Association of Perfluoroalkyl and Polyfluoroalkyl Substances With Premature Ovarian Insufficiency in Chinese Women

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Context: Perfluoroalkyl and polyfluoroalkyl substances (PFASs), a group of ubiquitous environmental chemicals with properties of endocrine disruption, are often detectable in humans.

Objective: The current study investigated the association between exposure to PFAS and primary ovarian insufficiency (POI).

Design, Patients, Interventions, and Main Outcome Measures: Levels of plasma PFAS were measured in 120 Chinese women with overt POI and 120 healthy control subjects from 2013 to 2016. Associations between PFAS levels and odds of POI, as well as hormonal profiles, were evaluated using multiple logistic regression and multiple linear regression models.

Results: Levels of perfluorooctanate (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexanesulfonate (PFHxS) were positively associated with the risks of POI (highest *vs.* lowest tertile, PFOA: OR, 3.80; 95% CI, 1.92–7.49; PFOS: OR, 2.81; 95% CI, 1.46–5.41; PFHxS: OR, 6.63; 95% CI, 3.22–13.65). In patients with POI, levels of PFOS and PFHxS exposure were positively associated with FSH (PFOS: adjusted β, 0.26; 95% CI, 0.15 to 0.38; PFHxS: adjusted β, 0.16; 95% CI, 0.04 to 0.28) and negatively associated with estradiol (PFOS: adjusted β, −0.30; 95% CI, −0.47 to −0.12; PFHxS: adjusted β, −0.19; 95% CI, −0.37 to −0.02). Exposure to PFOS and PFOA was associated with elevation of prolactin (PFOS: adjusted β, 0.17; 95% CI, 0.06 to 0.29; PFOA: adjusted β, 0.16; 95% CI, 0.01 to 0.30) or with a decrease of free triiodothyronine (PFOS: adjusted β, −0.88; 95% CI, −1.64 to −0.09; PFOA: adjusted β, −0.90; 95% CI, −1.88 to 0.09) and thyroxine (PFOS: adjusted β, −2.99; 95% CI, −4.52 to −1.46; PFOA: adjusted β, −3.42; 95% CI, −5.39 to −1.46).

Conclusion: High exposure to PFOA, PFOS, and PFHxS is associated with increased risk of POI in humans. (***J Clin Endocrinol Metab*** **103: 2543–2551, 2018)**

Primary ovarian insufficiency (POI) is characterized by a loss of ovarian function before the age of 40 and is a major cause of female infertility (1). POI becomes clinically (primary amenorrhea or secondary amenorrhea for >4 months) and biochemically evident [high FSH, low estradiol (E2)] when the ovarian follicular reserve is

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Abbreviations: AFC, antral follicle count; E2, estradiol; FT3, free triiodothyronine; FT4, free thyroxine; IQR, interquartile range; PFAS, perfluoroalkyl and polyfluoroalkyl substance; PFBS, perfluorobutane sulfonate; PFDeA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexanesulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanate; PFOS, perfluorooctane sulfonate; PFUA, perfluoroundecanoic acid; POI, primary ovarian insufficiency; PRL, prolactin; T3, triiodothyronine; T4, thyroxine; TGAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone.

already severely depleted (2). POI encompasses a wide spectrum of causes, including genetic, autoimmune, infectious, or iatrogenic (chemotherapy or radiation, surgical). The cause remains unknown in >75% of cases (3).

For most women from industrialized countries, menopause occurs at a median age of 50 to 52 (4). Early menopause occurs in ~10% of women before 45 years of age and in 1% to 2% before 40 years of age (5). Environmental factors seem to be the major determinants in ovarian reserve or premature menopause during the prenatal period or adult life (6). Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are persistent synthetic chemicals that are widely used in industrial applications and often detectable in humans (7). Perfluorohexanesulfonate (PFHxS), perfluorononanoate (PFNA), perfluorooctanoate (PFOA), and perfluorooctanesulfonate (PFOS) are used as stain, water, and grease repellents in a wide range of consumer products (8). Human exposure to PFAS mainly occurs orally via the intake of contaminated food, water, and dust (9). Because of the combination of carbon and fluorine atoms in the aliphatic carbon backbone of these substances, in which fluorine has replaced hydrogen atoms, PFAS can resist degradation and persist in extreme environmental and biological condition (10). The mean half-lives of PFOA, PFOS, and PFHxS in human serum have been estimated to be 3.8, 5.4, and 8.5 years, respectively (11). The relationship between PFAS and fertility has been examined in a relatively large number of epidemiologic studies. Exposure to PFOA and PFOS in the general Danish population may cause irregular menstrual periods and increased time until pregnancy (12). A recent epidemiologic study suggested that increased exposure to PFOA, PFOS, PFNA, and PFHxS is associated with higher odds of irregular and long menstrual cycles in women who plan to become pregnant (13). In addition, a National Health and Nutrition Examination Survey evaluation of the US population has revealed the associations between high exposure to PFAS and an increased incidence of subclinical and overt thyroid disease (14). In 202 human serum samples, the levels of PFOS, PFOA, and PFHxS were negatively correlated with triiodothyronine (T3) and thyroxine (T4) (15). Patients with hypothyroidism often show hyperprolactinemia (16). Hyperprolactinemia is a major neuroendocrine-related cause of reproductive disturbances in women, leading to menstrual abnormalities, infertility, and pregnancy loss (17). The aim of this study was to assess the current human epidemiologic evidence of the association between exposure to PFAS and the odds of POI in Chinese women and to explore the potential underlying mechanisms.

