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分担研究報告書

Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on Environment and Children's Health

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研究要旨

Perfluoroalkyl substances (PFASs) are synthetic chemicals that persist in the environment and in humans. There is a possible association between prenatal PFASs exposure and both neonate adipokines and birth size, yet epidemiological studies are very limited. The objective of this study was to examine associations of prenatal exposure to PFASs with cord blood adipokines and birth size. We conducted birth cohort study, the Hokkaido Study. In this study, 168 mother-child pairs were included. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in maternal blood were determined by liquid chromatography tandem mass spectrometry. Cord blood adiponectin and leptin levels were measured by ELISA and RIA, respectively. Birth weight and ponderal index (PI) were obtained from birth record. The median maternal PFOS and PFOA were 5.1 and 1.4 ng/mL, respectively. The median total adiponectin and leptin levels were 19.4 μg/mL and 6.2 ng/mL, respectively. Adjusted linear regression analyses found that PFOS level was positively associated with total adiponectin levels (β=0.12, 95% CI:0.01, 0.22), contrary was negatively associated with PI (β=-2.25, 95% CI: -4.01, -0.50). PFOA level was negatively associated with birth weight (β=-197, 95% CI: -391, -3). Leptin levels were not associated with PFASs levels. PFOS and adiponectin levels showed marginal dose-response relationship and both PFOS and PFOA and birth size showed significant dose-response relationships. Mediation analysis suggested that cord blood adiponectin was a mediator that could account for association between PFOS levels and PI. Results from this study suggested that prenatal PFASs exposure may alter cord blood adiponectin levels and may decrease birth size.

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A．研究目的

Perfluoroalkyl substances (PFASs) are widely used in the industry including textile impregnation, furnishings, non-stick housewares, and food packaging (Lau et al. 2007) and found in the environment, animals, and humans. The main exposure pathway to PFASs in human occurs orally via intake of contaminated food and water. (Fromme et al. 2009). Even though the use

of PFOS has been diminishing globally since they were included in Annex B of the Stockholm Convention on persistent organic pollutants in 2009 (UNEP 2007), due to their bioaccumulation and presence in older products, PFOS and PFOA are still detectable in human and environmental samples (Olsen et al. 2012; Okada et al. 2013). Since PFASs can cross the placental barrier and can be transferred from mother to fetus (Inoue et al. 2004; Midasch et al. 2007), studies in prenatal exposure to PFASs and its adverse health effects on fetus are warranted.

Adiponectin and leptin are hormones produced by adipocyte and have been used as biomarkers of metabolic function. The known roles of these hormones are metabolic homeostasis and regulation (Farooqi and O'Rahilly 2014; Fiaschi et al. 2014). Child adiponectin levels at birth and birth weight have been examined in the previous studies however, were inconsistent. Volberg et al. reported no relations (Volberg et al. 2013), while the others reported positive association between cord blood adiponectin levels with birth weight (Mantzoros et al. 2009) and the association between lower adiponectin and small for gestational age (SGA) and preterm birth (Palcevska-Kocevska et al. 2012; Yeung et al. 2015). A progressively significant negative association between adiponectin and BMI at 2, 5, and 9 years of age has been reported (Volberg et al. 2013). In adults, low adiponectin levels are the implication of obesity, metabolic syndrome and type 2 diabetes (DM2) (Mather and Goldberg 2014). Studies have suggested that both too high and too low leptin in fetus result in non-optimal fetal growth phenotypes that subsequently increase long term obesity risk (Ornoy 2011). High cord blood leptin levels have been known to positively associated with birth weight (Karakosta et al., 2011), while low cord blood leptin levels have been associated with SGA (Ren and Shen 2010).

Importance of investigating adipokine levels at birth have been suggested from the studies that found cord blood leptin levels may modify child growth trajectory (Parker et al. 2011; Kaar et al. 2014; Karakosta et al. 2016). There have been reported that cord blood adiponectin levels were negatively correlated with body weight at one year, weight gain after one year and with BMI at one year (Mazaki-Tovi et al. 2011) and that cord serum adiponectin levels were significant predictors of BMI Z-score gain from birth to 3 years of age (Nakano et al. 2012). Thus alternation of cord blood adiponectin levels may cause adverse effects on early childhood growth.

Previous epidemiological studies including our group have found that reduction of birth weight in association with prenatal exposure to PFASs (Olsen et al. 2009; Washino et al. 2009; Verner et al. 2015). In addition to birth weight, our group has reported that prenatal exposure to PFASs could results in disrupting various hormones balance including reproductive, thyroid and steroid hormone of neonates. PFOS were inversely associated with testosterone/estradiol, progesterone (P4) and inhibin B among boys and with P4 and prolactin among girls (Itoh et al. 2016). PFOS, but not PFOA were inversely correlated with maternal TSH and positively associated with infant serum TSH (Kato et al. 2016). Similarly, PFOS, but not PFOA was negatively associated with glucocorticoids in cord blood (Goudarzi et al. 2017).

