51	91.1 ± 0.8	0.691	90.5 ± 1.4	0.981
No	122	90.3 ± 0.5		89.9 ± 0.9	
Alcohol intake during pregnancy (g/day)		rs = 0.041	0.585	rs = −0.003	0.968
Caffeine intake during pregnancy (mg/day)		rs = −0.023	0.762	rs = −0.188	**0.013**
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	95	90.6 ± 0.5	0.686	90.1 ± 1.0	0.898
More than 1–2 times/week	78	90.4 ± 0.6		90.0 ± 1.1	
Deep-sea fish					
Less than 1–2 times/month	81	90.2 ± 0.6	0.575	89.8 ± 1.1	0.603
More than 1–2 times/week	92	90.8 ± 0.6		90.3 ± 1.0	
Blood sampling period					
During pregnancy	131	90.4 ± 0.5	0.381	89.3 ± 0.8	0.076
After delivery	42	90.8 ± 0.8		92.4 ± 1.5	
Child characteristics					
Sex					
Male	83	91.0 ± 0.6	0.234	89.2 ± 1.1	0.483
Female	90	90.0 ± 0.6		90.8 ± 1.0	
Type of delivery					
Vaginal	146	90.7 ± 0.4	0.313	90.6 ± 0.8	0.062
Cesarean section	27	89.2 ± 1.1		87.3 ± 1.9	
Gestational age (days)		rs = 0.144	0.058	rs = 0.245	**0.001**
Birth weight (g)		rs = 0.182	**0.016**	rs = 0.153	**0.044**
Birth length (cm)		rs = 0.142	0.061	rs = 0.157	**0.038**
Head circumference (cm)		rs = 0.101	0.182	rs = 0.051	0.503
Feeding					
Breast-feeding	72	91.7 ± 0.6	0.052	91.4 ± 1.1	0.113
Mix	79	89.4 ± 0.6		88.6 ± 1.1	
Bottle-feeding	6	92.8 ± 2.3		93.1 ± 4.0	
Breast-feeding (month)					
<3	71	90.4 ± 0.6	0.688	89.5 ± 1.2	0.317
≥3	102	90.5 ± 0.5		90.5 ± 1.0	
Index of child care environment		rs = −0.004	0.951	rs = −0.107	0.158

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.
Bold values indicate significance at p-value.

neurodevelopmental effects of PFCs at 18 months of age (data not shown).

4. Discussion

This study is one of few reports examining the effects of prenatal exposure to PFCs on neurodevelopment in early life. Median concentrations for maternal PFOS and PFOA in the current study were 5.7 and 1.2 ng/mL, respectively, which are one of the lowest levels reported among pregnant women compared to the median of those in the US (PFOS: 8.2, PFOA: 2.9 ng/mL) (Stein et al., 2012), Canada (PFOS: 16.6, PFOA: 2.1 ng/mL) (Monroy et al., 2008), Denmark (PFOS: 21.5, PFOA: 3.7 ng/mL) (Halldorsson et al., 2012), and Korea (PFOS: 9.3, PFOA: 2.6 ng/mL) (Lee et al., 2013). In this study, we examined the association between prenatal low exposure levels of PFOS/PFOA and neurodevelopment at 6 and 18 months of age using the BSID-II. We found an inverse association between prenatal exposure to PFOA and MDI scores only among female infants at 6 months of age; also quartile PFOA dose–response trend analysis showed a significant decrease in MDI scores at 6 months in female infants. We did not find an association between prenatal PFOA exposure and BSID II scores at 18 months of age. Prenatal exposure to PFOS was not associated with any of the measured neurodevelopmental scores at 6 and 18 months of age. Our study suggests that low levels of in utero exposure to PFOA may affect neurodevelopment in early infancy by sex differences.

The effects of PFCs on neurodevelopment in infancy and early childhood are not well understood. A group in Taiwan examined the association of PFC levels in cord blood plasma and neurodevelopment using a Taiwanese questionnaire (the Comprehensive Developmental Inventory for Infants and Toddlers) at 2 years of age (n = 239). The whole test contained 5 domains: motor (gross and fine), cognitive, language, social and self-help. Each item of these domains was scored 0 or 1, where indicates success in evaluation through direct testing, observation and self-reporting. In contrast to our results, PFOS but not PFOA levels in cord blood plasma were inversely associated with the overall test results and especially the gross-motor domain (Chen et al., 2013). In this

Table 5
Characteristics of mother–infant pairs in relation to 18-month old MDI and PDI (n = 133).

Characteristics	No.	MDI		PDI	
		Mean ± SD	p-Value	Mean ± SD	p-Value
Maternal characteristics					
Age (years)		rs = −0.068	0.436	rs = −0.090	0.301
Prepregnancy BMI (kg/m^2)		rs = −0.120	0.166	rs = −0.223	**0.009**
Parity (times)					
0	65	83.0 ± 1.4	0.550	84.8 ± 1.3	0.115
≥1	68	85.0 ± 1.4		87.7 ± 1.3	
Educational level (years)					
≤12	48	82.6 ± 1.7	0.540	85.9 ± 1.5	0.966
≥13	85	84.8 ± 1.3		86.5 ± 1.1	
Annual income (million yen)					
<5	85	81.7 ± 1.2	**0.012**	84.4 ± 1.1	**0.007**
≥5	48	88.1 ± 1.6		89.7 ± 1.5	
Smoking during pregnancy					
Yes	11	83.7 ± 3.6	0.964	86.4 ± 3.3	0.937
No	122	84.1 ± 1.0		86.3 ± 0.9	
Alcohol intake during pregnancy					
Yes	40	84.3 ± 1.9	0.945	87.2 ± 1.7	0.652
No	93	83.9 ± 1.2		86.0 ± 1.1	
Alcohol intake during pregnancy (g/day)		rs = 0.010	0.902	rs = 0.046	0.596
Caffeine intake during pregnancy (mg/day)		rs = −0.053	0.544	rs = 0.008	0.922
Fish intake during pregnancy					
Inshore fish					
Less than 1–2 times/month	73	83.6 ± 1.4	0.608	87.9 ± 1.2	0.091
More than 1–2 times/week	60	84.5 ± 1.5		84.4 ± 1.3	
Deep-sea fish					
Less than 1–2 times/month	64	83.5 ± 1.5	0.745	87.2 ± 1.3	0.418
More than 1–2 times/week	69	84.5 ± 1.4		85.5 ± 1.3	
Blood sampling period					
During pregnancy	106	84.3 ± 1.1	0.383	86.2 ± 1.0	0.813
After delivery	27	83.0 ± 2.3		86.8 ± 2.1	
Child characteristics					
Sex					
Male	66	81.2 ± 1.4	**0.007**	83.3 ± 1.2	**0.001**
Female	67	86.8 ± 1.4		89.2 ± 1.2	
Type of delivery					
Vaginal	110	85.1 ± 1.1	0.058	86.5 ± 1.0	0.652
Cesarean section	23	79.1 ± 2.4		85.4 ± 2.2	
Gestational age (days)		rs = 0.181	**0.036**	rs = 0.092	0.289
Birth weight (g)		rs = 0.125	0.151	rs = 0.050	0.567
Birth length (cm)		rs = 0.087	0.315	rs = 0.052	0.547
Head circumference (cm)		rs = −0.061	0.482	rs = 0.005	0.951
Feeding					
Breast-feeding	59	83.4 ± 1.5	0.852	86.7 ± 1.4	0.776
Mix	66	84.1 ± 1.4		85.4 ± 1.3	
Bottle-feeding	5	83.8 ± 5.4		86.4 ± 4.8	
Breast-feeding (month)					
<3	50	84.2 ± 1.7	0.881	86.6 ± 1.5	0.788
≥3	83	83.9 ± 1.3		86.1 ± 1.2	
Index of child care environment[a]		rs = 0.155	0.134	rs = −0.040	0.695

Statistical analysis is performed using Spearman rank correlation coefficient (rs), and Mann–Whitney *U*-test and Kruskal–Wallis test.
Bold values indicate significance at p-value.
[a] Not available for 39 subjects.

Taiwanese report, PFCs were examined in cord blood samples, not maternal blood samples, with medians of 7.0 and 2.5 ng/mL for PFOS and PFOA, respectively. No negative effects of low PFOS levels on infant neurodevelopment in our study may be due to the small sample size and low power. Inoue et al. (2004) reported that the mean PFOS concentration ratio in maternal blood to cord blood is 0.32, which indicates higher exposure levels of PFOS in the Taiwanese cohort compared to our study. Although we did not measure exposure levels of PFCs in cord blood samples, previous studies have determined that PFOA has higher transplacental transfer efficiency than PFOS (Beesoon et al., 2011; Lee et al., 2013), which may partially explain why we found a reverse association between only prenatal PFOA and developmental scores of infants in our study. In a Danish nationwide cohort study, Fei et al. (2008) investigated the associations between prenatal exposure to PFCs (PFOS and PFOA) and maternally reported developmental milestones at 6 and 18 months of age using a structured questionnaire with a large sample size (6 months, n = 1336; 18 months, n = 1255). The mothers were asked to recall at what time their infants developed motor (gross and fine motor) and mental skills. However, they did not find convincing associations between prenatal PFCs and neurodevelopmental milestones in early infancy. In addition, this group reported no association between prenatal PFC levels and behavioral or motor coordination problems in 7 year old children in the same cohort using the Strengths and Difficulties Questionnaire (SDQ) and the Developmental Coordination Disorder Questionnaire (DCDQ) (Fei and Olsen, 2011). In these two studies of the same cohort, maternal plasma PFOA and PFOS levels were 4–6 times higher than those in our study, although subtle effects of PFCs may not be detected by questionnaire-based examinations.

We selected confounders in the multiple linear regression model based on findings in this study and well-known factors important in infant neurodevelopment such as smoking and alcohol consumption during pregnancy. Additionally, our group reported the negative association between prenatal exposure to some congeners of dioxins

Table 6
The association between prenatal exposure to PFCs and 6-month old MDI and PDI (n = 173).