Materials and Methods

Study population

This study was designed to assess the correlation between serum PFAS and POI in a Chinese case-control study. Women aged 20 to 40 years who came to the First Affiliated Hospital of Nanjing Medical University for irregular menstruation or amenorrhea from January 2013 to October 2016 were potentially included in this study. Subjects with chromosomal abnormalities a history of radiotherapy or chemotherapy, ovarian surgery, thyroid-related diseases, or thyroid medications were excluded. According to the European Society of Human Reproduction and Embryology guidelines, POI is defined as an elevated FSH level >25 IU/L on two occasions >4 weeks apart and oligo/amenorrhea for at least 4 months (18). A total of 120 women diagnosed with POI and secondary amenorrhea were included. Normal menstruation was the specific matching criterion for control subjects, defined as cycle length of 21 to 35 days and no use of hormonal contraceptives within the past 6 months. A total of 120 participants, 20 to 40 years of age, were randomly chosen (2013 to 2016) as control subjects. Written informed consent was acquired from each subject. The protocol of our study was approved by the Ethics Committees of the First Affiliated Hospital of Nanjing Medical University.

Data collection

Each participant was given an in-person interview by a trained interviewer using a standardized questionnaire to collect information regarding demographic factors (age, occupation, education, and household income), menarche age, menstrual and reproductive history, family history of POI, and lifestyle behaviors (alcohol consumption and smoking). Medical information, including anthropometric variables (height and weight); history of radiotherapy, chemotherapy, surgery, and contraception; chromosome analysis; and gynecological examination was obtained from medical records. A 10-mL blood sample was collected from each participant at the time of enrollment. All blood specimens were centrifuged at 4000 rpm for 10 min. The plasma and serum samples were separated and stored at −80°C until analysis.

Plasma PFAS measurements

Plasma PFAS was measured at the Key Laboratory of Children's Environmental Health in Shanghai, China. The experimental materials and methods have been described by Zhou *et al.* (13) and Wang *et al.* (19). Concentrations of PFAS were detected from 100 μL of plasma using high-performance liquid chromatography/tandem mass spectrometry (Agilent 1290-6490; Agilent Technologies Inc.). After thawing at 4°C, the plasma sample was vortexed with 10 μL of 50 ng/mL internal standard solution ($^{13}C_8$-PFOA) for 30 seconds. Methanol (150 μL) was added before the second vortex. The third vortex was carried out after 150 μL of acetonitrile of 1% formic acid was added. Then, the mixture was sonicated for 20 minutes and centrifuged at 12,000 rpm for 10 minutes. The supernatant was collected and filtered through a 0.22-μm nylon syringe filter into a 1.5-mL auto-sampler vial. The calibration standard and quality control material were prepared by spiking blank fetal bovine serum with the standard mixture of the 10 analytes. The quality control samples were indistinguishable from the plasma

samples, and the laboratory technicians were blinded to subject information. The within-batch coefficients of variation for PFAS concentrations ranged from 0.79% to 8.48%, and the interassay CVs were 1.72% to 8.36%. The limits of detection were 0.09 ng/mL for PFOA and PFOS; 0.02 ng/mL for PFNA, PFHxS, perfluorodecanoic acid (PFDeA), and perfluoroundecanoic acid (PFUA); and 0.009, 0.05, 0.03, and 0.12 ng/mL, respectively, for perfluorobutane sulfonate (PFBS), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), and perfluorooctane sulfonamide. The results obtained from the two independent measurements were averaged.

Measurement of hormones

Blood samples were collected on days 2 to 4 of the menstrual cycle or randomly in noncycling women because the basal levels of FSH and E2 obtained in early follicular phase of the menstrual cycle can be used to evaluate the ovarian reserve (20). Concentrations of serum FSH, luteinizing hormone, E2, prolactin (PRL), and testosterone were detected by chemiluminescence immunoassay (Roche Diagnostics, Germany). Free T3 (FT3), free T4 (FT4), and thyroid-stimulating hormone (TSH) levels were measured by enzyme-linked immunosorbent assay (Kangrun Biotech, China). Thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TGAb) were measured on a Cobas Eless 601 module immunology analyzer (Roche Diagnositics, Switzerland) using the electrochemiluminescence immunoassay method. TPOAb positivity was classified as levels $\geq$35 IU/mL, and TGAb positivity was classified as levels $\geq$40 IU/mL. The intra-assay and interassay coefficients of variation were $<$10%. All hormone assays were repeated three times.