Animal studies have suggested that

developmental exposure to PFOS may contribute to lipid metabolic disorder in adulthood in rats (Lv et al. 2013). There was only one study in human that found inverse association between PFOS exposure and polyunsaturated fatty acid levels in pregnant women (Kishi et al. 2015). Developmental exposure to lower levels of PFOA induced elevated serum leptin and overweight in mid-life in female mice through increasing of fatty acid metabolism by activation of proliferator-activated receptors (PPAR)-alpha (Hines et al. 2009). However, findings from animal data may not be applicable to humans. To our knowledge, there has been only a few prospective cohort studies that examined associations between early life exposure to PFASs and metabolic function such as adipokine levels (Halldorsson et al. 2012; Fleisch et al. 2016; Ashley-Martin et al. 2017). One study found no evidence of an adverse effect of PFASs exposure on metabolic function in mid-childhood (Fleisch et al. 2016) and contrary, the other study suggested that prenatal PFOA exposure significantly associated with leptin and adiponectin levels in female at age of 20 years (Halldorsson et al. 2012). These studies only investigated postnatal adipokine levels at childhood and early adulthood, but not examined adipokine levels at birth. The recent study in Canada (MIREC Study) is the only one to examine associations between maternal PFAS concentrations and birth weight and cord blood concentrations of leptin and adiponectin (Ashley-Martin et al. 2017), which found null associations.

The fetal time period is critical window of adipocyte development and thus, exposures to PFASs during fetal period may change postnatal growth trajectory and increase the risk of obesity and metabolic disorders later in life (Grun and Blumberg 2009; Hatch et al. 2010). Though prenatal exposure to PFASs and birth outcomes such as birth size have been studied, adipokines at birth, the metabolic related biomarkers have not been well investigated and understood.

The objectives of this study was to examine the association between prenatal exposure to PFASs and neonatal adipokines including adiponectin and leptin levels in cord blood along with birth size.

B．研究方法

Study population and questionnaire

This prospective birth cohort study was based on the Sapporo Cohort, the Hokkaido Study on Environment and Children's Health (Kishi et al. 2011; Kishi et al. 2013). The Sapporo Cohort is an ongoing cohort study that began in 2002. Briefly, pregnant women at 23–35 weeks of gestation were recruited between July 2002 and October 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. 514 women agreed to participate in the cohort study. All participants were residents in Sapporo City or surrounding areas.

The participants completed the self-administered questionnaire including baseline information such as their dietary habits, exposure to chemical compounds in their daily life, smoking history, alcohol consumption, caffeine intake, family income, educational levels of themselves and partners. Maternal anthropometric measurement data and medical history were obtained from medical record and birth weight and length were collected from birth records. We used the following criteria to include the participants into the analyses; singleton baby born at term (37–42 weeks of gestation). Participants with no PFASs

measurement (n=22) or those with blood collected after delivery (n=124) were excluded since PFOS and PFOA concentrations were significantly lower in post-delivery blood samples (Goudarzi et al. 2016; Itoh et al. 2016). Finally, 168 mother-child pairs who had both PFASs and adipokine measurements were included into the statistical analyses (Fig 1). This study was conducted in accordance with the Declaration of Helsinki, and the protocol used in this study was approved by the Institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environment and Health Sciences. This study was conducted with the informed consent of all participants in written forms.

Maternal serum PFASs measurements

PFOS and PFOA concentrations in maternal serum were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Detailed methods for the PFOS and PFOA measurements can be found in our previous reports (Nakata et al. 2009; Kishi et al. 2015). The limit of detection (LOD) of PFOS and PFOA was 0.50 ng/mL. PFOS was detected in all samples, and for samples with PFOA below LOD, we used a value of half the LOD (0.25 ng/mL). Nine samples were below LOD for PFOA measurement.

Cord blood adipokine measurements

Total and high molecular weight (HMW) adiponectin and leptin levels in cord blood were measured. Adiponectin levels were determined by Enzyme Linked ImmunoSorbent Assay (ELISA) using Human Adiponectin Assay kit from Sekisui Medical Co. Ltd (Tokyo, Japan). Leptin levels were determined by Radioimmunoassay (RIA) using Human Leptin RIA kit from Linco Research Inc. (St. Charles, MO, USA). All the analyses were conducted at LSI Medience (Tokyo, Japan) according to the operation manual. Analyses were repeated for all samples with coefficient of variation (CV) greater than 15 %. The LODs of adiponectin was 0.39 μg/mL and of leptin was 0.5 ng/mL. All samples were in the range of detection. Intra- and inter-assay CVs for total adiponectin were < 9.1% and < 10.1%, for HMW adiponectin were < 9.2% and < 11.6% and for leptin were < 5.3% and < 8.1%, respectively.

Statistical analyses

PFOS and PFOA levels in relation to maternal and infant characteristics were examined by Spearman's correlation test and Mann-Whitney U test or Kruskal-Wallis test. Similarly, cord blood adipokine levels in relation to maternal and infant characteristics were examined by Spearman's correlation test and Mann-Whitney U test or Kruskal-Wallis test. Associations of maternal PFOS and PFOA levels with cord blood adipokines and birth size were analyzed by multiple linear regression analyses. Maternal PFOS and PFOA levels did not distribute normally, thus these levels were log10 transformed for linear regression analyses. Total and HMW adiponectin, leptin levels were also log10 transformed. PI which was calculated as follows; PI (kg/m3) = Birth weight (kg) / (Birth length (m))3. To assess dose-response relationships, PFAS levels were categorized into tertiles and the least square means (LSMs) and lower and upper 95 % CI were calculated. P for trend was obtained from dose-response analysis.

