The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study

Perfluorinated chemicals (PFCs) disrupt homeostasis of cholesterol, which is a substrate of steroid hormones. Steroid hormones such as glucocorticoids and androgenic hormones mediate several vital physiologic functions; however, the in utero effects of PFCs exposure on the homeostasis of these steroid hormones are not well understood in humans. We examined the relationship between prenatal exposure to perfluorooctane sulfonate (PFOS)/perfluorooctanoate (PFOA) and cord blood levels of glucocorticoid and androgenic hormones.

Methods. We conducted a hospital-based birth cohort study between July 2002 and October 2005 in Sapporo, Japan (n=514). In total, 251 mother-infant pairs were included in this study. The prenatal PFOS and PFOA levels were measured in maternal serum samples by liquid chromatography-tandem mass spectrometry (LC-MS-MS). Cord blood levels of glucocorticoid (cortisol and cortisone) and androgenic hormones (dehydroepiandrosterone (DHEA) and androstenedione) were also measured by LC-MS-MS. We found a dose-response relationship of prenatal PFOS exposure, but not PFOA, with glucocorticoid levels after adjusting for potential confounders. Cortisol and cortisone concentrations were -28.70 (95% confidence interval (CI): -46.10, -11.30; p for trend <0.001) and -77.93 ng/mL (95% CI: -130.97, -24.89; p for trend <0.001) lower, respectively, in infants with prenatal PFOS in the fourth quartile compared with those in the first quartile. The highest quartile of prenatal PFOS exposure was positively associated with a 0.99 ng/mL higher DHEA level compared with the lowest quartile (95% CI: 0.15, 1.83; p for trend=0.013), whereas PFOA showed a negative association with DHEA levels (quartile 4 vs 1: -0.98 ng/mL, 95% CI: -1.72, -0.23; p for trend=0.011). We observed no significant association between PFCs and androstenedione levels. Our results indicated that prenatal exposure to PFC levels were significantly associated with glucocorticoid and DHEA levels in cord blood.

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Perfluorinated chemicals (PFCs) are persistent and ubiquitous chemicals that have been widely used in different industries. PFCs have long elimination half-lives; serum elimination of PFOS and PFOA in human sera is estimated to take 5.4 and 3.8 years, respectively (Olsen et al. 2007). These result in the bioaccumulation of PFCs in the human body.

Fetuses are exposed to PFCs because of maternofetal passage during organ development (Inoue et al. 2004). Some

epidemiological studies in the general population suggest that these compounds are associated with poor birth outcomes such as reduced birth size (Apelberg et al. 2007; Washino et al. 2009). Cholesterol is a substrate of all steroid hormones. Previous human studies have reported that PFCs may change the cholesterol profile in pregnant (Starling et al. 2014) and non-pregnant people (Frisbee et al. 2010; Winquist et al. 2014). Joensen et al. (2013) reported an inverse association between PFOS and testosterone levels in serum samples of adult men. Previously, our group has reported a negative association between prenatal PFOS and progesterone hormone levels of cord blood samples in male and female infants. In addition, PFOS was negatively associated with testosterone/estradiol in male infants, whereas prenatal PFOA was positively associated with progesterone levels in cord blood samples of both sexes (Itoh et al. 2014). However, the effects of PFCs on glucocorticoid hormones and androgenic hormones (the main substrates of testosterone and estrogen) are not well understood in humans.

We investigated whether prenatal exposure to PFOS and PFOA was associated with cortisol and cortisone levels in cord blood samples in a birth cohort using a prospective design. In addition to glucocorticoids, to gain a better understanding of the effects of PFCs on steroidogenesis, we assessed the association of PFCs with DHEA and androstenedione as androgenic hormones in cord blood and assessed the balance of glucocorticoids and androgenic hormones in infants.

This study was part of the Hokkaido Study on the Environment and Children's Health that was conducted between July 2002 and October 2005 (n=514). The details of this study have been described previously (Kishi et al. 2011 and 2013). A self-administered questionnaire survey was completed after the second trimester of pregnancy that contained information related to previous medical history, socioeconomic status, and habits during pregnancy. 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, was obtained from participant medical records.