	MDI		PDI	
	Beta	(95% CI)	Beta	(95% CI)
Total (n = 173)				
PFOS				
Crude	0.035	(−3.32 to 5.40)	−0.007	(−8.05 to 7.29)
Model 1	0.015	(−4.33 to 5.21)	0.018	(−7.01 to 8.85)
Model 2	0.018	(−4.52 to 5.59)	0.039	(−6.38 to 10.37)
PFOA				
Crude	0.005	(−2.86 to 3.08)	−0.042	(−6.69 to 3.74)
Model 1	−0.039	(−4.15 to 2.59)	−0.013	(−6.09 to 5.13)
Model 2	−0.045	(−4.33 to 2.56)	−0.006	(−5.93 to 5.50)
Boys (n = 83)				
PFOS				
Crude	−0.084	(−8.74 to 3.88)	−0.020	(−10.70 to 8.85)
Model 1	−0.117	(−10.39 to 3.66)	0.124	(−4.44 to 15.47)
Model 2	−0.141	(−11.26 to 3.45)	0.120	(−5.24 to 15.60)
PFOA				
Crude	0.091	(−2.32 to 5.62)	−0.013	(−6.54 to 5.78)
Model 1	0.101	(−2.95 to 6.62)	0.055	(−5.27 to 8.36)
Model 2	0.110	(−3.31 to 7.14)	0.068	(−5.56 to 9.26)
Girls (n = 90)				
PFOS				
Crude	0.136	(−2.11 to 10.03)	0.002	(−11.63 to 11.90)
Model 1	0.093	(−3.93 to 9.34)	0.012	(−11.52 to 12.88)
Model 2	0.072	(−5.19 to 9.38)	0.031	(−11.66 to 15.09)
PFOA				
Crude	−0.094	(−6.56 to 2.48)	−0.055	(−10.97 to 6.34)
Model 1	−0.276	(−11.48 to −0.393)	0.068	(−7.59 to 13.25)
Model 2	−0.296	(−11.96 to −0.682)	0.055	(−8.37 to 12.93)

Model 1: adjusted for gestational age, parity, maternal age, smoking during pregnancy, alcohol consumption during pregnancy, caffeine during pregnancy, maternal education level, blood sampling period, breast feeding.
Model 2: model 1 + total dioxin levels (TEQ, WHO 2005).

and the neurodevelopment of infants at 6 months (Nakajima et al., 2006). We also found the same results in the current study. Therefore, we included dioxin levels in the fully adjusted model, although it did not change the results. In this study, PFOA and PFOS levels were modestly correlated (rs = 0.333), and mutual adjustment did not change the results in any consistent way. We have also adjusted this association for other potential confounders including the index of child care environment and birth weight, but the results did not change. Due to association of PFOS levels with type of delivery, we included type of delivery into fully adjusted models, and the results remained consistent.

At age 18 months, we did not find any association between PFCs and neurodevelopment. Fewer infants were examined at 18 months than at 6 months of age (n = 133 vs n = 173). Also, the significant correlation of birth weight and length with BSID II scores disappeared at 18 months, whereas we found a significant correlation of MDI and PDI scores at 18 months of age with higher annual income during pregnancy. Previous studies reported that socioeconomic status is associated with neurological functions including language, memory, cognition and social development (Hackman and Farah, 2009). We did not observe association between annual income and neurodevelopment at 6 months of age. However, we found a significant differences of MDI (high income vs low income: 88.1 vs 81.7, p = 0.012) and PDI (high income vs low income: 89.7 vs 84.4, p = 0.007) scores at 18 months of age according to annual income during pregnancy. Infants during first 6 months of life usually feed exclusively by breastfeeding, and they are not able to crawl, walk, talk, and interact with environment as much as 18-month

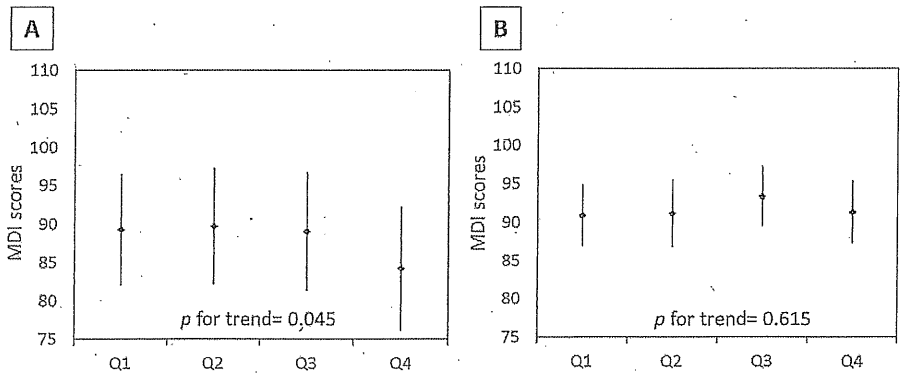

Fig. 1. The dose–response relationship between the quartiles of PFOA and reduced MDI scores among female (A) and male (B) infants at 6 months of age. For female infants (n = 90), values for the first (n = 26), second (n = 19), third (n = 27), and fourth (n = 18) quartiles, respectively, were as follows: <limit of detection (LOD) to 0.70, 0.70 to 1.15, 1.15 to 1.60, and 1.60 to 3.10 μg/mL. For male infants (n = 83), values for the first (n = 21), second (n = 17), third (n = 23), and fourth (n = 22) quartiles, respectively, were: <LOD to 0. 0.80, 0.80 to1.30, 1.30 to 1.80, 1.80 to 4.3. LSMs were adjusted for gestational age, parity, maternal age, smoking and alcohol consumption during pregnancy, caffeine intake during pregnancy, maternal education level, blood sampling period, breast feeding and total dioxin levels (TEQ, WHO 2005). LSMs are indicated in black circles and the error bars depict the upper and lower 95% CI. Q: quartile.

Table 7
The association between prenatal exposure to PFCs and 18-month old MDI and PDI (n = 133).

	MDI		PDI	
	Beta	(95% CI)	Beta	(95% CI)
PFOS				
Crude	0.090	(−5.27 to 16.94)	−0.026	(−11.65 to 8.54)
Adjusted[a]	0.052	(−9.91 to 16.66)	−0.023	(−13.45 to 10.72)
PFOA				
Crude	−0.098	(−11.20 to 3.03)	−0.072	(−9.19 to 3.72)
Adjusted[a]	−0.078	(−11.74 to 5.28)	0.002	(−7.66 to 7.85)

[a] Adjusted for gestational age, parity, maternal age, smoking and alcohol consumption during pregnancy, caffeine intake during pregnancy, maternal education, blood sampling period, breast feeding, and total dioxin levels (TEQ, WHO 2005).

infants. Families with higher annual income can provide better supplementary food and nutrition, educational resources, physical and psychosocial environment especially when infants start interaction with home environment and it helps infants for better neurodevelopment. It may partly explain why annual income affect neurodevelopment of infants at 18 months significantly compared to 6 months of age. Therefore, a negative association between exposure and mental scores at 6 months of age becomes difficult to find at 18 months of age because of the strong impact of annual income on neurodevelopment at 18 months of age. Another explanation for null impact of PFCs on neurodevelopment at 18 months of age could be postnatal environmental factors.

In this study, Bayley scores at 6 months were almost similar between girls and boys; however we found higher MDI and PDI scores at 18 months of age in girls. Girls have earlier cognitive changes than boys and this change occurs between 14 and 20 months of age (Reznick et al., 1997). It may be due to more sensitivity of girls to the environment and negative association of male testosterone with language skills (Christiansen and Knussmann, 1987). Previous epidemiological studies using BSID-II reported higher MDI and PDI scores among girls after first year of life but not at 6 months of age, however boys had steadier neurodevelopment trajectory (Lung et al., 2009; Augustyniak et al., 2013). These findings are consistent with our results and indicate role of sex on neurodevelopment.

The mechanistic effects of PFCs on neurodevelopment are not well understood. Recent research suggests that the endocrine-disrupting properties of PFCs, which can perturb metabolic endpoints including glucose homeostasis, thyroid hormone and sex hormone balance in animals may be the mechanism behind the adverse effects of PFCs (Seacat et al., 2003; Thibodeaux et al., 2003). Prenatal and postnatal exposure to PFCs interfere with thyroid hormone balance in humans resulting in higher thyroid stimulating hormone (TSH), decreased thyroxine (T4), and triiodothyronine (T3) (Ji et al., 2012; Wang et al., 2014; Berg et al., 2015), which may be responsible for the effects of these chemicals on the neurodevelopment of humans. Our results suggest that an infant's sex modifies the association between in utero PFOA exposure and neurodevelopment at 6 months of age. PFCs reduce serum testosterone and increase estradiol levels in rodents (Lau et al., 2007). Epidemiological studies suggest that PFCs are positively and negatively associated with estradiol and testosterone levels, respectively (Knox et al., 2011, Vested et al., 2013). A previous study in a highly PFOA-exposed population in the US showed that childhood PFOA levels has favorable association with neurodevelopment among boys but adverse association among girls (Stein et al., 2014). This result is in line with our results in terms of adverse effects of PFOA exposure on neurodevelopment among girls. However, more studies should be conducted to elucidate the sex differences of PFCs effect on neurodevelopment.

It has been shown that PFCs are associated with an increased risk of miscarriage (Darrow et al., 2014), preeclampsia (Stein et al., 2009), pregnancy-induced hypertension (Darrow et al., 2013), premature birth (Chen et al., 2012), and birth defects (Stein et al., 2009; Liew et al., 2014). In this study, we excluded participants with pregnancy-induced hypertension (n = 11), premature infants (n = 30), and infants with malformations (n = 1); infants born with these complicated pregnancies are more susceptible to neurodevelopmental problems. Therefore, we may underestimate the effects of prenatal exposure to PFCs on the neurodevelopment of infants due to the exclusion of these susceptible groups.