Potential confounding variables were

considered based on the previous literatures (Itoh et al. 2016; Kato et al. 2016; Goudarzi et al. 2017). Medical record and questionnaires were used for obtaining data. The final linear regression model was adjusted for maternal body mass index (BMI), maternal smoking status during pregnancy, parity, maternal blood sampling period (gestational weeks in categories, 23-31, 32-34 and 35-41), infant sex, and gestational age (days).

All the analyses were conducted for boys and girls combined as well as boys and girls separately. Results were considered significant at $p < 0.05$. All analyses were conducted using SPSS Version 22.0 J (Chicago, IL, USA). Additionally, mediation analysis was performed by SPSS PROCESS, a macro implemented in SPSS (Hayes 2013) to examine indirect effect of prenatal exposure to PFASs on birth size through cord blood adipokines. The indirect effect and the bias-corrected and accelerated confidence intervals of the indirect effect were determined by bootstrapping with 5000 iterations. The effect size was determined by using percent mediation (PM) method (Preacher and Hayes 2008).

This prospective birth cohort study was based on the Sapporo Cohort, Hokkaido Study on Environment and Children's Health [30, 31]. Briefly we recruited pregnant women at 23-35 weeks of gestation between July 2002 and October 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. All subjects were resident in Sapporo City or surrounding areas. The participants completed the self-administered questionnaire survey after the second trimester during their pregnancy. The questionnaire contained baseline information including their dietary habits, exposure to chemical compounds in their daily life, smoking history, alcohol consumption, caffeine intake, family income, educational levels of themselves and partners. The prenatal information of the mothers and their neonates was collected from their medical records. This study was conducted with the informed consent of all participants in written forms. This study was conducted in accordance with the Declaration of Helsinki, and the protocol used in this study was approved by the Institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environment and Health Sciences.

C．研究結果

The characteristics of both mothers and infants is shown in Table 1. Among 168 participants included in this study, the median concentrations of maternal PFOS and PFOA were 5.1 ng/mL (interquartile range [IQR]:3.7-6.7 ng/mL) and 1.4 ng/mL (IQR: 0.9-2.2 ng/mL), respectively. PFOS and PFOA levels were modestly correlated (Spearman's rho=0.287). Table 2 shows maternal PFOS and PFOA levels in relation to characteristics of mothers and infants. PFOS and PFOA levels were significantly higher among primiparous women. PFOS and PFOA levels were significantly lower among smokers. Caffeine intake during pregnancy was negatively correlated with PFOA levels. PFOS and PFOA levels were negatively correlated with blood sampling period (gestational weeks). The mean PFOA level was higher among boys compared to girls. PFOS level was negatively correlated with PI and PFOA level was negatively correlated with both birth weight and PI.

Concentrations of cord blood adiponectin

and leptin are shown in Table 3. The detection rate of both adiponectin and leptin was 100%.

Table 4 shows cord blood adipokine levels in relation to characteristics of mothers and infants. None of the maternal characteristics were significantly associated with either adiponectin or leptin levels. Adiponectin and leptin levels were significantly higher in girls than in boys. Total adiponectin level was negatively correlated with gestational age, contrary positively correlated with PI. Leptin level was positively correlated with birth weight, length and PI.

Associations of maternal PFOS and PFOA levels with cord blood adiponectin and leptin levels, birth weight and PI are shown in Table 5. PFOS level was positively associated with total adiponectin level (β = 0.12; 95% confidence interval [CI]: 0.01, 0.22). Contrary, PFOS level was negatively associated with PI (β = -2.25; 95% CI: -4.01, -0.50). PFOA levels were negatively associated with birth weight (β = -197; 95% CI: -391, -3) and marginally negatively associated with PI (β = -1.32; 95% CI: -2.66, 0.02). Stratification by infant sex found that positive association between PFOS and adiponectin and negative association between PFOS and PI were more significant in boys (Table S1). PFASs and sex interaction was examined and found to be not significantly associated except PFOS and sex interaction on leptin levels (p=0.008) (Table S1).

We also PFASs levels into tertiles and examined the dose-response relationships between PFASs and cord blood adipokines (Figure 2 and Tables S2 and S3). The tertile analysis with adjustment showed that the highest tertile of PFOS was associated with 2.91 μg/mL increase in total adiponectin compared to the lowest tertile and p for trend was 0.095. Similarly, the highest tertile of PFOS was associated with 1.99 μg/mL increase in HMW adiponectin compared to the lowest tertile and p for trend was 0.072. The highest tertile of PFOS was associated with 1.16 kg/m3 decrease in PI compared to the lowest tertile and p for trend was significant (Ptrend = 0.003). The PFOA level was associated with decreased birth weight and PI with clear dose-response relationships. P for trend for birth weight was 0.021 and for PI was 0.002, respectively.

D．考察

We have previously reported that decreased birth weight among girls in association with in utero exposure to PFOS with significance in this population (Washino et al. 2009). Similarly, our previous report of both PFOS and PFOA levels and PI were inversely associated (Kobayashi et al. 2016). Our results provided a new evidence of association between relatively lower levels of prenatal PFASs exposure and neonatal birth size and cord adipokines. In addition to our previous findings of inverse association between PFOS exposure and polyunsaturated fatty acids levels of mothers (Kishi et al. 2015), this study suggested PFOS exposure may associate with disruption of fetal metabolic function.