A 40-mL blood sample was taken from the maternal peripheral vein after the second trimester of pregnancy to measure PFOS and PFOA levels. PFOS and PFOS levels were measured in maternal serum samples using column-switching liquid chromatography -tandem mass spectrometry (LC-MS-MS) (Nakata et al. 2009). A blood sample (10–30 mL) was collected from the umbilical cord at delivery. Concentrations of cortisol, cortisone, DHEA, and androstenedione were measured in cord blood samples using LC–MS/MS (Yamashita et al. 2007a, 2007b).

The following subjects were excluded from the analysis of associations between maternal PFCs and glucocorticoids: 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 the mentioned subjects, 428 mother–infant pairs had available PFOS and PFOA concentrations. Of those, 251 mother-infant pairs had available cord blood samples and included in current analysis. Because of the skewed distributions, we treated the levels

of PFCs, glucocorticoid and androgenic hormones 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. The same statistical analyses were performed to find associations between steroid hormone levels and participants' characteristics. We performed multiple-regression analysis to examine the association between glucocorticoid and androgenic hormones and the levels of PFCs in maternal serum samples. Potential confounders that affected exposure and/or outcome levels including maternal age (year), parity (0/≥1), gestational age (continuous), caffeine intake (continuous), smoking during pregnancy (yes/no), and blood sampling period (before and after delivery). To assess a dose-response relationship, we divided PFC levels into four quartiles and least square means (LSMs) and 95% confidence intervals (CI) were calculated. To calculate a p value for the trend, we used linear contrast coefficients of -3, -1, +1, and +3 assigned to quartiles 1, 2, 3, and 4, respectively. We performed all of the statistical analyses using JMP clinical 5 (SAS Institute Inc., NC, USA) and results were considered significant when p < 0.05.

All participants provided written informed consent and the study protocol was approved by the institutional ethical board for epidemiological studies at the Graduate School of Medicine and the Center for Environmental and Health Sciences, Hokkaido University.

The average age of the mothers at birth was 29.9 years (standard deviation (SD) 4.8); 49.9 % of mothers were nulliparous (Table 1). Among pregnant women, 15.5 % smoked and 33.1 % consumed alcohol during pregnancy. The mean (±SD) of birth weight was 3119.2 g (±332.7), and 44.2% of newborns were boys. PFOS levels were detected in all of the samples, however PFOA levels were not detected in 16 maternal serum samples (6.3% of participants). The median (25-75 percentile) values of PFOS and PFOA were 5.0 ng/mL (3.2 to 6.8 ng/mL) and 1.4 ng/mL (0.9 to 1.9 ng/mL), respectively (Table 2). We observed statistically significant differences in mean PFOS concentrations by parity and blood sampling period. Additionally, there were significant differences in mean PFOA concentrations by parity, blood sampling period, and smoking and caffeine intake during pregnancy. Median (25-75 percentile) values of cortisol, cortisone, DHEA and androstenedione in cord blood samples were 37.9 (22.3-63.0), 93.5 (68.9-123.3), 2.2 (1.7-3.0), and 0.46 (0.36-0.59) ng/mL, respectively (Table 3). Cortisol and cortisone were detected in 97.2 % and 94.4 % of samples, respectively. The detection rates of DHEA and androstenedione were both 100%.

Cortisol and cortisone levels in cord blood showed a negative association with maternal age. Glucocorticoid levels in cord blood of infants with multiparous mothers were significantly lower compared with those in infants with nulliparous mothers. Gestational age had a significant positive correlation with cortisol levels and a non-significant positive correlation with cortisone levels. DHEA and androstenedione

levels did not show any association with maternal or infant characteristics (data not shown).