Examination of ovarian antral follicles

Gynecological examination and transvaginal ultrasonography were routinely conducted for each participant. Antral follicle counts (AFCs) were recorded after pelvic ultrasonography. AFC was defined as the number of follicles 2 to 10 mm in length in the early follicular phase.

Statistical analysis

Comparisons of descriptive statistics and baseline characteristics by case status were performed with the Student t test and Pearson χ^2 test. Hormone concentrations or AFC were compared using binary logistic regression analysis. The percentages of TGAb- and TPOAb-positive subjects were compared with the Pearson χ^2 test. We analyzed the distribution of PFAS in control subjects and patients with POI using the median and interquartile range (IQR) (19). Differences of PFAS concentrations were compared between control subjects and patients with POI using the binary logistic regression analysis. To explore the correlation of plasma PFAS levels and POI, unconditional logistic regression models were performed to calculate the OR and 95% CI. Because the relationship between exposure of PFAS and POI may not be monotonically linear, PFAS concentrations were categorized into tertiles to model each PFAS analyte as a set of indicator variables, with the lowest tertile considered as the referent (19). To test the trend across categories of PFAS concentrations, a continuous variable coded as the median tertile concentration of each exposure category was included in the adjusted logistic regression model (19). Using the multiple linear regression models, we investigated the association between PFAS exposure and hormone levels. Concentrations of PFAS and sexual hormone were treated as a continuous variable after log transformation.

Based on previous studies and our univariate analysis, we identified the following variables as potential confounders and adjusted them in the multivariate logistic regression and multiple linear regression models: age, body mass index, education, household income, sleep, and parity. The vast majority of women were nonsmokers and nondrinkers, so these two variables were not considered for adjustment. Because only one control subject and three case subjects had a family history of POI, this factor was not adjusted as a confounder. Because consumption of oral contraceptives may be a consequence rather than a determinant of POI, we did not include oral contraceptives as a covariate to avoid inducing potential overadjustment. All statistical analyses were performed with SAS software 9.2 (SAS Institute, Inc., Cary, NC). A P value $<$0.05 was considered statistically significant.

Results

Basic characteristics

Basic characteristics of patients with POI (n = 120) and control subjects (n = 120) are shown in Table 1. The average age between patients with POI and control subjects was generally similar without changes in abnormal sleep quality and history of smoking or drinking. There were no significant differences in age at menarche, body mass index, parity, income, and education between patients with POI and control subjects.

Endocrine profiles and ovarian follicular reserve

With ultrasound examination, the number of follicles of 2 to 10 mm in the early follicular phase was recorded. The AFC was zero in 32 patients with POI. The mean and standard error of the AFC was 2.53 $\pm$ 0.21 and 16.44 $\pm$ 0.28 in patients with POI and healthy control subjects, respectively.

The levels of serum FSH ($P < 0.001$) and luteinizing hormone ($P < 0.001$) in patients with POI were significantly elevated, whereas the level of E2 ($P < 0.001$) was decreased compared with control subjects (Table 2). Additionally, serum PRL level ($P = 0.041$) in patients with POI was higher than that in the control subjects.

In comparison with the control subjects, patients with POI showed decreases in the levels of FT3 ($P < 0.001$) and FT4 ($P < 0.001$), which was associated with an increase in the level of TSH ($P < 0.001$). There were no significant differences in TGAb-positive percentage ($P = 0.640$) and TPOAb-positive percentage ($P = 0.511$) between the two groups.

Distribution of plasma PFAS concentrations

The concentrations of plasma PFAS in patients with POI and control subjects are shown in Table 3. The detection rates were 91.7% for PFBS, 99.2% for PFDoA,

Table 1. Baseline Characteristics of Patients With POI and Control Subjects

	POI (n = 120)	Control (n = 120)	*P* Value
Age, y (mean ± SD)	28.9 ± 5.6	29.7 ± 4.6	0.279[a]
BMI, kg/m (mean ± SD)	21.9 ± 3.6	21.3 ± 1.9	0.138[a]
Age at menarche, y (mean ± SD)	14.0 ± 1.5	13.7 ± 1.3	0.067[a]
Parity			0.366[b]
Nulliparous, n (%)	66 (55.0)	59 (49.2)	
Parous, n (%)	54 (45.0)	61 (50.8)	
Sleep			0.382[b]
Occasional sleeplessness, n (%)	98 (81.7)	103 (85.5)	
Frequent insomnia	22 (18.3)	17 (14.2)	
Education, y			0.502[b]
Lower than college, n (%)	46 (38.3)	41 (34.2)	
Completed college, n (%)	74 (61.7)	79 (65.8)	
Income (10^3 CNY)			0.405[b]
<10, n (%)	38 (31.7)	41 (34.2)	
10–15, n (%)	45 (37.5)	53 (44.2)	
15–30, n (%)	29 (24.2)	19 (15.8)	
>30, n (%)	8 (6.7)	7 (5.8)	
Alcohol consumption			0.472[b]
Never or occasional, n (%)	117 (97.5)	115 (95.8)	
Regular, n (%)	3 (2.5)	5 (4.2)	
Smoking			0.561[b]
Never or occasional, n (%)	118 (98.3)	119 (99.2)	
Regular, n (%)	2 (1.7)	1 (0.8)	
Family history of POI			0.313[b]
No	117 (97.5)	119 (99.2)	
Yes	3 (2.5)	1 (0.8)	