Median concentrations of maternal PFOS and PFOA in this study were 5.1 and 1.4 ng/mL, respectively, which were comparable to the recent report from Canada (PFOS: 4.6, PFOA: 1.7 ng/mL) (Ashley-Martin et al. 2017), however, lower than previous reports from Korea (PFOS: 9.3, PFOA: 2.6 ng/mL) (Lee et al. 2013)., the United States (PFOS: 8.2, PFOA: 2.9 ng/mL) (Stein et al. 2012), Denmark (PFOS: 21.5, PFOA: 3.7 ng/mL)

(Halldorsson et al. 2012), Norway (PFOS: 13, PFOA: 2.2 ng/mL) (Starling et al. 2014). Adiponectin and leptin levels in this study were comparable to those from Japanese study (Nakano et al. 2012) and other studies in Asian countries (Chou et al. 2011; Kim et al. 2016). Contrary, cord blood adiponectin in our study showed lower level compared to the previous studies from North America and Europe (Brynhildsen et al. 2013; Lagiou et al. 2013; Luo et al. 2013; Ashley-Martin et al. 2017). Similarly, compared to Canadian study, leptin level in our study was lower. (Ashley-Martin et al. 2014; Ashley-Martin et al. 2017). Relatively lower levels of adiponectin and leptin in our study was consistent with previously reported observations that showed differences in these adipokine levels among ethnicities (Mente et al. 2010; West et al. 2014).

Two of the previous birth cohort studies (Halldorsson et al. 2012; Fleisch et al. 2016) only examined associations between maternal levels of PFASs and adipokine levels of mid-childhood and early adulthood, however, there were lacking information at birth. Besides exposure levels were relatively high in those two studies whereas our study could assess relatively lower level exposures to PFASs on metabolic related outcomes. The recent Canadian birth cohort study found overall null associations between maternal PFAS levels and cord blood adiponectin and leptin and birth weight z score (Ashley-Martin et al. 2017). The maternal PFAS levels in their study were similar to ours and our findings partially agreed to their results. Regression coefficients in our study were also comparable to their results and both of the studies found no association between maternal PFOS and PFOA levels and cord blood leptin levels.

The mediation analysis found a significant indirect effect of maternal PFOS levels on PI through cord blood total adiponectin levels (Figure 3). The results showed that cord blood adiponectin as a mediator could account for ~27% of the total effect (PM=0.27). Cross-sectional studies reported a negative association between cord blood PFOS levels and PI (Apelberg et al. 2007) and an inverse relationship between neonatal adiponectin levels and PI (Mantzoros et al. 2004). Our study added the evidence that prenatal exposure to PFOS were associated with both increased cord blood adiponectin levels and reduced PI. Result from the mediation analysis suggested that mediatory effect of cord blood adiponectin may partially be responsible for the observed relationship between prenatal exposure to PFOS and PI. However, the result should be cautiously interpreted. There are possibilities that other unmeasured factors including other types of adipokines and hormones that are responsible for fetal growth can account for our result. Observed null association between maternal PFOA and cord blood adipokine levels indicated that prenatal PFOA exposure’s adverse effects on birth size was not likely to occur through adiposity-related pathways.

The mechanisms behind observed association between prenatal PFOS exposures and cord blood adiponectin levels are not fully understood. Mutual adjustment to see whether PFOS and PFOA have additive effects on outcomes was performed, however, the regression coefficient did not change. The possible pathway could be interaction of PFASs with PPAR-alpha, which were involved in lipid metabolism in adipocytes (Takacs and Abbott 2007; Hines

et al. 2009). Yet, why only PFOS showed inverse association with adiponectin levels remain unclear. PFOA can pass placenta more efficient than PFOS (Gutzkow et al. 2012) may explain our observed association between PFOA and reduced birth weight and PI.

Accumulating evidences from epidemiological studies indicated that reduced birth size was a risk factor for a range of metabolic problems including high adult BMI, insulin resistance, increased visceral adiposity, and impaired glucose tolerance (Calkins and Devaskar 2011). Thus our finding of reduced birth size in association with prenatal exposure to PFASs may also be responsible for adverse metabolic outcomes in later life. Continuous follow-up of cohort participants is required to determine whether altered adipokine levels at birth persist and reduced birth size relates to metabolic dysfunction.

The limitations of this study should be considered. The participants included into the statistical analyses were limited to those who with available prenatal PFASs exposure and cord blood adipokines measurements (n=168), which may have led to potential selection bias. We should note that cord blood samples for adipokine measurements were available only from those who had vaginal delivery. Compared to the whole population, participants included in this study showed higher prevalence of primipara, higher rate of smoking during pregnancy, lower family income (< 5million yen/year) and longer gestational age (Table S4). However, maternal age, pre-pregnancy BMI, alcohol intake during pregnancy and maternal education of participants in this study are similar to those in the whole population. In the statistical analysis, variables differed between this study population and the whole population were adjusted, thus potential influence of these variables were considered to be null. Although the number of participants were limited, we included only those who had blood samples during pregnancy for PFASs exposure measurements, which enabled accurate reflection of prenatal exposures. There might be a possibility of the influence of unmeasured co-exposures and confounders.

Recently, our group has reported that maternal MEHP levels were associated with alternation of adiponectin and leptin levels in cord blood in sex-specific manner (Minatoya et al. 2017). Cord blood adipokine levels can be investigated in association with these environmental chemical exposures in our future work. As a strength of prospective birth cohort study, we have longitudinal follow-up data including childhood anthropometric measurements and metabolic related health outcomes at different ages. The follow-up data together with exposure assessment and cord blood adipokines and birth size can be used for further investigation of associations between prenatal exposures and metabolic related outcomes in later life.