As shown in Table 4, after controlling for potential confounders, prenatal PFOS concentration was inversely associated with cortisol levels (β = -0.284; 95% CI: -1.05, -0.397; p-value < 0.001). Similarly, we observed a significant negative association between PFOS and cortisone levels (β = -0.268; 95% CI: -1.37, -0.471; p value <0.001). In addition, prenatal PFOS concentrations were positively associated with DHEA levels (β = 0.181; 95% CI: 0.067, 0.436; p-value = 0.007). We found a non-significant positive association between PFOA and cortisol (β =0.117; 95% CI: -0.053, 0.525; p-value = 0.109) and cortisone levels (β = 0.136; 95% CI: -0.024, 0.761; p-value=0.066). Prenatal exposure to PFOA was negatively associated with DHEA levels (β = -0.219; 95% CI: -0.396, -0.085; p value = 0.002). In addition, we assessed the association of PFCs with the cortisol to DHEA ratio and the glucocorticoid to androgenic hormones ratio. PFOS was significantly negatively associated with the ratios of cortisol/DHEA and glucocorticoid/androgenic hormones. However, PFOA showed a positive and significant association with these ratios.

For further assessment, we also divided maternal PFC levels into quartiles and examined the dose-response relationship between PFCs and steroid hormones (Figure 1). The quartile analysis after full adjustment showed that the highest quartile of PFOS was associated with a -28.70 ng/mL (95% CI: -46.10, -11.30; p for trend < 0.001) in cortisol and -77.93 ng/mL (95% CI: -130.97, -24.89; p for trend < 0.001) in cortisone levels compared with the lowest quartile.

PFOA did not show any significant trend for glucocorticoid levels. In addition, we found significant increases in DHEA levels across PFOS quartiles (quartile 4 vs 1 difference: 0.99 ng/mL, 95% CI: 0.15, 1.83; p for trend = 0.012), but significant decreases in DHEA levels among PFOA quartiles (quartile 4 vs 1 difference= -0.98 ng/mL, 95% CI: -1.72, -0.23; p for trend = 0.011). We did not observe a dose-response relationship between PFCs and androstenedione levels.

To the best of our knowledge, this study is the first to address the association of PFCs with cord blood glucocorticoid and androgenic hormone levels in a prospective birth cohort. In this study, we found a significant negative association of prenatal PFOS levels with cortisol and cortisone levels in cord blood samples. In addition, we found a non-significant association of prenatal PFOA with cortisol and cortisone levels. We observed a positive association between PFOS and DHEA levels, whereas PFOA was inversely associated with DHEA levels. Our results provide new evidence regarding the association of exposure to low levels of PFCs in utero with the concentration of glucocorticoid and androgenic hormones in the next generation.

The values we obtained for glucocorticoids in cord blood samples in our study are comparable with those in cord blood samples both in and outside of Japan (Hasegawa et al. 2010; Anderson et al. 2010). There is a physiologic hypercortisolism during pregnancy, and glucocorticoids are essential for regulating and/or modulating normal physiologic functions in metabolism, growth, neurodevelopment, the immune system, blood pressure maintenance, and fluid and electrolyte homeostasis (Reynolds

2010; Braun et al. 2013). Moreover, glucocorticoids have a crucial role in late gestational lung and heart maturation, and insufficient or excess amounts of these hormones have lifelong adverse effects on the cardiovascular system (Rog-Zielinska et al. 2014; Ishimoto and Jaffe 2011). In addition, cord blood cortisol is lower in infants with intrauterine growth retardation compared with infants with appropriate growth for their gestational age (Strinic et al. 2007). Our findings suggest that dyshomeostasis of glucocorticoids and DHEA at birth are associated with in utero PFCs exposure, and this may have adverse effects on the hypothalamic-pituitary-adrenal (HPA) axis and steroid hormone homeostasis later in life. Therefore, in utero PFC exposure may be a public health concern and longer observations of these effects are warranted.

The fetal adrenal uses large amounts of progesterone supplied by the placenta for cortisol synthesis (Mastorakos and Ilias 2003). PFOS can inhibit the secretion of progesterone in a concentration-dependent manner in human placental syncytiotrophoblasts (Zhang et al. 2015). In addition, we reported that prenatal exposure to PFOS was inversely associated with progesterone levels in cord blood of male and female infants in the same cohort. In contrast, prenatal PFOA levels were positively associated with cord blood progesterone levels in male and female infants (Itoh et al. 2014). Therefore, this may partly explain the negative association of PFOS but not PFOA with glucocorticoids in the current study.