Abbreviations: BMI, body mass index; SD, standard deviation.

[a]Student *t* test.

[b]Pearson χ^2 test.

97.5% for PFHpA, and 100% for other compounds. Because the detection rate of perfluorooctane sulfonamide in our participants was only 2.9%, it not further analyzed.

The median and IQR of PFOA [11.1 (7.60 to 14.45) ng/mL], PFOS [8.18 (5.50 to 13.51) ng/mL], and PFHxS [0.38 (0.29 to 0.67) ng/mL] in patients with POI were higher than PFOA [8.35 (6.27 to 11.31) ng/mL, $P < 0.001$], PFOS [6.02 (4.24 to 9.11) ng/mL, $P < 0.001$], and PFHxS [0.29 (0.22 to 0.37) ng/mL, $P = 0.001$] in the control subjects. The concentrations of plasma PFBS ($P = 0.958$), PFHpA ($P = 0.813$), PFDeA ($P = 0.376$), PFUA ($P = 0.212$), PFNA ($P = 0.119$), and PFDoA ($P = 0.675$) were not significantly different between patients with POI and control subjects.

Table 2. Concentrations of Sex and Thyroid Hormones and AFC in Patients With POI and Control Subjects

Characteristics	POI[a] (n = 120)	Control[a] (n = 120)	*P* Value	Normal Range
FSH, IU/L	71.65 ± 2.99	6.18 ± 0.11	0.000[b]	3.85–8.78
LH, IU/L	31.77 ± 1.57	5.83 ± 0.13	0.000[b]	2.12–10.89
E2, pmol/L	55.28 ± 3.66	211.87 ± 5.83	0.000[b]	99.1–447.7
PRL, mIU/L	295.50 ± 11.90	263.88 ± 9.83	0.041[b]	70.8–566.4
T, nmol/L	1.05 ± 0.05	1.17 ± 0.11	0.079[b]	0.35–2.60
FT3, pmol/L	3.66 ± 0.12	4.26 ± 0.07	0.000[b]	3.10–6.80
FT4, pmol/L	14.87 ± 0.25	16.01 ± 0.19	0.000[b]	12.00–22.00
TSH, mIU/L	3.56 ± 0.14	2.40 ± 0.08	0.000[b]	0.27–4.20
TGAb positive, n (%)	9 (7.5)	11 (9.2)	0.640[c]	<40 IU/mL
TPOAb positive, n (%)	10 (8.3)	13 (10.8)	0.511[c]	<35 IU/mL
AFC	2.53 ± 0.21	16.44 ± 0.28	0.000[b]	>5–7

Abbreviations: AFC, antral follicle counts; LH, luteinizing hormone.

[a]Values are mean ± standard error.

[b]Binary logistic regression analysis.

[c]Pearson χ^2 test.

Table 3. Concentrations of PFAS in Patients With POI and Control Subjects

			POI (n = 120)			Control (n = 120)			
PFAS	LOD (ng/mL)	Percent > LOD (%)	25%	50%	75%	25%	50%	75%	*P* Value[a]
PFOA	0.09	100	7.60	11.10	14.45	6.27	8.35	11.31	0.000
PFOS	0.09	100	5.50	8.18	13.51	4.24	6.02	9.11	0.000
PFNA	0.02	100	1.34	2.07	3.02	1.32	1.81	2.69	0.119
PFHxS	0.02	100	0.29	0.38	0.67	0.22	0.29	0.37	0.001
PFDeA	0.02	100	1.04	1.80	2.95	1.02	1.74	2.56	0.376
PFUA	0.02	100	0.86	1.42	2.21	0.83	1.26	1.91	0.212
PFBS	0.009	91.7	0.02	0.06	0.10	0.04	0.05	0.12	0.958
PFDoA	0.05	99.2	0.11	0.17	0.25	0.12	0.17	0.24	0.675
PFHpA	0.03	97.5	0.11	0.19	0.30	0.14	0.20	0.29	0.813

Abbreviation: LOD, limit of detection.

[a]Binary logistic regression analysis.