E．結論

Our findings provided some evidences of possible adverse effects of prenatal exposure to PFASs on metabolic function at birth and birth size. PFOS and adiponectin levels showed marginal dose-response relationship and both PFOS and PFOA and birth size showed significant dose-response relationships. Additionally, our result suggested mediatory effect of adiponectin on relationship between PFOS exposure and PI. Further investigation is required to

determine whether prenatal exposure to PFASs continue to associate with growth and metabolic related outcomes such as obesity and DM2 in later life. Additionally, potential sex-specific influence of exposure to PFASs on metabolic related outcomes should be further investigated for better understanding of mechanism behind observed findings. Future follow-up study in the Hokkaido Study will enable to explore associations between prenatal exposures and childhood growth.

Ｆ．研究発表

1.論文発表

Machiko Minatoya, Sachiko Itoh, Chihiro Miyashita, Atsuko Araki, Seiko Sasaki, Ryu Miura, Houman Goudarzi, Yusuke Iwasaki, Reiko Kishi. Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on Environment and Children's Health. Environ Res submitted.

2.学会発表

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Ｇ．知的財産権の出願・登録状況（予定を含む。）

該当なし

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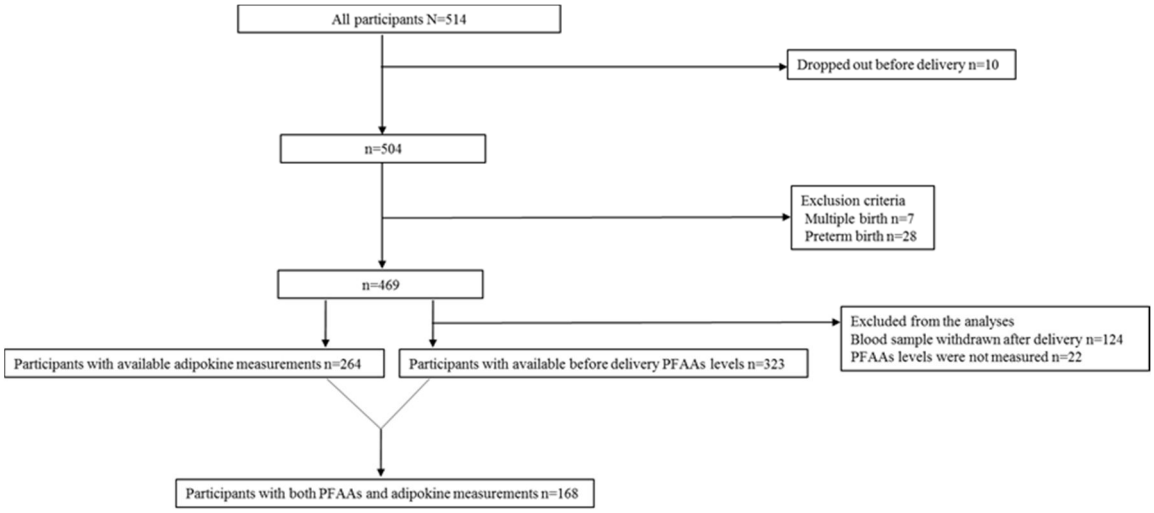
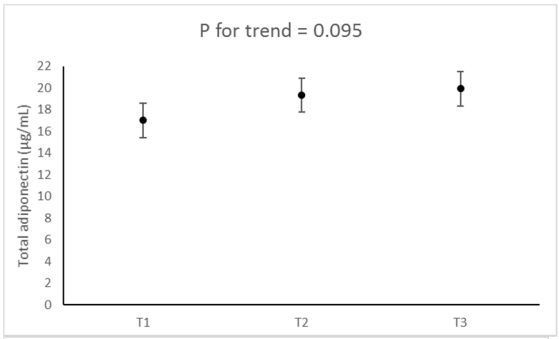
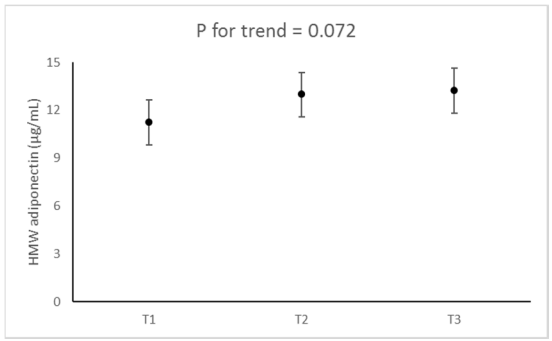
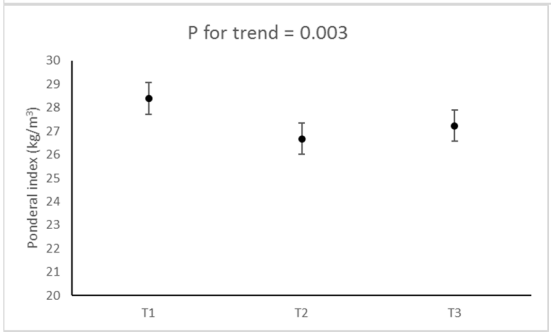


Figure 1. Flowchart of participants' selection.

