

Plasma Perfluoroalkyl and Polyfluoroalkyl Substances Concentration and Menstrual Cycle Characteristics in Preconception Women

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BACKGROUND: Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are persistent synthetic chemicals that are widely used in industrial applications and often detectable in humans. In rats, PFASs can interfere with the estrous cycle. In humans, menstruation has been viewed as a proxy of female fecundity, and periodic menstruation plays a critical role in endometrial sloughing in the absence of pregnancy and in preparing for embryo implantation.

OBJECTIVES: We investigated the association between PFAS exposure and menstrual cycle characteristics in women who plan to become pregnant.

METHODS: Plasma level of 10 PFASs was measured in 950 women who were attempting to become pregnant and recruited in two preconception care clinics in Shanghai, China, from August 2013 to April 2015. Information on menstrual cycle characteristics was collected by questionnaires. Associations between PFAS levels and menstrual cycle regularity, length, and bleeding volume were examined using multiple logistic regression models.

RESULTS: Pre-pregnant women with higher levels of log-transformed perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexanesulfonate (PFHxS) had increased odds of self-reported history of irregular menstrual cycle [PFOA-adjusted odds ratio (OR) = 1.52 (95% CI: 1.08, 2.15); PFOS OR = 1.29 (95% CI: 0.98, 1.70); PFNA OR = 1.50 (95% CI: 1.03, 2.07); PFHxS OR = 1.80 (95% CI: 1.17, 2.77)] and long menstrual cycle [PFOA OR = 1.50 (95% CI: 1.06, 2.10); PFOS OR = 1.34 (95% CI: 1.02, 1.75); PFNA OR = 1.49 (95% CI: 1.05, 2.11); PFHxS OR = 1.73 (95% CI: 1.13, 2.65)]. Log-transformed PFOA, PFOS, PFNA, and PFHxS levels were negatively associated with self-reported history of menorrhagia [PFOA OR = 0.37 (95% CI: 0.21, 0.65); PFOS OR = 0.57 (95% CI: 0.37, 0.90); PFNA OR = 0.47 (95% CI: 0.26, 0.86); PFHxS OR = 0.14 (95% CI: 0.06, 0.36)].

CONCLUSIONS: Certain PFASs are associated with abnormal menstruation in humans. <https://doi.org/10.1289/EHP1203>

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are persistent man-made chemicals that have been produced since the 1950s. These compounds are characterized by hydrophobicity and oleophobicity and are extensively used in a wide range of consumer and industrial applications, such as surfactants, adhesives, repellents, food packaging, and fire-fighting foams (Butenhoff et al. 2006). Some PFASs have a long half-life in human body. For example, the half-life of perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexanesulfonate (PFHxS) has been estimated at 3.8, 5.4, and 8.5 y, respectively (Olsen et al. 2007). Although studies have shown a decrease in body burdens of PFOA and PFOS after restriction of usage in some countries since the early 2000s (Haug et al. 2009; Sundström et al. 2011), the production of PFOS and PFOA has

continued in China. Human health concerns regarding exposure to low-level PFASs continue.

The toxicity of PFASs has been extensively studied in experimental animals, with developmental toxicity, carcinogenicity, hepatotoxicity, immunotoxicity, and hormonal effects identified as the effects of most concern (Kennedy et al. 2004; Lau et al. 2007). PFASs may also interfere with reproductive functions, but epidemiologic studies are still limited and inconsistent. Vélez et al. (2015) reported that exposure to PFOA and PFHxS at the levels found in the general Canada population may reduce fecundity, indicated by increased time to pregnancy (TTP) and risks of infertility. A Danish National Birth Cohort also linked maternal serum concentrations of PFOS and PFOA with subfecundity (Fei et al. 2009). In the study conducted by Buck Louis et al. (2013), perfluorooctane sulfonamide (PFOSA) exposure was associated with 18% reduced fecundability {adjusted odds ratio for fertility = 0.82 [95% confidence interval (CI): 0.71, 0.95]} albeit only 10% of the samples had PFOSA levels above the detection limit. However, other studies observed no association between PFASs and TTP or subfecundity (Bach et al. 2015; Vestergaard et al. 2012; Whitworth et al. 2012). It should be pointed out that some studies were retrospective (Bach et al. 2015; Fei et al. 2009; Vélez et al. 2015; Whitworth et al. 2012), whereas others were prospective (Buck Louis et al. 2013; Vestergaard et al. 2012).

In addition, some studies stratified their analysis by parity or focused only on nulliparous women. They found that positive results could only be observed in parous, not nulliparous women (Bach et al. 2015; Vestergaard et al. 2012; Whitworth et al. 2012). Reverse causality was postulated for this phenomenon (Bach et al. 2015). Previous births may result in lower PFAS levels, and parous women with longer TTP would have had more time to reaccumulate PFASs. However, Vélez et al. (2016) argued that adjusting or stratifying on parity is redundant and would cause over-adjustment, as parity is the result, among other factors, of proven fecundability.