Associations of PFAS levels with POI

The results shown in Table 4 indicate that the concentrations of PFOA, PFOS, and PFHxS were associated with increased risks of POI. Trend tests indicated that there were dose-response patterns for PFOA ($P_{trend} < 0.01$), PFOS ($P_{trend} < 0.01$), and PFHxS ($P_{trend} < 0.01$). In the adjusted model, a unit increase in PFOA, PFOS, and PFHxS was associated with significantly increased odds of POI (PFOA, second *vs*. lowest tertile: OR, 1.34; 95% CI, 0.70 to 2.58; highest *vs*. lowest tertile: OR, 3.80; 95% CI, 1.92 to 7.49; PFOS, second *vs*. lowest tertile: OR, 1.75; 95% CI, 0.91 to 3.38; highest *vs*. lowest tertile: OR, 2.81; 95% CI, 1.46 to 5.41; PFHxS, second *vs*. lowest tertile: OR, 2.04; 95% CI, 1.03 to 4.04; highest *vs*. lowest tertile: OR, 6.63; 95% CI, 3.22 to 13.65). No meaningful relationships were found between the other six PFASs (PFBS, PFHpA, PFDeA, PFUA, PFNA, PFDoA) and the odds of POI. Thus, we have presented detailed results only in the former three PFAS.

Associations between PFAS levels and sex hormones

According to adjusted multiple linear regression, the levels of PFOS and PFHxS exposure in patients with POI were positively associated with FSH concentration (PFOS: adjusted β, 0.26; 95% CI, 0.15 to 0.38; PFHxS: adjusted β, 0.16; 95% CI, 0.04 to 0.28) and negatively associated with the E2 level (PFOS: adjusted β, −0.30; 95% CI, −0.47 to −0.12; PFHxS: adjusted β, −0.19; 95% CI, −0.37 to −0.02) (Table 5). In addition, each log-unit increase in PFOA and PFOS exposure was associated with the high concentration of PRL in patients with POI (PFOA: adjusted β, 0.16; 95% CI, 0.01 to 0.30; PFOS: adjusted β, 0.17; 95% CI, 0.06 to 0.29). The concentrations of PFOS, PFOA, and PFHxS in the control group did not show an association with sexual hormones (Supplemental Table 1).

Associations between PFAS and thyroid hormones

Table 6 shows that, in patients with POI, each log-unit increase in PFOA and PFOS exposure was associated with the decreased concentration of FT3 (PFOA: adjusted β, −0.90; 95% CI, −1.88 to 0.09; PFOS: adjusted β, −0.88; 95% CI, −1.64 to −0.09) and FT4 (PFOA: adjusted β, −3.42; 95% CI, −5.39 to −1.46; PFOS: adjusted β, −2.99; 95% CI, −4.52 to −1.46), which were associated with the increase in TSH (PFOA: adjusted β, 1.39; 95% CI, 0.18 to 2.59; PFOS: adjusted β, 1.57; 95% CI, 0.65 to 2.50). The increase of plasma PFOA or PFOS was positively associated with TSH concentration in the control subjects (PFOA: adjusted β, 1.65; 95% CI, 0.86 to 2.44; PFOS: adjusted β, 0.67; 95% CI, 0.08 to 1.26) (Supplemental Table 2). However, the levels of plasma PFHxS in patients with POI and control subjects were not associated with FT3 concentration (POI: adjusted β, −0.05; 95% CI, −0.82 to 0.73; control: adjusted β, −0.29; 95% CI, −0.97 to 0.38), FT4 concentration (POI: adjusted β, −0.55; 95% CI, −2.15 to 1.04; control: adjusted β, −0.38; 95% CI, −2.12 to 1.36), and TSH (POI: adjusted β, 0.88; 95% CI, −0.06 to 1.82; control: adjusted β, 0.33; 95% CI, −0.39 to 1.06).

Discussion

This study examined PFAS exposure in relation to the clinical characteristics and ovarian follicular reserve of patients with POI. In comparison with control subjects, the levels of plasma PFOA, PFOS, and PFHxS in patients with POI were significantly increased, whereas the levels of PFBS, PFHpA, PFDeA, PFUA, PFNA, PFDoA were not. In 120 patients with POI, primary amenorrhea; chromosomal abnormalities; and history of radiotherapy, chemotherapy, or ovarian surgery had been excluded. Thus, the results obtained from this study indicate that high exposure to PFOA, PFOS, and PFHxS may be associated with increased risks of POI.