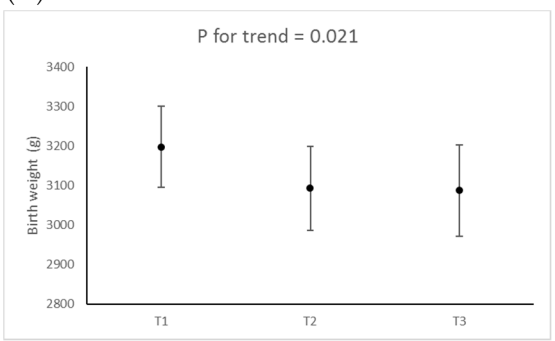
(A) PFOS







(B) PFOA



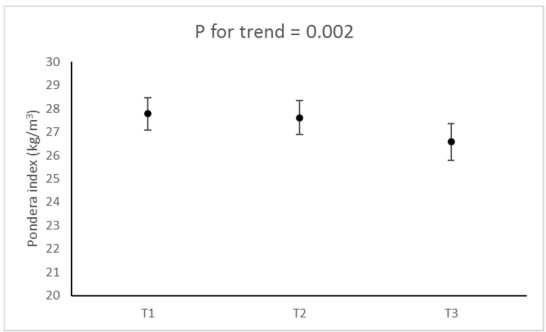


Figure 2. The dose-response relationships of PFOS (A) and PFOA (B) tertiles with adipokine levels and birth size. The LSMs were adjusted for maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age and infant sex.
PFOS; T1:1.5-4.0 ng/mL, T2: 4.1-6.2 ng/mL, T3: 6.3-14.7 ng/mL.
PFOA; T1:<LOD-1.10 ng/mL, T2: 1.20-1.80 bg/mL, T3: 1.90-5.30 ng/mL.
The error bars show the lower and upper 95% confidence intervals.
LSM: least square mean, LOD: limit of detection.

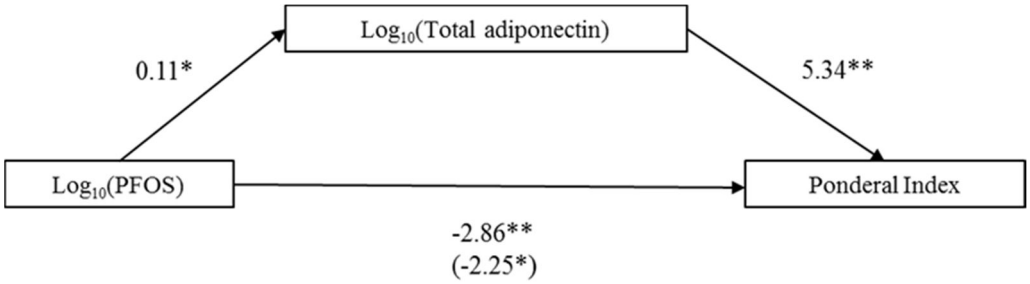


Figure 3. Mediation analysis of the association between PFOS and total adiponectin and between PFOS and PI. Regression coefficients of each path are described alongside with arrows. The total effect of PFOS on PI is described in the parentheses. A significant indirect effect of PFOS on PI through total adiponectin was observed (indirect effect=0.61; bias-corrected and accelerated confidence interval: 0.04, 1.50). The total adiponectin as a mediator can account for roughly 30% of total effect (percent mediation=0.27). Adjusted for maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age and infant sex. *p<0.05 and **P<0.01.

Table 1 Characteristics of participants (n=168).

Characteristics		N (%) or mean ± S.D.
Mother		
Age (years)		30.0 ± 4.6
Pre-pregnancy BMI (kg/m^2)		21.2 ± 3.3
Parity	0	90 (53.6)
	≧ 1	78 (46.4)
Educational level (years)	≦ 12	76 (45.2)
	≧ 13	92 (54.8)
Family income (million yen)	≦ 5	121 (72.0)
	> 5	47 (28.0)
Smoking during pregnancy	Yes	31 (18.5)
	No	137 (81.5)
Alcohol intake during pregnancy	Yes	56 (33.3)
	No	112 (66.7)
Caffeine intake during pregnancy (mg/day)		139.5 ± 127.9
Blood sampling period	23-31 weeks	64 (38.1)
	32-34 weeks	39 (23.2)
	34-41 weeks	65 (38.7)
Infant		
Sex	Boy	78 (46.4)
	Girl	90 (53.6)
Gestational age (days)		279.0 ± 6.6
Birth weight (g)		3150 ± 330
Birth length (cm)		48.6 ± 1.6
Ponderal index (kg/m^3)		27.5 ± 2.2

Table 2 Maternal PFOS and PFOA levels in relation to characteristics of participants.