In this study, we measured prenatal PFC levels in a prospective birth cohort, which provides strong causality between exposure levels and outcomes in infants. In addition, we assessed the infants' neurodevelopment through expert staff experienced in the field of developmental disabilities and therefore avoiding measurement bias and recall bias from mother-reported neurodevelopmental milestones. The limitations of this study need to be considered. The small sample size precluded estimation of the subtle effects of PFCs on infant neurodevelopment. Among subjects with available PFC levels in original cohort (n = 428), a subpopulation of those had neurodevelopment assessment at 6 and 18 months of age in the current study, it may be a potential source of selection bias. The participants in the current study had higher maternal education, higher annual income and lesser smoking rate during pregnancy compare to the original cohort. However, the characteristics of subjects in original cohort were similar to participants of the current study in terms of PFC levels, maternal age, prepregnancy BMI, parity, and gestational age. We did not assess postnatal PFC exposure, and this may introduce some uncontrolled confounders, particularly for the assessment done at 18 months of age.

Previously, our group reported temporal trends of 11 types of PFCs between 2003 and 2011 in plasma samples of pregnant women in Hokkaido (Okada et al., 2013). The results indicated that PFOS and PFOA concentrations declined, whereas long-chain PFCs (including PFNA and PFDA) levels increased. In future studies, assessment of the effects of pre- and postnatal exposure to PFCs with longer carbon chains on the neurodevelopment of infants and children with bigger sample sizes, different battery tests and longer observation periods is necessary.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.10.017.

Conflict of interest

The authors declare they have no actual or potential competing financial interests.

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Effects of *in utero* exposure to polychlorinated biphenyls, methylmercury, and polyunsaturated fatty acids on birth size

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HIGHLIGHTS

- The risk of small for gestational age by weight decreased with increasing hair mercury concentration.
- The concentrations of mercury in maternal hair had no association with birth weight.
- The concentrations of polychlorinated biphenyls in maternal blood had no association with birth size.

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Polyunsaturated fatty acids

ABSTRACT

The adverse effects of *in utero* exposure to polychlorinated biphenyls (PCBs) or methylmercury (MeHg), and the beneficial effects of nutrients from maternal fish intake might have opposing influences on fetal growth. In this study, we assessed the effects of *in utero* exposure to PCBs and MeHg on birth size in the Japanese population, which is known to have a high frequency of fish consumption. The concentrations of PCBs and polyunsaturated fatty acids in maternal blood, and the total mercury in hair (as a biomarker of MeHg exposure) were measured during pregnancy and at delivery. Maternal intakes of fish (subtypes: fatty and lean) and shellfishes were calculated from a food frequency questionnaire administered at delivery. Newborn anthropometric measurement data were obtained from birth records. The associations between chemical exposures and birth size were analyzed by using multiple regression analysis with adjustment for confounding factors among 367 mother–newborn pairs. The birth weight was 3073 ± 37 g (mean ± SD). The incidence of babies small for gestational age (SGA) by weight was 4.9%. The median concentrations of total PCBs and hair mercury were 108 ng/g lipid and 1.41 μg/g, respectively. There was no overall association between mercury concentrations and birth weight, birth length, chest circumference, and head circumference. We observed that the risk of SGA by weight decreased with increasing mercury concentration in regression analyses with adjustment for polyunsaturated fatty acids. Our results suggest that the beneficial effect of essential nutrition may mask the adverse effects of MeHg on birth size. The concentrations of PCBs had no association with birth size.

1. Introduction

Newborn anthropometric measurements (weight, length, and head and chest circumference) reflect fetal growth *in utero*, and are reported to predict infant survival, growth, morbidity, and neurobehavioral performance in early life (Kajantie et al., 2005; Barker, 2006). In Japan, public health concerns have been raised about a marked increase in the prevalence of babies with low birth weight, from 4.2% to 8.3% between 1980 and 2000 (Takimoto et al., 2005). Birth cohort studies reported

Abbreviations: BMI, body-mass index; B, partial regression coefficient; CI, confidence interval; FFQ, food-frequency questionnaire; Hg, mercury; MeHg, methylmercury; ND, not detectable; OR, odds ratio; SGA, small for gestational age; PCB, polychlorinated biphenyl; TEQ, toxic equivalent.

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discrepant findings about the association between maternal intake of fish/seafood during pregnancy and birth size: some found a significant positive association (Olsen et al., 1990, 1993; Olsen and Secher, 2002; Thorsdottir et al., 2004; Drouillet-Pinard et al., 2010; Brantsaeter et al., 2012; Leventakou et al., 2014), whereas others found a null or negative association (Rylander et al., 2000; Oken et al., 2004; Guldner et al., 2007; Halldorsson et al., 2007; Mendez et al., 2010; Heppe et al., 2011).

A plausible explanation is that fish/seafood is a nutrient source of polyunsaturated fatty acids for the mother and, at the same time, exposes the fetus to polychlorinated biphenyls (PCBs) (Grandjean et al., 2001; Halldorsson et al., 2008; Papadopoulou et al., 2013) and methylmercury (MeHg) (Drouillet-Pinard et al., 2010; van Wijngaarden et al., 2014; Vejrup et al., 2014). The adverse effects of *in utero* exposure to environmental contaminants and the positive effects of the nutrients from fish might have opposing influences on fetal growth (Grandjean et al., 2001; Halldorsson et al., 2008; Papadopoulou et al., 2013). PCBs are classified as persistent organic pollutants as they are lipophilic, stable, and show widespread contamination in the environment, food web, and human tissues (Sonneborn et al., 2008). Hg in fish muscle is mostly present in the form of MeHg, which is bioconcentrated up through the aquatic food web, eventually resulting in exposure through the human diet (van Wijngaarden et al., 2014). Fetal exposure to PCBs and MeHg *in utero* has the potential for serious health concerns because these pollutants can cross the placental and blood–brain barriers to reach the immature fetal organs and tissues, which are particularly susceptible to the effects of these toxins (Zahir et al., 2005; National Research Council, 2000; Wojtyniak et al., 2010; Casas et al., 2015).

The toxic mechanism of action of PCBs has not yet been fully elucidated; however, it is suspected that their estrogenic activity may play a role (Decastro et al., 2006). Experimental studies have demonstrated that PCBs display endocrine-disrupting effects in their ability to stimulate estrogen and can also function as xenoestrogens (Bonefeld-Jorgensen et al., 2001; Cooke et al., 2001). Estrogenic and antiestrogenic PCBs may have opposite associations with infant anthropometrics (Cooke et al., 2001). Other adverse effects induced by PCBs include dioxin-like activities such as activation of aryl hydrocarbon receptors (Van den Berg et al., 2006), and the potential toxic effects induced by dioxin-like PCB congeners may be stronger than those of non-dioxin-like (NDL) congeners (Giesy and Kannan, 1998). On the other hand, in our previous study, we found that fish/seafood consumption was associated with the concentration of NDL congeners (Miyashita et al., 2015). PCB 153 has been the most frequently used indicator of the effects on fetuses of exposure to PCBs in epidemiological studies. In previous studies, specific PCB congeners 153, 156, 118, 74, and 77 had potential estrogenic and antiestrogenic activities (Cooke et al., 2001; Decastro et al., 2006) and significant associations with birth size (Wojtyniak et al., 2010; Casas et al., 2015).

Epidemiological studies have previously reported inconsistent findings about the effect of prenatal exposure to PCBs at background levels on birth weight: some found significant inverse associations (Patandin et al., 1998; Rylander et al., 1998; Karmaus and Zhu, 2004; Sagiv et al., 2007; Halldorsson et al., 2008; Sonneborn et al., 2008; Tan et al., 2009; Brucker-Davis et al., 2010; Papadopoulou et al., 2013), whereas others found a null or positive association (Vartiainen et al., 1998; Grandjean et al., 2001; Gladen et al., 2003; Longnecker et al., 2005; Givens et al., 2007; Khanjani and Sim, 2007; Wolff et al., 2007; Murphy et al., 2010; Lopez-Espinosa et al., 2011; Kezios et al., 2012; Lignell et al., 2013; Hisada et al., 2014). In populations exposed to relatively high MeHg levels because of high consumption of contaminated seafood or accidental poisoning, epidemiologic studies have reported that prenatal MeHg exposure can lead to harmful effects on children's health such as impaired neurobehavioral development, congenital malformations, and restriction of fetal growth (National Research Council, 2000). However, limited epidemiological studies reported no conclusive evidence on the effects of low-level MeHg exposure on birth size (Drouillet-Pinard et al., 2010; Gundacker et al., 2010; Ramirez et al., 2000; Ramon et al., 2009; van Wijngaarden et al., 2014; Vejrup et al., 2014; Zahir et al., 2005).

Moreover, a balance of the opposite effects of contaminants and fish/seafood intakes across populations consuming different types of fish/seafood may have resulted in the discrepant finding among the previous birth cohort studies (Mahaffey, 2004; Halldorsson et al., 2008; Ramon et al., 2009). A meta-analysis study including 19 European cohorts described that the most pronounced effect on birth weight was observed for fatty fish, which is known to be a main source of long-chain polyunsaturated fatty acids (LCPUFAs) (Leventakou et al., 2014). Systematic reviews have suggested that maternal intake of omega-3 fatty acid supplements during pregnancy is associated with small but significant increases in infant birth size (Makrides et al., 2006; Szajewska et al., 2006; Salvig and Lamont, 2011). However, in some Asian countries, including Japan, where there is a high frequency of fish consumption (Miyashita et al., 2015), there is insufficient evidence about the effect of *in utero* exposure to PCBs and MeHg on birth size.

Thus, the aim of this study is to assess the effects of prenatal exposure to PCBs and MeHg on newborn anthropometric measurements, as well as the incidence of babies born small for gestational age (SGA), taking into account the biomarker of LCPUFAs among Japanese pregnant women.

2. Materials and methods

2.1. Study population

The subjects in this study were all currently enrolled in the Hokkaido Study on Environment and Children's Health. A total of 514 pregnant Japanese women were recruited at the Sapporo Toho Hospital in Hokkaido, Japan, from July 2002 to September 2005 (Kishi et al., 2013). An overview of this study is shown in Fig. 1. During their last trimester, the subjects completed a self-administered questionnaire on demographic characteristics, socioeconomic status, tobacco smoking and alcohol habits, and frequency of consumption during pregnancy of food items such as shoreline fish (*e.g.*, saury, Pacific herring, or mackerel), pelagic fish (*e.g.*, tuna, bonito, or salmon), beef, pork, chicken, milk, and eggs. The medical records for 504 mother–newborn pairs were used to gather information on delivery characteristics, including maternal height, maternal prepregnancy weight, pregnancy complications, gestational age, infant sex, parity, congenital anomalies, and newborn anthropometric measurements.