Most of the epidemiological research on PFAS has centered on PFOS and PFOA because they are the two PFASs present in the highest concentrations in humans

Table 4. Concentrations of PFAS and Odds of POI (N = 240)

PFAS	Tertiles (ng/mL)	POI, n (%)	Control, n (%)	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
PFOA	First (1.65–7.56)	30 (25.0)	50 (41.7)	1.00 (reference)	1.00 (reference)
	Second (>7.56–11.7)	36 (30.0)	44 (36.7)	1.36 (0.73–2.56)	1.34 (0.70–2.58)
	Third (>11.7–116.7)	54 (45.0)	26 (21.6)	3.46 (1.81–6.64)	3.80 (1.92–7.49)
	P trend[b]			<0.01	<0.01
PFOS	First (1.03–5.46)	30 (25.0)	50 (41.7)	1.00 (reference)	1.00 (reference)
	Second (>5.46–9.10)	40 (33.3)	39 (32.5)	1.71 (0.91–3.22)	1.75 (0.91–3.38)
	Third (>9.10–47.8)	50 (41.7)	31 (25.8)	2.69 (1.42–5.08)	2.81 (1.46–5.41)
	P trend[b]			<0.01	<0.01
PFNA	First (0.34–1.47)	39 (32.5)	41 (34.2)	1.00 (reference)	1.00 (reference)
	Second (>1.47–2.45)	36 (30.0)	43 (35.8)	0.88 (0.47–1.64)	0.96 (0.50–1.85)
	Third (>2.45–9.56)	45 (37.5)	36 (30.0)	1.31 (0.71–2.44)	1.34 (0.70–2.54)
	P trend[b]			0.33	0.34
PFHxS	First (0.09–0.27)	25 (20.8)	53 (44.2)	1.00 (reference)	1.00 (reference)
	Second (>0.27–0.39)	37 (30.8)	43 (35.8)	1.82 (0.96–3.49)	2.04 (1.03–4.04)
	Third (>0.39–8.46)	58 (48.4)	24 (20.0)	5.12 (2.62–10.04)	6.63 (3.22–13.65)
	P trend[b]			<0.01	<0.01
PFDeA	First (0.19–1.24)	38 (31.7)	41 (34.2)	1.00 (reference)	1.00 (reference)
	Second (>1.24–2.42)	38 (31.7)	42 (35.0)	0.98 (0.52–1.82)	1.03 (0.54–1.96)
	Third (>2.42–18.1)	44 (36.6)	37 (30.8)	1.28 (0.69–2.39)	1.36 (0.71–2.60)
	P trend[b]			0.39	0.33
PFUA	First (0.16–0.97)	40 (33.4)	40 (33.4)	1.00 (reference)	1.00 (reference)
	Second (>0.97–1.66)	37 (30.8)	43 (35.8)	0.86 (0.46–1.60)	0.95 (0.50–1.82)
	Third (>1.66–8.08)	43 (35.8)	37 (30.8)	1.16 (0.63–2.16)	1.28 (0.67–2.46)
	P trend[b]			0.58	0.43
PFBS	First (0.000–0.039)	44 (36.7)	37 (30.8)	1.00 (reference)	1.00 (reference)
	Second (>0.039–0.091)	37 (30.8)	42 (35.0)	0.74 (0.40–1.38)	0.84 (0.44–1.60)
	Third (>0.091–4.684)	39 (32.5)	41 (34.2)	0.80 (0.43–1.49)	0.92 (0.48–1.76)
	P trend[b]			0.60	0.90
PFDoA	First (0.03–0.13)	41 (34.2)	37 (30.8)	1.00 (reference)	1.00 (reference)
	Second (>0.13–0.21)	41 (34.2)	41 (34.2)	0.90 (0.49–1.68)	0.90 (0.48–1.71)
	Third (>0.21–1.35)	38 (31.6)	42 (35.0)	0.82 (0.44–1.53)	0.85 (0.45–1.62)
	P trend[b]			0.53	0.63
PFHpA	First (0.003–0.148)	44 (36.7)	35 (29.1)	1.00 (reference)	1.00 (reference)
	Second (>0.148–0.257)	34 (28.3)	47 (39.2)	0.58 (0.31–1.08)	0.63 (0.33–1.20)
	Third (>0.257–4.972)	42 (35.0)	38 (31.7)	0.88 (0.47–1.64)	0.97 (0.51–1.85)
	P trend[b]			0.77	0.97

[a] Adjusted for age (continuous), body mass index (continuous), education (categorical), income (categorical), sleep (categorical), and parity (categorical).
[b] *P* value for test of trend across tertiles.

(21). The levels of plasma PFOA, PFOS, and PFHxS in the general U.S. population (2013 to 2014) were reported to be 1.66 (1.48 to 1.87) ng/mL, 3.96 (3.60 to 4.35) ng/mL, and 1.01 (0.91 to 1.12) ng/mL, respectively (22). An American study from the DuPont Washington Works Plant near Parkersburg, West Virginia, which is known as a heavily polluted area (23), reported that the median PFOA and PFOS levels were 17.6 and 15.0 ng/mL, respectively, in women aged 18 to 42 years (n = 13,458, 2005 to 2006). The median and interquartile range of PFOA, PFOS, and PFHxS in women of reproductive age in China (2000 to 2015, n = 178) were reported to be 12.09 (7.33 to 22.59) ng/mL, 6.60 (3.92 to 13.54) ng/mL, and 0.32 (0.24 to 0.49) ng/mL, respectively (19). The median and IQR of PFOA, PFOS, and PFHxS in women preconception in Shanghai, China (2013 to 2015, n = 950) were recently reported to be 13.84 (10.08 to 18.83) ng/mL, 10.49 (7.55 to 15.37) ng/mL, and 0.69 (0.56 to 0.88) ng/mL, respectively (13). The median and IQR of PFOA, PFOS, and PFHxS in women of Nanjing, China, were 8.35 (6.27 to 11.31) ng/mL, 6.02 (4.24 to 9.11) ng/mL, and 0.29 (0.22 to 0.37) ng/mL, respectively, from all our participants.