Characteristics		PFOS mean ± S.D. or correlation	p-value	PFOA mean ± S.D. or correlation	p-value
Mother					
Age (year)		ρ=-0.048	0.534	ρ=-0.021	0.789
Pre-pregnancy BMI (kg/m^2)		ρ=-0.105	0.177	ρ=-0.060	0.439
Parity	0	5.86 ± 2.56	0.009	1.95 ± 0.99	< 0.001
	≧1	4.90 ± 2.34		1.24 ± 0.79	
Educational level (years)	≦12	5.27 ± 2.10	0.846	1.58 ± 1.02	0.295
	≧13	5.54 ± 2.80		1.66 ± 0.93	
Family income (million yen)	≦5	5.30 ± 2.47	0.350	1.63 ± 1.03	0.674
	>5	5.71 ± 2.59		1.60 ± 0.81	
Smoking during pregnancy	Yes	4.57 ± 2.23	0.047	1.29 ± 0.80	0.023
	No	5.61 ± 2.53		1.69 ± 0.99	
Alcohol intake during pregnancy	Yes	5.37 ± 2.43	0.968	1.62 ± 0.85	0.819
	No	5.44 ± 2.55		1.62 ± 1.02	
Caffeine intake during pregnancy (mg/day)		ρ=-0.144	0.141	ρ=-0.183	0.018
Blood sampling period (weeks)	23-31	6.06 ± 2.18	< 0.001	1.82 ± 0.90	0.007
	32-34	5.85 ± 3.08		1.61 ± 1.14	
	35-41	4.53 ± 2.16		1.43 ± 0.90	
Infant					
Sex	Boys	5.85 ± 2.63	0.054	1.77 ± 0.90	0.013
	Girl	5.04 ± 2.33		1.49 ± 1.01	
Gestational age (days)		ρ=0.055	0.483	ρ=0.093	0.233
Birth weight (g)		ρ=-0.048	0.539	ρ=-0.156	0.044
Birth length (cm)		ρ=0.131	0.091	ρ=0.071	0.364
Poderal index (kg/m^3)		ρ=-0.232	0.003	ρ=-0.259	0.001

Mann-Whitney U test or Kruskal-Wallis test. Spearman's rho.

Table 3 Concentration of cord blood adipokines.

		N	Median (IQR)
Total adiponectin (μg/ml)	All	168	19.4 (15.7-22.6)
	Boy	78	18.7 (14.8-21.1)
	Girl	90	20.4 (16.8-23.7)
HWM adiponectin (μg/ml)	All	168	12.9 (9.9-15.6)
	Boy	78	11.6 (9.7-14.6)
	Girl	90	13.6 (10.3-16.7)
Leptin (ng/ml)	All	165	6.2 (3.9-10.1)
	Boy	78	5.0 (3.4-6.8)
	Girl	87	8.2 (4.8-13.0)

Detection rate was 100% for all the adipokines.

Table 4 Cord blood adipokines in relation to characteristics of participants.

Characteristics		Total adiponectin mean ± S.D. or correlation	p-value	HMW adiponectin mean ± S.D. or correlation	p-value	Leptin mean ± S.D. or correlation	p-value
Mother							
Age (year)		ρ=0.005	0.954	ρ=-0.018	0.821	ρ=-0.046	0.554
Pre-pregnancy BMI (kg/m^2)		ρ=0.119	0.125	ρ=0.113	0.143	ρ=0.153	0.050
Parity	0	20.0 ± 5.7	0.445	13.3 ± 4.6	0.642	8.3 ± 5.9	0.398
	≧1	19.0 ± 5.4		12.8 ± 4.6		7.2 ± 5.1	
Educational level (years)	≦12	20.2 ± 5.6	0.108	13.8 ± 4.8	0.052	8.6 ± 6.2	0.071
	≧13	19.0 ± 5.5		12.4 ± 4.3		7.1 ± 4.9	
Family income (million yen)	≦5	19.9 ± 5.9	0.239	13.4 ± 4.9	0.175	8.1 ± 5.7	0.246
	>5	18.5 ± 4.4		12.1 ± 3.6		7.0 ± 5.1	
Smoking during pregnancy	Yes	19.6 ± 7.0	0.920	13.3 ± 5.6	0.781	7.9 ± 6.1	0.869
	No	19.5 ± 5.2		13.0 ± 4.3		7.7 ± 5.5	
Alcohol intake during pregnancy	Yes	19.5 ± 5.5	0.946	12.9 ± 4.5	0.874	7.7 ± 5.7	0.757
	No	19.6 ± 5.6		13.1 ± 4.6		7.8 ± 5.5	
Caffeine intake during pregnancy (mg/day)		ρ=-0.036	0.645	ρ=-0.002	0.978	ρ=-0.037	0.638
Infant							
Sex	Boys	18.4 ± 4.5	0.017	12.1 ± 3.6	0.015	6.0 ± 4.5	<0.001
	Girl	20.5 ± 6.2		13.9 ± 5.1		9.4 ± 6.0	
Gestational age (days)		ρ=-0.157	0.042	ρ=-0.138	0.075	ρ=0.139	0.076
Birth weight (g)		ρ=0.131	0.090	ρ=0.116	0.133	ρ=0.366	<0.001
Birth length (cm)		ρ=-0.060	0.442	ρ=-0.073	0.348	ρ=0.155	0.048
Poderal index (kg/m^3)		ρ=0.259	0.001	ρ=0.264	0.001	ρ=0.294	<0.001

Mann-Whitney U test or Kruskal-Wallis test. Spearman's rho.

Table 5 Association of maternal PFASs levels with cord blood adipokines and birth size.

	PFOS		PFOA	
All	β (95% CI)	p-value	β (95% CI)	p-value
Total adiponectin	0.12 (0.01, 0.22)	0.028	0.04 (-0.04, 0.11)	0.377
HMW adiponectin	0.12 (-0.01, 0.25)	0.075	0.03 (-0.07, 0.13)	0.575
Leptin	-0.05 (-0.27, 0.18)	0.691	0.02 (-0.15, 0.19)	0.830
Birth weight (g)	-29 (-289, 232)	0.828	-197 (-391, -3)	0.047
Ponderal index (kg/m^3)	-2.25 (-4.01, -0.50)	0.012	-1.32 (-2.66, 0.02)	0.054

Both PFASs levels and adipokine levels were $\log_{10}$ transformed.
Adjusted for maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age and infant sex.