We found that PFOS is associated with a decrease in the cortisol/DHEA ratio and glucocorticoid/androgenic hormone ratio, indicating that PFOS may shift steroidogenesis to androgenic hormones. Additionally, PFCs, especially PFOS, inhibit the activity of several enzymes in the pathway of steroidogenesis in human cells, such as 3β-hydroxysteroid dehydrogenase (HSD3B), that convert pregnenolone to progesterone and DHEA to androstenedione (Zhao et al. 2010). Therefore, these modified enzyme activities may disrupt the balance of C19-steroids (androgenic hormones) and C21-steroids (glucocorticoids). In contrast, PFOA increased these ratios. In this study, we found that the direction of PFOS and PFOA effects on steroids are different. Further studies are necessary to replicate these findings and clarify the mechanistic effects of these PFCs on steroidogenesis.

Our results indicated that prenatal exposure to PFC levels were significantly associated with glucocorticoid and DHEA levels in cord blood.

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    Not yet.

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Table 1. Characteristics of the subjects participating in the Hokkaido Study on Environment and Children's Health, Sapporo, Japan, 2002-2005 (n = 251).

Characteristics	N (%) or mean± SD
Maternal characteristics	
Age (years)	29.9± 4.8
Pre-pregnancy BMI (kg/m^2)	20.8± 2.7
Parity (times)[a]	
0	125 (49.9)
≥1	126 (50.1)
Educational level (years)	
≤12	118 (47.0)
≥13	133 (53.0)
Annual household income (million yen)[a]	
less than 5	180 (72.2)
more than 5	69 (27.8)
Smoking during pregnancy	
Yes	39 (15.5)
No	212 (84.5)
Alcohol intake during pregnancy	
Yes	83 (33.1)
No	168 (66.9)
Caffeine intake during pregnancy (mg/day)	149.6± 123.6
Blood sampling period	
during pregnancy	185 (73.7)
after delivery	66 (26.3)
Gestational age (days)	278.0 ± 7.0
Infant characteristics	
Sex	
Male	111 (44.2)
Female	140 (55.8)

[a]Missing data: annual household income (n=2).

Table 2. Maternal blood PFOS and PFOA levels (ng/mL) in relation to the characteristics of the subjects participating in the Hokkaido Study on Environment and Children's Health, Sapporo, Japan, 2002-2005 (n = 251).

Characteristics	N (%)	PFOS mean ± SD, median (25–75 percentile), or correlation[a] (p-value)	p-Value	PFOA mean ± SD, median (25–75 percentile), or correlation[a] (p-value)	p-Value
Mean (±SD)	251 (100)	5.4 (2.7)		1.5 (0.8)	
Median (25-75 percentile)	251 (100)	5.0 (3.2-6.8)		1.4 (0.9-1.9)	
Maternal characteristics					
Age (years)[a]		$\rho = -0.093$	0.138	$\rho = -0.055$	0.377
Pre-pregnancy BMI (kg/m^2)[a]		$\rho = -0.033$	0.594	$\rho = -0.060$	0.338
Parity (times)[b]					
0	125	6.08 ± 0.24	<0.001	1.89 ± 0.07	<0.001
≥1	126	4.73 ± 0.24		1.12 ± 0.07	
Educational level (years)[b]					
≤12	118	5.27 ± 0.25	0.480	1.44 ± 0.08	0.254
≥13	133	5.52 ± 0.24		1.56 ± 0.07	
Annual household income (million yen)[b, d]					
less than 5	180	5.36 ± 0.20	0.866	1.49 ± 0.06	0.630
more than 5	69	5.43 ± 0.33		1.55 ± 0.10	
Smoking during pregnancy [b]					
Yes	39	4.68 ± 0.44	0.078	1.23 ± 0.14	0.034
No	212	5.53 ± 0.19		1.55 ± 0.06	
Alcohol intake during pregnancy [b]					
Yes	83	5.28 ± 0.30	0.625	1.51 ± 0.09	0.985
No	168	5.46 ± 0.21		1.50 ± 0.06	
Caffeine intake during pregnancy (mg/day)[a]		$\rho = -0.102$	0.104	$\rho = -0.190$	0.010
Blood sampling period [b]					
during pregnancy	185	5.78 ± 0.20	<0.001	1.60 ± 0.06	0.003
after delivery	66	4.34 ± 0.33		1.23 ± 0.10	