Our results show that patients with POI had a higher exposure to PFOA [11.1 (7.60 to 14.45) ng/mL], PFOS [8.18 (5.50 to 13.51) ng/mL], and PFHxS [0.38 (0.29 to 0.67) ng/mL] than control subjects. Because samples prior to ceased or disrupted menstruation were not collected, we could not compare the levels of PFAS before and after amenorrhea to determine whether the high exposure of PFAS caused their amenorrhea or was a result of it. Regardless, the INUENDO cohort enrolled 1623 pregnant women in three countries (Greenland, Poland, and Ukraine), and reported serum PFOA was positively associated with long menstrual cycles (24). The association between PFOA or PFOS and menstrual

Table 5. Associations Between PFAS and Sex Hormones in Patients With POI (n = 120)

	PFOA[a]		PFOS[a]		PFHxS[a]	
Sex Hormones	Crude β (95% CI)	Adjusted[b] β (95% CI)	Crude β (95% CI)	Adjusted[b] β (95% CI)	Crude β (95% CI)	Adjusted[b] β (95% CI)
FSH						
Continuous[a]	0.10 (−0.06 to 0.25)	0.09 (−0.07 to 0.25)	0.28 (0.16 to 0.39)[c]	0.26 (0.15 to 0.38)[c]	0.18 (0.06 to 0.30)[c]	0.16 (0.04 to 0.28)[c]
LH						
Continuous[a]	−0.19 (−0.40 to 0.02)	−0.20 (−0.42 to 0.02)	−0.14 (−0.30 to 0.03)	−0.13 (−0.31 to 0.04)	−0.06 (−0.22 to 0.11)	−0.07 (−0.24 to 0.10)
E2						
Continuous[a]	−0.10 (−0.32 to 0.13)	−0.08 (−0.31 to 0.16)	−0.32 (−0.49 to −0.14)[c]	−0.30 (−0.47 to −0.12)[c]	−0.22 (−0.39 to −0.04)[c]	−0.19 (−0.37 to −0.02)[c]
PRL						
Continuous[a]	0.15 (0.00 to 0.29)[c]	0.16 (0.01 to 0.30)[c]	0.17 (0.06 to 0.28)[c]	0.17 (0.06 to 0.29)[c]	0.10 (−0.01 to 0.21)	0.11 (−0.01 to 0.22)
Testosterone						
Continuous[a]	−0.13 (−0.29 to 0.04)	−0.11 (−0.29 to 0.06)	−0.03 (−0.16 to 0.11)	−0.02 (−0.16 to 0.12)	−0.04 (−0.17 to 0.09)	−0.03 (−0.16 to 0.11)

Data are presented as ng/mL.

Abbreviation: LH, luteinizing hormone.

[a]Log-transformed PFAS and sex hormones as continuous variables.

[b]Adjusted for age (continuous), body mass index (continuous), education (categorical), income (categorical), sleep (categorical), and parity (categorical).

[c]$P < 0.05$, multiple linear regression models.

irregularity and length has been reported in a subset of 1240 pregnant women randomly selected from the Danish National Birth Cohort (12). Similarly, the pre-pregnant women exposed to high levels of PFOA, PFOS, and PFHxS have an increased odds of irregular menstrual cycles (13). Vélez *et al.* (25) reported that exposure to PFHxS in the general Canadian population may increase the time to pregnancy and the risk of infertility. Moreover, a cross-sectional analysis of women from the C8 Health Project (n = 25,957) revealed associations between higher PFOA and PFOS exposure and earlier menopause (23). This body of literature and our results indicate that high exposure to PFOA, PFOS, and PFHxS is associated with increased risk of POI and the irregular cyclicity reported with the condition.

PFOS is known to have weak estrogenic activities, whereas it exerts an antiestrogenic effect when coadministered with estradiol (26). Our data obtained from patients with POI showed that the levels of PFOS and PFHxS were negatively associated with E2 and positively associated with FSH. There was no association between the levels of PFBS, PFHpA, PFDeA, PFUA, PFNA, and PFDoA and the level of E2 or FSH (Supplemental Table 3). PFOS concentration is negatively associated with serum estradiol in perimenopausal and menopausal women (23). PFOS has been reported to decrease production of estradiol in women of reproductive age (27) and to alter steroidogenesis (28). The exposure of mice to PFOS (0.1 mg/kg) through selectively reducing histone acetylation of ovarian steroidogenic acute regulatory promoter suppresses the ovarian hormone production and impairs follicular development and ovulation (29), suggesting that high exposure to PFOA and PFOS may reduce the ovarian follicular reserve.