Table S1 Association of maternal PFASs levels with cord blood adipokines and birth size stratified by child sex and interaction between PFASs and child sex.

PFOS	Boys	Girls	
	β (95% CI)	β (95% CI)	$p_{interaction}$
Total adiponectin	0.13 (0.00, 0.26)	0.11 (-0.06, 0.27)	0.563
HMW adiponectin	0.13 (-0.04, 0.30)	0.12 (-0.08, 0.33)	0.598
Leptin	0.01 (-0.28, 0.30)	-0.14 (-0.49, 0.21)	0.008
Birth weight (g)	190 (-162, 543)	-251 (-645, 143)	0.201
Ponderal index (kg/m^3)	-2.46 (-4.74, -0.18)	-2.11 (-4.86, 0.64)	0.658
PFOA			
Total adiponectin	0.06 (-0.13, 0.13)	0.05 (-0.05, 0.16)	0.811
HMW adiponectin	-0.01 (-0.19, 0.16)	0.05 (-0.09, 0.18)	0.885
Leptin	-0.14 (-0.42, 0.15)	0.08 (-0.15, 0.31)	0.591
Birth weight (g)	-276 (-619, 67)	-169 (-419, 81)	0.160
Ponderal index (kg/m^3)	-1.02 (-3.33, 1.29)	-1.39 (-3.13, 0.36)	0.376

Adjusted for maternal BMI, parity, smoking during pregnancy, blood sampling period and gestational age. $P_{interaction}$ for PFOS or PFOA and child sex interaction.

Table S2 Adjusted least square means (LSM) for cord blood adipokines. and birth size by tertiles of PFASs.

PFOS	Total adiponectin			HMW adiponectin			Leptin		
	LSM	LCI	UCI	LSM	LCI	UCI	LSM	LCI	UCI
T1	17.02	15.41	18.63	11.23	9.81	12.65	7.44	5.58	9.31
T2	19.36	17.77	20.94	12.97	11.58	14.36	8.11	6.28	9.95
T3	19.93	18.34	21.53	13.22	11.81	14.62	7.09	5.22	8.97
P_{trend}	0.095			0.072			0.906		
PFOA									
T1	17.78	16.19	19.37	11.72	10.34	13.09	7.45	5.78	9.13
T2	21.24	19.60	22.89	14.67	13.24	16.09	7.89	6.15	9.63
T3	19.86	18.08	21.65	13.14	11.59	14.69	7.23	5.35	9.11
P_{trend}	0.644			0.796			0.789		

PFOS; T1:1.5-4.0 ng/mL, T2: 4.1-6.2 ng/mL, T3: 6.3-14.7 ng/mL.
PFOA; T1:<LOD-1.10 ng/mL, T2: 1.20-1.80 bg/mL, T3: 1.90-5.30 ng/mL.
Adjusted for maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age and infant sex.
LSM: least square mean, LCI: lower 95% confidence interval, UCI: upper 95% confidence interval, T: tertile, LOD: limit of detection.

Table S3 Adjusted least square means (LSM) for birth size by tertiles of PFASs.

PFOS	Birth weight			Ponderal index		
	LSM	LCI	UCI	LSM	LCI	UCI
T1	3196	3095	3298	28.39	27.71	29.06
T2	3076	2976	3176	26.68	26.02	27.34
T3	3158	3057	3258	27.23	26.57	27.90
P_{trend}	0.424			0.003		
PFOA						
T1	3197	3095	3300	27.78	27.08	28.48
T2	3092	2986	3199	27.61	26.89	28.34
T3	3087	2972	3203	26.58	25.79	27.37
P_{trend}	0.021			0.002		

PFOS; T1:1.5-4.0 ng/mL, T2: 4.1-6.2 ng/mL, T3: 6.3-14.7 ng/mL.
PFOA; T1:<LOD-1.10 ng/mL, T2: 1.20-1.80 bg/mL, T3: 1.90-5.30 ng/mL.
Adjusted for maternal BMI, parity, smoking during pregnancy, blood sampling period, gestational age and infant sex.
LOD: limit of detection.

Table S4 Comparison of basic characteristics between whole population (n=469) and participants included in this study (n=168).

Characteristics		**Whole population (n=469)**	**Participants included in this study (n=168)**
Mother		**% or mean (SD)**	
Age at delivery (years)		30.7 (4.9)	30.0 (4.6)
Pre-pregnancy BMI (kg/m^2)		21.1 (3.1)	21.2 (3.3)
Parity	0	48.0%	53.6%
	≧ 1	51.8%	46.4%
Smoking during pregnancy	No	83.3%	81.5%
	Yes	16.6%	18.5%
Alcohol intake during pregnancy	No	68.0%	66.7%
	Yes	32.0%	33.3%
Education (years)	≦ 12	45.0%	45.2%
	≧ 13	55.0%	54.8%
Family income (yen)	< 5M	68.9%	72.0%
	≧ 5M	31.1%	28.0%
Infant			
Sex	Boy	46.9%	46.4%
	Girl	53.1%	53.6%
Gestational age (days)		276.7 ± 7.8	279.0 ± 6.6