Within 5 days after delivery, the mothers completed a food frequency questionnaire (FFQ) to estimate their fish/seafood intake and history of synthetic hair waving ($n = 430$). The FFQ provided information about the frequency and portion size for maternal fish intake (Supplementary Table 1). The estimated daily fish intake (g/day) was calculated from the FFQ (Yasutake et al., 2003). We divided maternal fish intake to four subtypes: fatty fish, lean fish, shellfishes, and whole. The fatty fish group consisted of tuna, salmon, yellowtail, sardine, mackerel, saury, eel, Atka mackerel, shishamo smelt, pacific herring, and trout. The lean fish group included bonito, sea bream, flatfish, flounder, horse mackerel, carp, sweetfish, crucian carp, and Pacific cod. The shellfishes group included cuttlefish, octopus, crab, shrimp, shellfish, and fish products (Leventakou et al., 2014).

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

2.2. Exposure assessment

A 40-mL blood sample was taken from the maternal peripheral vein during the last trimester. In subjects with pregnancy-related anemia, the samples were taken during hospitalization immediately after delivery. Consequently, 356 samples were taken during pregnancy and 148

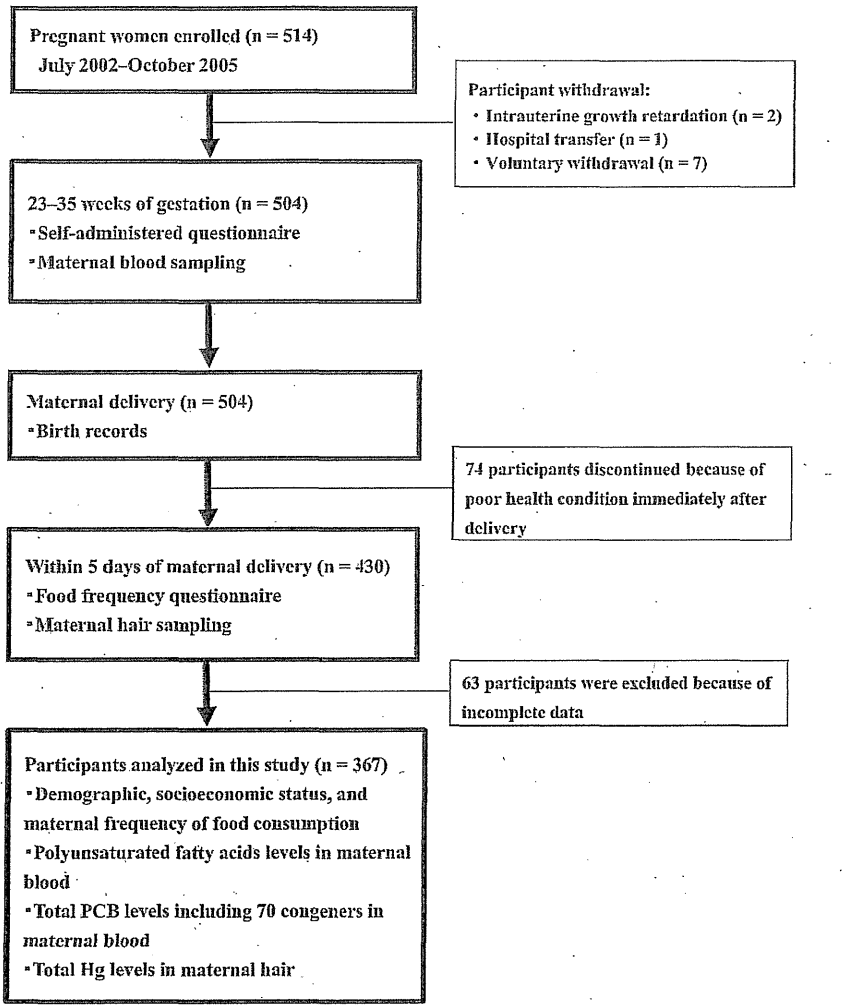

Fig. 1. Research overview.

samples were taken after delivery. All samples were stored at −80 °C until needed for analysis. The extraction, purification, and analysis of PCBs from whole blood specimens were performed by using a previously reported method (Iida and Todaka, 2003; Todaka et al., 2008a,2008b). The concentrations of PCBs were analyzed at the Fukuoka Institute of Health and Environmental Sciences by using high-resolution gas chromatography/high-resolution mass spectrometry of 5-g blood samples. To evaluate the accuracy and reliability of the PCB analysis, quality control studies were completed and compared against those done at three other laboratories. The average variation among the concentrations of PCBs in human blood samples was considered acceptable if it was within 10% (Kajiwara et al., 2008, 2009). The concentrations of 70 PCBs congeners were measured in 426 blood samples and adjusted for lipids (pg/g lipid). The sample values below the detection limit for the 70 PCBs congeners were assigned a value of one-half the detection limit. The remaining samples were not analyzed because of unavailable or insufficient sample volumes (<5 g) for measurement. PCB congeners were separated into four groups based on their suggested biological activities and the effect of exposure to them due to fish intake: estrogenic, antiestrogenic, dioxin-like, and NDL PCBs (Cooke et al., 2001). The estrogenic group included congeners 4, 10, 5, 8, 15, 17, 18, 31, 44, 47, 48, 52, 70, 99, 101, 136, 153, and 188. The antiestrogenic group included congeners 77, 110, 105, 114, 126, 156, 171, and 169. The dioxin-like PCBs included congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 (Van den Berg et al., 2006). NDL PCBs had 58 congeners excluding the 12 dioxin-like PCBs from all 70 congeners measured in our study (Supplementary Table 2) (Miyashita et al., 2015). Additionally, we used the specific PCB congeners 153 (main contributor), 156, 118, 74, and 77 as biomarkers of exposure to PCBs.

Maternal hair was collected within 5 days after delivery (n = 430). For the 1 cm of hair closest to the scalp, the concentrations of total Hg were determined by using the oxygen combustion-gold amalgamation method with the MD-1 atomic absorption detector (Nippon Instruments Co., Ltd., Osaka, Japan) at the National Institute for Minamata Disease (Yasutake et al., 2003). The total Hg concentration in hair was used as a convenient biomarker of MeHg exposure (van Wijngaarden et al., 2014) because >90% of the total Hg in hair is MeHg that is covalently bound to the cysteine residue of hair protein (National Research Council, 2000).

2.3. Maternal polyunsaturated fatty acid assessment

The fatty acid levels in maternal whole blood were determined by using gas chromatography–mass spectrometry (GC–MS) as described in detail in our previous study (Nakashima et al., 2013). Briefly, whole blood lipid was extracted from 25 μL blood (Folch et al., 1957), mixed with 1.2 mL methanol, 75 μL acetyl chloride, and 75 μL of 10 μg/100 μL tricosanoic acid ethyl ester/methanol (internal standard). After adding *n*-hexane (500 μL) and centrifugation of the sample, the upper organic layer was collected and transferred into another vial. The *n*-hexane

Table 1
Maternal and infant characteristics (n = 367).

Characteristics	n (%)
Maternal characteristics	
Age at delivery (years)	30.8 ± 4.8[a]
Height (cm)	158 ± 5.4[a]
Prepregnancy maternal weight (kg)	52.5 ± 8.0[a]
Parity	
0	180 (49.0)
1	146 (39.8)
2	35 (9.5)
3	6 (1.6)
Blood sampling period	
<28 weeks	21 (5.7)
28 to <36 weeks	148 (40.3)
≥36 weeks	78 (21.3)
After delivery	120 (32.7)
History of chemical hair waving	
No	260 (70.8)
Yes	107 (29.2)
Education level (years)	
≤9	7 (1.9)
10–12	147 (40.1)
13–16	208 (56.7)
≥17	5 (1.4)
Annual household income (million yen)	
<3	61 (16.6)
3 to <5	183 (49.9)
5 to <7	78 (21.3)
≥7	45 (12.3)
Tobacco smoking during pregnancy	
Nonsmoker	305 (83.1)
Smoker	62 (16.9)
Alcohol consumption during pregnancy	
No	255 (69.5)
Yes	112 (30.5)
Caffeine intake during pregnancy (mg/day)	120 (1.50, 646)[b]
Frequency of food consumption during pregnancy	
Shoreline fish	
<Once/week	198 (54.0)
≥Once/week	169 (46.0)
Pelagic fish	
<Once/week	171 (46.6)
≥Once/week	196 (53.4)
Beef	
<Once/week	274 (75.3)
≥Once/week	90 (24.7)
Pork	
<Once/week	274 (75.3)
≥Once/week	90 (24.7)
Chicken	
<Once/week	30 (8.2)
≥Once/week	337 (91.8)
Egg	
<Once/week	53 (14.4)
≥Once/week	314 (85.6)
Milk	
<Once/week	10 (2.7)
≥Once/week	356 (97.3)
Fish intake from food frequency questionnaires	
Fish intake (g/day)	38.8 (0.0, 400)[b]
Fatty fish	23.3 (0.0, 160)[b]
Lean fish	0.0 (0.0, 66.7)[b]
Shellfish	11.1 (0.0, 200)[b]
Whale	0.0 (0.0, 6.70)[b]
Infant characteristics	
Sex	
Male	173 (47.1)
Female	194 (52.9)
Type of delivery	
Vaginal birth	292 (79.3)
Cesarean section	76 (20.7)
Gestational age at birth (weeks)	39.0 ± 1.4[a]
Birth weight (g)	3073 ± 37[a]
Length (cm)	48.1 ± 1.9[a]
Chest circumference (cm)	31.5 ± 1.6[a]
Head circumference (cm)	33.3 ± 1.3[a]
SGA by weight	18 (4.9)
SGA by length	43 (11.7)

Table 2
Concentrations of LCPUFA (μg/mL) in maternal blood (n = 367).