Gestational age (days)[a]		$\rho = 0.073$	0.246	$\rho = 0.100$	0.111
Infant characteristics					
Sex[b]					
Male	111	5.74 ± 0.26	0.088	1.59 ± 0.08	0.161
Female	140	5.13 ± 0.23		1.43 ± 0.07	

P-values calculated by [a] Spearman's correlation (ρ), [b] Mann-Whitney U-test.
[d] annual household income (n=2).

Table 3. Concentrations (ng/mL) of steroid hormones in cord blood samples (n=251).

	n	mean	SD	Med	(25th-75th)	>LOD (%)
Cortisol	251	46.5	35.6	37.9	(22.3-63.0)	97.2
Cortisone	251	96.0	41.4	93.5	(68.9-123.3)	94.4
DHEA	251	4.1	9.2	2.2	(1.7-3.0)	100
Androstenedione	251	0.60	0.73	0.46	(0.36-0.59)	100

Table 4. Association of prenatal PFC levels with cord blood glucocorticoids and androgenic hormones (n=251).

	PFOS				PFOA		
	Std β	(95% CI)	p-value		Std β	(95% CI)	p-value
Cortisol							
Crude	-0.225	(-0.888, -0.266)	<0.001		0.145	(0.043, 0.540)	0.021
Adjusted [a]	-0.284	(-1.05, -0.397)	<0.001		0.117	(-0.053, 0.525)	0.109
Cortisone							
Crude	-0.248	(-1.26, -0.437)	<0.001		0.102	(-0.057, 0.613)	0.104
Adjusted [a]	-0.268	(-1.37, -0.471)	<0.001		0.136	(-0.024, 0.761)	0.066
DHEA							
Crude	0.204	(0.115, 0.455)	0.001		-0.128	(-0.276, -0.005)	0.041
Adjusted [a]	0.181	(0.067, 0.436)	0.007		-0.219	(-0.396, -0.085)	0.002
Androstenedione							
Crude	-0.010	(-0.140, 0.119)	0.868		-0.080	(-0.167, 0.035)	0.203
Adjusted [a]	-0.020	(-0.163, 0.119)	0.762		-0.111	(-0.211, 0.027)	0.130
Cortisol/DHEA ratio							
Crude	-0.235	(-1.30, -0.419)	<0.001		0.150	(0.077, 0.787)	0.017
Adjusted [a]	-0.267	(-1.45, -0.502)	<0.001		0.165	(0.062, 0.891)	0.024
Glucorticoid/androgenic hormones ratio							
Crude	-0.247	(-1.44, -0.496)	<0.001		0.131	(0.024, 0.786)	0.037
Adjusted [a]	-0.265	(-1.55, -0.526)	<0.001		0.171	(0.082, 0.972)	0.020

[a]Adjusted for maternal age, parity, smoking, and caffeine intake during pregnancy, blood sampling period, and gestational age.
Both exposure and outcome measures were $\log_{10}$ transformed.
Std β: standardized Beta

Figure 1. The dose-response relationship of prenatal PFOS (A) and PFOA (B) quartiles with glucocorticoid and DHEA levels in cord blood, Sapporo, Japan, 2002-2005 (n=251). The LSMs were adjusted for gestational age, maternal age, smoking, and caffeine intake during pregnancy, parity and the blood sampling period. The LSMs were back transformed from $\log_{10}$ to normal values and the error bars depict the upper and lower 95% CI. Q = quartile.