On the other hand, the levels of T3 and T4 in patients with POI were decreased in comparison with control subjects, although they did not exceed the normal range. The levels of PFOS and PFOA were negatively associated with FT3 and FT4 levels in patients with POI, but the levels of PFHxS, PFBS, PFHpA, PFDeA, PFUA, PFNA, and PFDoA were not (Supplemental Table 4). The most consistent effect of exposure to PFOA and PFOS is the occurrence of hypothyroidism, particularly in women and children (30). Exposure to PFOS reduced serum T4 and T3 in rats and monkeys (31, 32). PFOS and PFOA have been found to reduce the synthesis of thyroid

Table 6. Associations Between PFAS and Thyroid Hormones in Patients With POI (n = 120)

	PFOA[a]		PFOS[a]		PFHxS[a]	
Thyroid Hormones	Crude β (95% CI)	Adjusted[b] β (95% CI)	Crude β (95% CI)	Adjusted[b] β (95% CI)	Crude β (95% CI)	Adjusted[b] β (95% CI)
FT3						
Continuous	−0.84 (−1.80 to 0.12)	−0.90 (−1.88 to 0.09)	−0.84 (−1.61 to −0.07)[c]	−0.88 (−1.64 to −0.09)[c]	0.04 (−0.71 to 0.79)	−0.05 (−0.82 to 0.73)
FT4						
Continuous	−3.00 (−5.00 to −1.00)[c]	−3.42 (−5.39 to −1.46)[c]	−3.05 (−4.62 to −1.48)[c]	−2.99 (−4.52 to −1.46)[c]	−0.31 (−1.91 to 1.29)	−0.55 (−2.15 to 1.04)
TSH						
Continuous	1.22 (0.06 to 2.38)[c]	1.39 (0.18 to 2.59)[c]	1.54 (0.63 to 2.45)[c]	1.57 (0.65 to 2.50)[c]	0.75 (−0.16 to 1.66)	0.88 (−0.06, 1.82)

Data are presented as ng/mL.

[a]Log-transformed PFAS as continuous variables.

[b]Adjusted for age (continuous), body mass index (continuous), education (categorical), income (categorical), sleep (categorical), and parity (categorical).

[c]$P < 0.05$, multiple linear regression models.

hormone (33) and to enhance the metabolism and clearance of thyroid hormone (34). In addition, we observed a significant increase of serum PRL level in patients with POI, and exposure to PFOA and PFOS was positively associated with the level of PRL. PRL elevation was reported in 21% of patients with overt hypothyroidism and in 8% of patients with subclinical hypothyroidism (35). It is likely that the decline in thyroid hormone enhances thyroid-releasing hormone secretion, which promotes the release of TSH and PRL (36). However, the levels of PFOS and PFOA were positively associated with TSH concentration in patients with POI and in control subjects. There are conflicting reports describing a negative association of PFOS with TSH (37) or no association with TSH (38), which likely depends on length of time and level of exposure to one or more PFAS. Thus, further experiments are required to examine the direct role of PFOS and PFOA in the secretion of thyroid-releasing hormone and TSH. Hyperprolactinemia is a major cause of reproductive disturbances in women and female rats (36). During lactation, the high level of PRL suppresses follicular development and ovulation (39). However, because PRL level in patients with POI was within normal range, it is unlikely the increased PRL is solely involved in earlier menopause and decline of ovarian reserve.

Conclusion

The results in the current study show that high plasma concentrations of PFOA, PFOS, and PFHxS are associated with an increased risk of POI in Chinese women. Knox *et al.* (23) reported that levels of serum PFOS and PFOA were higher in women 40 to 55 years of age who had a hysterectomy compared with women who had not. Because in the current study we did not examine the timing of PFAS exposure relative to menopause in patients with POI, our ability to make a causal inference is limited. One possible causal explanation for an association between PFAS and POI is that high exposures to PFOA and PFOS suppress ovarian hormone production and impair follicular development, resulting in the loss of ovarian function and earlier menopause. Although studies have shown a decrease in body burdens of PFOA and PFOS after restriction of usage since the early 2000s (40), exposure to PFOS and PFOA has continued in China and other countries. Moreover, the reproductive health effect of PFHxS is an important concern because concentrations of the "newer" PFAS may be rising (22). The exposures to PFOS, PFOA, and PFHxS in this study occur as a mixture. Human blood contains several kinds of perfluorinated compounds, which are thought to have similar effects (13, 41). Therefore, the findings in this study may have important public health implications for female reproductive health.

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