	Minimum	Percentile 25th	50th	75th	Maximum
EPA + DHA	3.0	20.5	32.2	47.8	163
AA	2.8	43.5	61.2	89.7	219
Omega-3 fatty acids	4.1	28.2	43.4	63.9	188
Omega-6 fatty acids	16.1	581	798	1030	2840

LCPUFA: long-chain polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; Omega-3 fatty acids: EPA, DHA, α-linolenic acid (ALA); Omega-6 fatty acids: AA, linoleic acid (LA).

extraction was repeated once, and then the concentration of fatty acid methyl ester in the *n*-hexane layer was measured with GC–MS. Finally, nine fatty acid species were measured including the omega-6 fatty acids, palmitoleic and oleic acids, linoleic acid, and arachidonic acid (AA), and the omega-3 fatty acids, α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The detection rates for eight fatty acids were >99.0% and that for EPA was 97.8% (Kishi et al., 2015). We used EPA + DHA, AA, omega-3 fatty acids, and omega-6 fatty acids as biomarkers of maternal LCPUFAs (van Wijngaarden et al., 2014; Vejrup et al., 2014).

2.4. Statistical analyses

Some subjects were excluded from analyses because of pregnancy-induced hypertension (n = 11), diabetes mellitus (n = 1), fetal heart failure (n = 1), and multiple births (n = 7). The final study population comprised 367 mother–newborn pairs with completed questionnaire data and birth records, whose PCB and hair Hg concentrations were measured (Fig. 1). SGA by weight was defined as a birth weight less than the 10th percentile for the gestational age at delivery, based on growth charts specific for newborn sex and maternal parity for birth size standards by gestational age for Japanese neonates. SGA by length was defined as birth length less than the 10th percentile for the gestational age at delivery, based on growth charts for birth size standards by gestational age for Japanese neonates (Itabashi et al., 2014). Associations between subject characteristics and concentrations of PCBs and hair Hg were evaluated by using the Mann–Whitney *U*-test and Spearman's rank correlation coefficient. Associations between subject characteristics and birth size were evaluated by using Student's *t*-test, Pearson correlation, Spearman's rank correlation coefficient, and one-way analysis of variance. For linear regression analyses, we used $\log_{10}$-transformed values for concentrations of PCBs and hair Hg, as well as LCPUFAs, because these variables displayed a skewed distribution. Associations between PCBs or hair Hg (expressed as continuous concentrations) and newborn anthropometric measurements were evaluated by using linear regression analyses. For logistic regression analyses, we used concentrations of PCBs and hair Hg, divided into quartiles, to evaluate potential nonlinear relationships. The associations between PCBs or hair Hg and the incidence of babies born SGA by weight and length were evaluated by using logistic regression analyses. All regression analyses were conducted with or without adjustment of factors—chosen for their significant associations with exposure and birth size in this study ($p < 0.05$)—and possible confounding factors as reported in previous studies (Drouillet-Pinard et al., 2010; Halldorsson et al., 2008; Ramon et al., 2009; Papadopoulou et al., 2013; van Wijngaarden et al., 2014; Vejrup et al., 2014). Specifically, the adjusted factors included maternal age (continuous), height (continuous), prepregnancy weight (continuous), smoking during pregnancy (yes/no), alcohol consumption during

Notes to Table 1
SGA: small for gestational age.
[a] Mean ± SD.
[b] Median (minimum, maximum).

pregnancy (yes/no), household income (less than or greater than 5 million Yen annually), blood sampling period (during pregnancy or after delivery), birth order (first-born or later children) reported as maternal parity, infant sex, gestational age, maternal LCPUFAs, and total 70 PCBs or hair Hg. The logistic regression analysis for SGA by weight was not adjusted for birth order, infant sex, and gestational age, because SGA by weight was defined based on growth charts for birth size standards by gestational age specific for newborn sex and maternal parity. Furthermore, the logistic regression analysis for SGA by length was not adjusted for gestational age, because SGA by length was defined based on growth charts for birth size standards by gestational age.

A p-value of <0.05 was considered statistically significant. Statistical analyses were performed by using the Statistics Package for Social Sciences (version 19.0 J; IBM, Armonk, NY, USA) software for Windows.

3. Results

The subjects' characteristics are described in Table 1. The percentage of babies born SGA by weight was 4.9% and that of babies SGA by length was 11.7%. Table 2 shows the distribution of maternal biomarkers of fatty acid. The median concentration of the total 70 PCBs in the maternal blood was 108 ng/g lipid (Supplementary Table 2). The distributions of PCB concentrations are shown in Table 3. The geometric mean concentrations of estrogenic, antiestrogenic, dioxin-like, and NDL PCBs were 27.9, 3.98, 10.9, and 93.8 ng/g lipid, respectively, and that of hair Hg was 1.34 μg/g. The concentrations of total PCBs significantly increased with maternal age and intake of fish, EPA + DHA, and omega-3 fatty acids during pregnancy. The concentrations of hair Hg significantly increased with fish intake during pregnancy (Table 4). The concentrations of the total 70 PCBs and hair Hg in subjects with no history of parity; high household income; frequent consumption of pelagic fish, beef, or milk (≥once/week); or for non-SGA babies by weight were significantly higher than those in subjects with a history of parity; low income; infrequent consumption of pelagic fish, beef, or milk; or SGA babies by weight, respectively (Table 4). The newborn anthropometric measurements significantly increased with maternal height, prepregnancy weight, male sex, birth by vaginal delivery, and increasing gestational age (Supplementary Table 3). Incidences of SGA babies by weight and length significantly reduced with increased maternal prepregnancy weight and male sex (Supplementary Table 4).

We found no associations between the concentrations of estrogenic PCBs, antiestrogenic PCBs, dioxin-like PCBs, NDL PCBs, or hair Hg and newborn anthropometric measurements of birth weight, length, chest circumference, and head circumference in the multiple linear regression models with or without adjustment for factors (Supplementary Table 5). As shown in Table 5, we found no significant associations of SGA by weight with any quartile of estrogenic, antiestrogenic, dioxin-like, or NDL PCB levels, for all models. We also found no significant associations between the incidence of SGA by length and levels of estrogenic PCBs, antiestrogenic PCBs, dioxin-like PCBs, NDL PCBs, and hair Hg in all models. The adjusted odds ratios (ORs) for SGA by weight among the third (OR: 0.12, 95% confidence interval [95% CI]: 0.02–0.68), and fourth quartiles (OR: 0.17, 95% CI: 0.04–0.79) for hair Hg significantly reduced as compared with those in the first quartile (reference) with a significant trend (Table 5). The overall results analyzed by using regression analyses remained statistically significant after adjusting for omega-3 fatty acids (Table 5, Supplementary Table 5), and EPA + DHA, AA, omega-6 fatty acids, fish intake, fatty fish intake, and frequent consumption of pelagic fish, beef, and milk (data not shown). Additionally, we found no interaction effect of PCBs or Hg and omega-3 fatty acids on SGA risk (Table 5), as well as EPA + DHA, AA, and omega-6 fatty acids on birth weight, birth length, chest circumference, head circumference, and SGA risk (data not shown).

PCB 153, 156, 118, and 74 were detected in all subjects, and PCB 77 was detected in 64% of the subjects. The median concentrations of PCB 153, 156, 118, 74, and 77 were 21.4, 1.95, 5.78, 3.12, and 0.011 ng/g lipid, respectively. The contribution rates of PCB 153, 156, 118, 74, and 77 according to total PCBs were 20.3%, 1.8%, 5.4%, 3.0%, and 0.01%, respectively. PCB 153 was the main contributor to PCB exposure in this study (Supplementary Table 2). In congener-specific analyses, after sample values below the detection limit were assigned a value of one-half the detection limit, associations between PCB 153, 156, 118, 74, or 77 and birth size were evaluated by regression analyses with adjustment for confounding factors. There were no associations between concentrations of specific PCB congeners and newborn anthropometric measurements or the incidence of babies born SGA in any of the regression analyses (data not shown).

Table 3
Concentrations of polychlorinated biphenyls in maternal blood (PCBs; ng/g lipid) and hair mercury (μg/g) in maternal samples (n = 367).

	Percentile				
	Minimum	25th	50th	75th	Maximum
Estrogenic PCBs[a]	3.88	19.5	28.7	40.0	147
Antiestrogenic PCBs[b]	0.63	2.75	4.13	5.60	21.7
Dioxin-like PCBs[c]	1.74	7.51	11.2	15.6	49.8
Non-dioxin-like PCBs	16.0	64.8	95.7	133	445
Hair Hg	0.24	0.96	1.41	1.89	4.73

[a] PCB 52, 49, 47, 44, 70, 95, 101, 99, 110, and 153 (Cooke et al., 2001).
[b] PCB 37, 77, 81, 126, 169, 114, 105, and 156 (Cooke et al., 2001).
[c] PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 (Van den Berg et al., 2006).

4. Discussion

4.1. Prenatal exposure to PCBs and birth size

We found that prenatal exposure to PCBs, including antiestrogenic PCBs as well as specific PCB congeners, has no association with newborn anthropometric measurements at birth, or the incidence of babies born SGA after adjusting for confounding factors, including hair Hg, demographic characteristics, socioeconomic status, and maternal level of LCPUFAs. Similar results were obtained when examining only subjects with a normal birth weight and gestation period.

Median concentrations of PCB 153 have been reported with a wide range, from 10.7 ng/g lipid weight in a Poland cohort to 450 ng/g lipid in the maternal serum of a Faroe Island cohort (Grandjean et al., 2001; Hertz-Picciotto et al., 2005; Sonneborn et al., 2008; Wojtyniak et al., 2010). Concerning the exposure levels among the general population in Japan, the maternal PCB 153 level of 21.0 ng/g lipid in this study seemed to be comparable to that of 15.9 ng/g lipid (Nakamura et al., 2008) and 16.0 ng/g lipid (Hisada et al., 2014) measured in pregnant women in previous studies. Hisada et al. (2014) described that no association was observed between prenatal exposure to PCBs and birth size, and the levels of PCB exposure among the general population in this study was considerably lower than that among European (Wojtyniak et al., 2010) and American populations (Hertz-Picciotto et al., 2005), in which a significant negative association with prenatal exposure to PCBs and birth size was found. Therefore, one of the reasons for the inconsistent results may be the difference in PCB exposure level. Murphy et al. (2010) reported no association between prenatal exposure to antiestrogenic PCBs and birth weight of newborns of fish anglers, which is consistent with our findings. The estrogenic/antiestrogenic activities of PCBs have been demonstrated in *in vitro* and *in vivo* models; however, their affinity for estrogens and xenoestrogens are two to five times lower than that of natural hormones (Decastro et al., 2006). This suggests that the concentrations of estrogenic/antiestrogenic PCBs in our study may not be at levels too low to see any adverse effects on birth size but rather indicate a true biological effect.

A European meta-analysis with a pooled dataset including populations with a low PCB exposure described that birth weight reduced because of PCB 153 in cord serum (El Majidi et al., 2012; Casas et al., 2015).

Table 4
Total polychlorinated biphenyls (PCBs) and hair mercury (Hg) levels in relation to maternal and infant characteristics and polyunsaturated fatty acids (n = 367).

Characteristics		Total PCBs (ng/g lipid)		Hair Hg (μg/g)	
		r	Median (min, max)	r	Median (min, max)
Maternal characteristics					
Age at delivery (years)		0.415[a**]		0.094[a]	
Height (cm)		0.079[a]		−0.055[a]	
Prepregnancy weight (kg)		0.016[a]		−0.029[a]	
Parity	0		115 (19.6, 495)[*]		1.41 (0.30, 3.73)[*]
	≥1		102 (17.8, 354)		1.38 (0.24, 4.73)
Blood sampling period	During pregnancy		110 (17.8, 363)		1.41 (0.24, 4.73)
	After delivery		104 (27.4, 495)		1.40 (0.30, 4.30)
History of chemical hair waving	No		108 (17.8, 495)		1.37 (0.24, 4.35)
	Yes		109 (19.6, 362)		1.46 (0.30, 4.73)
Education level (years)	≤12		99.0 (17.8, 363)		1.33 (0.24, 4.35)
	>12		111 (19.6, 495)		1.42 (0.30, 4.73)
Annual household income (million yen)	<5		102 (17.8, 362)[*]		1.28 (0.24, 4.73)[*]
	≥5		123 (27.4, 495)		1.47 (0.30, 4.33)
Tobacco smoking during pregnancy	Nonsmoker		110 (17.8, 362)		1.41 (0.30, 4.35)
	Smoker		95.4 (19.6, 495)		1.39 (0.24, 4.73)
Alcohol consumption during pregnancy	No		100 (17.8, 354)		1.33 (0.31, 4.03)
	Yes		113 (27.8, 495)		1.42 (0.24, 4.73)
Caffeine intake (mg/day)		0.017[a]		−0.005[a]	
Frequency of food consumption during pregnancy					
Shoreline fish	<Once/week		101 (17.8, 362)		1.31 (0.31, 4.35)
	≥Once/week		113 (19.6, 495)		1.46 (0.24, 4.73)
Pelagic fish	<Once/week		106 (17.8, 362)		1.24 (0.24, 4.03)[**]
	≥Once/week		109 (27.4, 495)		1.49 (0.32, 4.73)
Beef	<Once/week		108 (17.8, 363)		1.34 (0.24, 4.73)[*]
	≥Once/week		107 (19.6, 495)		1.51 (0.30, 3.69)
Pork	<Once/week		85.9 (19.6, 302)		1.54 (0.66, 4.03)
	≥Once/week		109 (17.8, 495)		1.39 (0.24, 4.73)
Chicken	<Once/week		108 (31.3, 362)		1.30 (0.37, 4.03)
	≥Once/week		108 (17.8, 495)		1.41 (0.24, 4.73)
Egg	<Once/week		102 (59.0, 213)		1.28 (1.19, 1.49)
	≥Once/week		108 (17.8, 495)		1.41 (0.24, 4.73)
Milk	<Once/week		74.9 (30.2, 354)[**]		1.24 (0.45, 3.09)
	≥Once/week		111 (17.8, 495)		1.42 (0.24, 4.73)
Food frequency questionnaires at delivery					
Fish intake (g/day)		0.187[a**]		0.215[a**]	
Fatty fish (g/day)		0.141[a**]		0.210[a**]	
Shellfish (g/day)		0.087[a]		0.084[a]	
LCPUFA in maternal blood					
EPA + DHA		0.182[a**]		0.056[a]	
AA		0.048[a]		−0.077[a]	
Omega-3 fatty acids		0.155[a**]		0.022[a]	
Omega-6 fatty acids		0.073[a]		−0.018[a]	
Infant characteristics					
Sex	Male		111 (27.4, 362)		1.41 (0.24, 4.35)
	Female		104 (17.8, 495)		1.39 (0.30, 4.73)
Type of delivery	Vaginal birth		109 (17.8, 363)		1.43 (0.24, 4.73)
	Cesarean section		97.0 (19.6, 495)		1.24 (0.30, 4.35)
Gestational age (weeks)		0.025[a]		0.017[a]	
SGA by weight	No		108 (17.8, 495)		1.42 (0.30, 4.73)[*]
	Yes		98.7 (51.0, 223)		0.92 (0.24, 2.62)
SGA by length	No		109 (17.8, 495)		1.41 (0.24, 4.73)
	Yes		97 (19.6, 247)		1.24 (0.46, 3.55)

LCPUFA: long-chain polyunsaturated fatty acids, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid.
[a] r: Spearman's rank correlation coefficient.
[*] $p < 0.05$ by Mann–Whitney *U*-test and Spearman's rank correlation test.
[**] $p < 0.01$ by Mann–Whitney *U*-test and Spearman's rank correlation test.

However, a systematic analysis of 20 epidemiological studies described that the observed discrepancies in the concentration–response relation between prenatal PCB exposure and birth weight could not be attributed conclusively to a difference in biological PCB levels (El Majidi et al., 2012). In fact, in Inuit children exposed to high concentrations of PCBs, a lack of association between PCB 153 in cord blood and birth size was observed (Dallaire et al., 2014). As one of the possible explanations, the beneficial nutrients from fish/seafood intake may have an opposite action to the toxic effects of PCBs (Mahaffey, 2004; Halldorsson et al., 2008; Ramon et al., 2009). In the Danish National Birth Cohort of subjects with 70 ng/g lipid of the median PCB 153 and 5 g/day of median fatty fish intake from the FFQ, inverse associations were observed between maternal PCB levels and birth weight (Halldorsson et al., 2008). In a Faroe Island cohort of subjects, higher concentrations of PCB 153 and PUFAs than that in our study were found, and a negative effect of maternal EPA and no effect of PCB exposure on birth weight were observed (Grandjean et al., 2001). We found no association between maternal levels of LCPUFAs and birth weight, birth length, chest circumference, and head circumference or SGA risk in this study. However, our previous study on the same cohort suggested that maternal EPA might affect infant chest circumference (Jia et al., 2014). It is difficult to compare our results with those of other studies because of substantial differences in the exposure levels, profiles of fish/seafood intake, and contribution rate of fish/seafood to the overall PCB exposure

Table 5
Odds ratios for babies born small for gestational age (n = 367).

		SGA by weight			SGA by length		
		Crude	Adjusted 1[a]	Adjusted 2[a]	Crude	Adjusted 1[b]	Adjusted 2[b]
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)
Estrogenic PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.41 (0.10–1.65)	0.51 (0.11–2.30)	0.56 (0.12–2.58)	1.21 (0.53–2.79)	1.48 (0.60–3.67)	1.57 (0.62–4.00)
	Quartile 3	0.27 (0.05–1.34)	0.40 (0.07–2.24)	0.42 (0.07–2.41)	0.38 (0.13–1.14)	0.36 (0.11–1.17)	0.37 (0.11–1.22)
	Quartile 4	0.85 (0.27–2.62)	1.95 (0.46–8.18)	1.88 (0.45–7.83)	1.00 (0.42–2.36)	0.81 (0.28–2.29)	0.68 (0.23–2.03)
	p for trend	0.662	0.694	0.696	0.509	0.334	0.197
	P for interaction			0.335			0.211
Antiestrogenic PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.99 (0.28–3.54)	1.16 (0.29–4.68)	1.31 (0.32–5.34)	1.31 (0.56–3.05)	1.44 (0.58–3.57)	1.53 (0.61–3.84)
	Quartile 3	0.57 (0.13–2.47)	0.99 (0.20–4.87)	1.07 (0.21–5.47)	0.50 (0.18–1.42)	0.52 (0.17–1.63)	0.50 (0.16–1.57)
	Quartile 4	1.00 (0.28–3.58)	1.95 (0.44–8.55)	1.89 (0.43–8.29)	1.10 (0.46–2.65)	1.07 (0.38–2.98)	0.94 (0.32–2.73)
	p for trend	0.824	0.523	0.511	0.71	0.718	0.550
	P for interaction			0.317			0.249
Dioxin-like PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	1.23 (0.36–4.18)	1.54 (0.39–6.05)	1.93 (0.48–7.77)	1.34 (0.57–3.13)	1.62 (0.64–4.09)	1.79 (0.70–4.56)
	Quartile 3	0.38 (0.07–2.02)	0.66 (0.11–4.06)	0.64 (0.10–4.01)	0.60 (0.22–1.62)	0.68 (0.22–2.07)	0.62 (0.20–1.94)
	Quartile 4	1.01 (0.28–3.62)	2.20 (0.48–10.1)	2.01 (0.44–9.19)	1.01 (0.42–2.47)	1.01 (0.35–2.90)	0.83 (0.28–2.48)
	p for trend	0.669	0.570	0.560	0.617	0.714	0.260
	P for interaction			0.155			0.096
Non-dioxin like PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.48 (0.12–1.97)	0.56 (0.12–2.59)	0.57 (0.12–2.65)	1.71 (0.73–3.99)	1.99 (0.79–5.01)	2.02 (0.79–5.17)
	Quartile 3	0.81 (0.24–2.77)	1.36 (0.32–5.69)	1.47 (0.34–6.42)	0.47 (0.15–1.42)	0.49 (0.15–1.64)	0.49 (0.14–1.66)
	Quartile 4	0.64 (0.18–2.36)	1.21 (0.24–6.22)	1.18 (0.23–5.96)	1.21 (0.50–2.97)	1.00 (0.33–3.05)	0.88 (0.28–2.76)
	p for trend	0.654	0.759	0.752	0.697	0.483	0.345
	P for interaction			0.417			0.461
Hair Hg	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.28 (0.07–1.04)	0.24 (0.06–1.00)	0.22 (0.05–0.94)*	0.68 (0.29–1.62)	0.69 (0.27–1.76)	0.71 (0.27–1.84)
	Quartile 3	0.18 (0.04–0.86)*	0.12 (0.02–0.68)*	0.11 (0.02–0.64)*	0.60 (0.25–1.47)	0.58 (0.22–1.54)	0.57 (0.21–1.55)
	Quartile 4	0.28 (0.07–1.05)	0.17 (0.04–0.79)*	0.16 (0.03–0.77)*	0.69 (0.29–1.64)	0.65 (0.24–1.76)	0.61 (0.22–1.73)
	p for trend	0.023	0.014	0.014	0.359	0.362	0.324
	P for interaction			0.965			0.562

The odds ratios (OR) and 95% confidence intervals (95% CI) for babies born small for gestational age (SGA) were calculated by using the first quartile as the reference category.
p for trend: linear trend across quartiles.
Adjusted 1[a]: adjusted for maternal age, maternal height, prepregnancy maternal weight, tobacco smoking during pregnancy, alcohol consumption during pregnancy, household income, blood sampling period, and total PCBs or hair Hg.
Adjusted 2[a]: adjusted for omega-3 fatty acids in addition to the adjusted factors in Adjusted 1[a]
Adjusted 1[b]: adjusted for maternal age, maternal height, prepregnancy maternal weight, tobacco smoking during pregnancy, alcohol consumption during pregnancy, household income, blood sampling period, parity, infant sex, and total PCBs or hair Hg.
Adjusted 2[b]: adjusted for omega-3 fatty acids in addition to the adjusted factors in Adjusted 1[b]
P for interaction: introduced for interaction terms of quartile PCBs or quartile Hg, and quartile omega-3 fatty acids, in addition to the adjusted factors in Adjusted 2[a] or Adjusted 2[b].
* $p < 0.05$.

level. However, we have provided additional data to support the finding that low exposure to PCBs is likely insufficient to cause a negative effect on fetal growth taking into account maternal LCPUFAs.

4.2. Prenatal exposure to MeHg and birth size

Our findings suggest that prenatal exposure to MeHg has no association with newborn anthropometric measurements, although the incidence of babies born SGA by weight may reduce with higher concentrations of Hg in hair. The maternal hair Hg level of 1.41 μg/g at delivery in our population was comparable to that of 1.96 μg/g (Suzuki et al., 2010) and 1.62 μg/g in pregnant women (Sakamoto et al., 2012), and that of 1.43 μg/g in nonpregnant women from the general population in Japan (Yasutake et al., 2003), in which the effect on birth size was not evaluated.

Our finding is consistent with the results of several epidemiological studies that also showed a lack of significant association between parental exposure to MeHg and birth weight (Drouillet-Pinard et al., 2010; Gundacker et al., 2010; Ramirez et al., 2000; Ramon et al., 2009; van Wijngaarden et al., 2014). However, two different studies described adverse effects from prenatal exposure to MeHg in relation to birth size taking into account maternal fish intake (Ramon et al., 2009; Vejrup et al., 2014). In a study in Spain in which the subjects had a mean total Hg of 9.4 μg/L in cord blood and a mean fish intake of 36 g/day, the concentrations of total Hg increased with reduced birth weight and increased the risk of being born SGA for length but not SGA for weight (Ramon et al., 2009). One possible explanation for the inconsistent findings is that the subjects of our study more frequently consumed fatty fish than the subjects of the Spanish study. Fatty fish is known to be the main source of PUFAs (Leventakou et al., 2014). In study in the Republic of Seychelles on subjects with a mean hair MeHg of 5.9 μg/g and a median omega-3 fatty acid level of 30 μg/mL, no association was observed between MeHg or PUFAs and birth weight (van Wijngaarden et al., 2014). In a Norwegian study of subjects with 1.45 μg/day median estimated dietary Hg and 6 g/day fatty fish intake, a positive effect of maternal fish/seafood intake and a negative effect of Hg exposure on birth weight were observed (Vejrup et al., 2014). Our study subjects had 23.3 g/day median fatty fish intake and 43 μg/mL median omega-3 fatty acids, which were higher than that found in the Seychelles and Norwegian studies. The beneficial effect of essential nutrition in our study may mask the adverse effects of MeHg on birth size, as observed in the Norwegian study.

On the other hand, our finding that the risk of SGA by weight reduced at higher concentrations of Hg in hair remained significant after adjustment for the concentrations of LCPUFAs. A plausible physiological mechanism underlying our findings should be investigated. To our knowledge, no previous studies have reported a reasonable assumption about the direct protective role of low MeHg exposure *in utero* on fetal growth. As another possible explanation, the association between higher Hg in hair and reduced risk of SGA by weight may be confounded

by an unobserved common factor. In fact, biochemical observations showed that selenium, one of the essential micronutrients for fetal growth, plays a protective role against Hg toxicity (Zahir et al., 2005; Chen et al., 2006). Because our findings of the impact of prenatal MeHg exposure on fetal growth even at low levels are not conclusive, we consider continuous risk assessment as important among our population in which the fourth quartile included subjects (n = 59) with hair Hg concentrations >2.2 μg/g, which corresponds to the provisionally tolerable MeHg intake level as set by the Food and Agriculture Organization and the World Health Organization in 2006 (1.6 μg/kg body weight/week) (FAO/WHO, 2006).

4.3. Strengths and limitations

The strengths of this study are as follows: (1) the assessment of biomarkers of LCPUFAs; (2) the detection of 70 congeners of PCBs that were reported as the most predominant congeners in the Japanese population (Todaka et al., 2008ab); (3) a high PCB detection rate of 98.8%, and the ability to group and analyze them based on bioactivities such as estrogen/antiestrogen, and dioxin-like effects; (4) various demographic, socioeconomic, behavioral, and dietary data were collected prospectively, minimizing recall error; and (4) evaluation with multiple linear models adjusted for confounding effects between demographic characteristics, socioeconomic status, maternal diet, and PCB or Hg contamination in fish/seafood. We propose that additional studies be conducted to assess whether exposure to PCBs and MeHg in the general population is at levels insufficient to cause impaired fetal growth in humans. The mothers included in this study were older at delivery, had heavier weight at prepregnancy, had lower smoking rate during pregnancy, and had a later sampling period than mothers who were not included in analysis. However, we considered that the potential selection bias was limited because we found no difference in PCB and Hg exposure levels between the mothers included and those not included in this study. The children included in this study had a higher gestational age, weight, length, chest circumference, and head circumference, and lower SGA for length at birth than those children who were not included in the analysis. A potential selection bias may have resulted from the effect on healthy children, in whom the influence of contaminants on birth size may have been underestimated. We cannot exclude the possibility that our findings occur by chance because of the small number of babies born SGA. A further study with a larger sample size is needed to evaluate the effects of prenatal exposure to PCBs and MeHg on the later growth of children.

5. Conclusion

No overall association was found between mercury concentrations and birth weight, length, chest circumference, and head circumference. We observed that the risk of SGA by weight reduced with increasing mercury concentration in hair in regression analyses with adjustment for polyunsaturated fatty acids. In Japanese pregnant women, who are known to have a high frequency of fish consumption, the beneficial effect of essential nutrition may mask the adverse effects of MeHg on birth size, as was observed in a previous European study. On the other hand, we cannot exclude the possibility that prenatal MeHg exposure may adversely influence fetal growth even at low levels; therefore, a follow-up study is needed to evaluate the effect of prenatal MeHg exposure on the later growth of children. The concentrations of estrogenic, antiestrogenic, dioxin-like, and NDL PCBs had no association with birth weight, length, chest circumference, head circumference, and SGA risk.

Conflicts of interest and source of funding

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Appendix A. Supplementary data

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The Association of Prenatal Exposure to Perfluorinated Chemicals with Maternal Essential and Long-Chain Polyunsaturated Fatty Acids during Pregnancy and the Birth Weight of Their Offspring: The Hokkaido Study

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Background: Fatty acids (FAs) are essential for fetal growth. Exposure to perfluorinated chemicals (PFCs) may disrupt FA homeostasis, but there are no epidemiological data regarding associations of PFCs and FA concentrations.

Objectives: We estimated associations between perfluorooctane sulfonate (PFOS)/perfluorooctanoate (PFOA) concentrations and maternal levels of FAs and triglyceride (TG) and birth size of the offspring.

Methods: We analyzed 306 mother–child pairs in this birth cohort between 2002 and 2005 in Japan. The prenatal PFOS and PFOA levels were measured in maternal serum samples by liquid chromatography–tandem mass spectrometry. Maternal blood levels of nine FAs and TG were measured by gas chromatography–mass spectrometry and TG E-Test Wako kits, respectively. Information on infants' birth size was obtained from participant medical records.

Results: The median PFOS and PFOA levels were 5.6 and 1.4 ng/mL, respectively. In the fully adjusted model, including maternal age, parity, annual household income, blood sampling period, alcohol consumption, and smoking during pregnancy, PFOS but not PFOA had a negative association with the levels of palmitic, palmitoleic, oleic, linoleic, α-linolenic, and arachidonic acids ($p < 0.005$) and TG (p-value = 0.016). Female infants weighed 186.6 g less with mothers whose PFOS levels were in the fourth quartile compared with the first quartile (95% CI: −363.4, −9.8). We observed no significant association between maternal levels of PFOS and birth weight of male infants.

Conclusions: Our data suggest an inverse association between PFOS exposure and polyunsaturated FA levels in pregnant women. We also found a negative association between maternal PFOS levels and female birth weight.

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Introduction

Perfluorinated chemicals (PFCs) are ubiquitous and stable chemicals widely detected in humans and environment. Contamination of drinking water, house dust, foods, and fish products are the possible major exposure pathways of humans to PFCs (Lau et al. 2007). The most widely studied and detected PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). In 2009, PFOS was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs) (United Nations Environment Programme 2007). PFOS and PFOA are being voluntarily phased out by several industries, and are being substituted by longer carbon-chain PFCs. Recently, we reported that plasma levels of PFOS and PFOA were generally decreasing in plasma of pregnant Japanese women; however, we observed an increased trend for PFCs with longer carbon chains (Okada et al. 2013). Additionally, PFOS and PFOA are still present in older products, and they are slowly eliminated from the human body, with mean half-lives of 5.4 and 3.8 years for PFOS and PFOA, respectively (Olsen et al. 2007).

Both PFOS and PFOA have been shown to have developmental and reproductive toxicity in animal studies, including early pregnancy loss, reduced fetal weight, and postnatal mortality (Abbott et al. 2007; Luebker et al. 2005). A strong correlation of these compounds has been demonstrated between maternal and cord blood samples in humans, indicating that neonates are exposed to PFCs via the placental passage (Inoue et al. 2004; Monroy et al. 2008). Some epidemiological studies have also reported an association between PFC exposure and poor birth outcomes including decreased birth size (Apelberg et al. 2007; Fei et al. 2007; Washino et al. 2009). In a prospective study, prenatal PFOA exposure was positively associated with the prevalence of overweight female offspring at 20 years of age (Halldorsson et al. 2012). However, Barry et al. (2014) reported no association between early-life PFOA exposure and overweight and obesity risk in adults 20–40 years of age.

Recent research has shown that PFCs perturb metabolic end points, including lipid metabolism, glucose homeostasis, and thyroid hormone balance, in animals (Seacat et al. 2003; Thibodeaux et al. 2003). Such effects might explain associations between PFCs and birth outcomes. Most epidemiological studies regarding the association between PFCs and lipids [triglyceride (TG) and cholesterol] have been conducted in nonpregnant participants. Although previous reports suggest a positive association between PFCs and cholesterol levels (Frisbee et al. 2010; Winquist and Steenland 2014), the reports regarding the association of PFCs and TG levels are inconsistent. In a targeted group of Inuit adults 18–74 years of age, Château-Degat et al. (2010) reported a significant negative association between high PFOS exposure levels and TG only in women. However, some groups reported no association between exposure to PFCs and TG levels (Fisher et al. 2013; Sakr et al. 2007) or even a positive association between exposure to PFCs and TG levels in nonpregnant women (Steenland et al. 2009). Therefore, the relevance of these findings is uncertain.

Fetal growth is dependent on maternal metabolic resources, and this is exemplified by the correlation between maternal and fetal

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TG and fatty acids (FAs) levels (Kitajima et al. 2001; Schaefer-Graf et al. 2011). Major physiologic changes in lipid metabolism take place during normal pregnancies. There is an increase in body fat depots during early pregnancy, whereas lipolysis of fat depots occurs in late pregnancy, resulting in hyperlipidemia. Maternal hyperlipidemia during late pregnancy facilitates the availability of lipid substrates to the fetus. Fetuses need essential fatty acids (EFAs) and long-chain polyunsaturated fatty acids (LCPUFAs) for growth and development, especially for nervous system development (Alvarez et al. 1996). EFAs include linoleic acid and α-linolenic acid, which are precursors of omega 6 and omega 3 LCPUFAs, respectively. These substances must be obtained from the maternal diet, and the maternal blood concentrations of EFAs determine the corresponding concentrations in the cord blood (Herrera and Ortega-Senovilla 2010).

PFCs resemble FAs structurally and they may disrupt the homeostasis of FAs (Hu et al. 2005). Physiologic hyperlipidemia in late pregnancy is essential for fetal growth. However, to our knowledge, the influence of PFCs on FA homeostasis in pregnant women has not been investigated. In the present analyses, we explored the relationship between PFOS/PFOA exposure and the levels of four families of FAs (saturated, monounsaturated, omega 3, and omega 6 polyunsaturated FAs) including nine FAs and TGs in the maternal blood samples and birth size of their offspring from a birth cohort study.

Methods

Study population. This study was a part of the Hokkaido Study on Environment and Children's Health conducted between July 2002 and October 2005, and the details have been previously described (Kishi et al. 2011, 2013). Of 1,796 potentially eligible women, the following subjects were excluded: women who decided to participate in the Japanese cord blood bank (22% of those approached), and women who decided to deliver at another hospital (3% of those approached). Of the remaining eligible subjects, 514 women (28.6% of those approached) agreed to participate in this study (Konishi et al. 2009). These pregnant women at 23–35 weeks of gestation registered during a routine gynecologic checkup and delivered at the Toho Hospital in Sapporo, Hokkaido, Japan. Ten registered women were excluded due to miscarriage and stillbirth (n = 2), relocation (n = 1), or voluntary withdrawal (n = 7) from the study before follow-up.

Questionnaires and medical records. A self-administered questionnaire survey was completed after the second trimester (Washino et al. 2009) containing information related to smoking, household income and educational levels, and alcohol and caffeine intake during pregnancy. Medical information including maternal age, maternal body mass index (BMI) before pregnancy, parity, gestational age, pregnancy complications, type of delivery, infant sex, and birth size (weight, length, chest, and head circumferences) were obtained from participant medical records. This study was conducted with the written informed consent of all participants, and the study protocol was approved by the institutional ethical board for epidemiological studies at the Graduate School of Medicine and Center for Environmental and Health Sciences, Hokkaido University.

Blood sampling and exposure assessments. A 40-mL blood sample was taken from a peripheral vein after the second trimester of pregnancy and was used to measure maternal serum levels of PFOS, PFOA, TG, and FAs. All samples were stored at −80°C until analysis. Detailed methods for the measurement of PFOS and PFOA have been described in our previous report (Nakata et al. 2009). In brief, serum samples (0.1 mL) were mixed with 0.2 mL internal standard ($^{13}C_4$-PFOS-Na^+ and $^{13}C_2$-PFOA) solution containing acetonitrile, centrifuged at 1,450 × g for 10 min, and the supernatant was transferred to a polypropylene tube. An aliquot of the filtered sample solution was subjected to column-switching liquid chromatography–tandem mass spectrometry. The PFOS values of all samples were detected, and for samples with PFOA levels below the detection limit (0.50 ng/mL) (n = 17, 5.5% of participants), we used a value of half the detection limit (0.25 ng/mL).

The TG and FA concentrations in maternal blood. The FA levels in nonfasting maternal blood specimens were determined by gas chromatography–mass spectrometry (GC-MS) as described previously in detail (Nakashima et al. 2013). Briefly, the FA levels in maternal blood were measured as follows: Lipid extracted from 25 µL of blood according to the method of Folch et al. (1957) was mixed with 1.2 mL methanol, 75 µL acetyl chloride, and 75 µL 10 µg/100 µL tricosanoic acid ethyl ester/methanol (internal standard). After adding n-hexane (500 µL) and centrifugation, the upper organic layer was collected and moved into another vial. The n-hexane extraction was repeated once more, and then the concentration of FA methyl ester contained in the n-hexane layer was measured by GC-MS. Finally, the nine FA species targeted for measurement, including the palmitic and stearic acids of saturated FAs, the palmitoleic and oleic acids of monounsaturated FAs, linoleic acid (LA) and arachidonic acid (AA) of the omega 6 FAs, and the α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) of omega 3 FAs. The detection limits were 2.4 µL/mL for palmitic acid, 1.3 µg/mL for stearic acid, 0.069 µg/mL for palmitoleic acid, 3.6 µg/mL for oleic acid, and 2.0 µg/mL for the others. The detection rates for all FAs were > 99.0% (except for EPA, with a detection rate of 97.8%). Nonfasting blood TG levels were measured using TG E-Test Wako kits (Wako, Osaka, Japan) after lipid extraction according to the methods described by Folch et al. (1957).

Data analysis. For the analysis of associations between maternal PFOS and PFOA levels and birth size, the following subjects were excluded: women with pregnancy-induced hypertension (n = 11), women with diabetes mellitus (n = 1), mother–infant pairs with fetal heart failure (n = 1), and twins (n = 7). After the exclusion of these subjects, 428 mother–infant pairs had available PFOS and PFOA concentrations. We excluded subjects whose blood samples were obtained after delivery (n = 105). Additionally, TG and/or FA levels were not available for 17 subjects. The available sample size for statistical analysis after considering the exclusion criteria was 306 subjects. Because of the skewed distributions, we treated the levels of PFOS, PFOA, and lipids as a continuous variable on a $\log_{10}$ scale.

We analyzed correlations between PFOS and PFOA concentrations and the characteristics of the mothers and infants using the Spearman correlation test, the Mann–Whitney U-test, and the Kruskal–Wallis test. The same statistical analyses were performed to find correlations between the maternal blood TG and FA levels and the characteristics of the mothers and infants. Additionally, we performed multiple regression analyses to determine the relationship between the maternal PFOS and PFOA levels and the lipid levels, and potential confounders selected according to the current results in this paper influencing exposure levels (maternal age, parity, smoking during pregnancy), lipid levels (alcohol intake during pregnancy), or both (blood sampling period). In addition, we included annual household income as an indicator of socioeconomic status. Therefore, the fully adjusted models included maternal age (years), smoking status and alcohol intake during pregnancy (yes/no), annual household income (categorical), parity (0/≥ 1), and the blood sampling period during pregnancy (categorical or continuous). The blood sampling period during pregnancy was categorized in model 1 as follows: 23–31 weeks of gestation (n = 137), 32–34 weeks of gestation (n = 82), and 35–41 weeks of gestation (n = 87) (Konishi et al. 2009). Due to the importance of the blood sampling time with respect to the PFC and TG/FA levels,