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Menstruation has long been viewed as a proxy of female fecundity (Buck Louis et al. 2011; Harlow et al. 2013). Dysfunction of menstrual cycle is a major cause of infertility (Harlow and Ephross 1995). Irregular and long cycles have been related to lower fecundity (Jensen et al. 1999; Mumford et al. 2012). Animal and human evidences suggest that PFASs affect steroidogenesis and hormone levels manifesting in altered menstrual cycles such as prolonged lengths (Barrett et al. 2015; Feng et al. 2015; Tsai et al. 2015). In animal studies, a 2-wk exposure to PFOS (10 mg/kg) has been reported to cause persistent diestrus in rats (Austin et al. 2003). In mice, the chronic exposure to a low-dose PFOS (0.1 mg/kg/d) has also resulted in estrous cyclicity disruption in adult females (Feng et al. 2015). A recent epidemiologic study suggested that menstrual cycles may be lengthened in women with the highest serum concentrations of PFOA in comparison to those with the lowest concentrations (Lyngsø et al. 2014). But evidence from Asia is still lacking. The objective of this study was to investigate this association in women who attempted to conceive in China.

Methods

Study Population

To reduce the incidence of birth defects and improve pregnancy outcomes, the Chinese government has been promoting free pre-conception care nationwide in recent years (Yang et al. 2015). Between August 2013 and April 2015, the Shanghai Birth Cohort Study enrolled women who came for the care at two preconception care clinics in Shanghai, China. Included were couples who were at least 20 y of age, registered residents of Shanghai with no plan to move out of Shanghai in the next 2 y, had stopped using contraception, and planned to conceive naturally and give birth in the collaborating hospitals. Women who had tried continuously to conceive spontaneously for more than 1 y without success or had sought medical assistance to conceive were excluded.

At the time of enrollment, the participants were interviewed by a trained research staff regarding demographic and lifestyle characteristics, environmental factors, and reproductive and medical history. Blood samples were collected. A total of 1,182 women were recruited. Some women did not provide blood samples. Thus, only 950 women had complete PFASs exposure data available. The study was approved by the local ethical committees.

Exposure to PFASs

Blood samples were collected from women at the time of their enrollment and then were stored in freezers at -80°C until tested. PFASs were measured at the Shanghai Key Laboratory of Children's Environmental Health, China. Altogether, 10 PFASs were analyzed, including PFOA, PFOS, perfluorononanoic acid (PFNA), PFHxS, perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUA), perfluorobutane sulfonate (PFBS), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), and PFOSA.

PFASs concentrations were measured from 100 μL of plasma using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS-MS) (Agilent 1290–6490; Agilent Technologies Inc., USA). After the sample was thawed at 4°C , 100 μL of plasma sample was vortexed with 10 μL of 50 ng/mL internal standard solution ($^{13}\text{C}_8$ -PFOA) for 30 sec. Then 150 μL of methanol was added before the second vortex. The third vortex was performed after adding 150 μL acetonitrile of 1% formic acid. The mixture was sonicated for 20 min and then centrifuged for 10 min at 12,000 rpm. The supernatant was collected and then filtered through a 0.22- μm nylon syringe filter into a

1.5-mL auto-sampler vial. Calibration standards and quality control materials were prepared by spiking blank fetal bovine serum with the standard mixture of the 10 analytes. Carbon-isotope labeled internal standards were added each time before extraction. Lab technicians were blinded to participant information, and the quality control samples were indistinguishable from the plasma samples. The within-batch coefficients of variation for PFASs concentrations ranged from 0.79% to 8.48%, and the interassay CVs from 1.72% to 8.36%.

All samples had PFOA, PFOS, PFNA, PFHxS, PFDeA, and PFUA above the limit of detection (LOD) (PFOA and PFOS: 0.09 ng/mL; PFNA, PFHxS, PFDeA, and PFUA: 0.02 ng/mL). The other six PFASs were detected in at least 97% of the samples. Any value below the LOD was assigned half of the LOD.

Outcome

Women were queried concerning their menstrual cycle characteristics in the past year. The following question was used to assess menstrual cycle characteristics “Is your menstrual cycle regular, i.e., variations month by month within 7 d?” If the answer was “No,” then “What were the longest and shortest menstrual cycles in the past year?” Specific numbers of days were recorded. If the answer was “Yes,” then ask, “What is the average length of menstrual cycle in the past year?” As to the amount of menstrual bleeding, women were given four choices: light, average, heavy, very heavy. “Light” was considered as hypomenorrhea, while “heavy and very heavy” as menorrhagia in this study. An irregular cycle was defined as variations in length for more than 7 d between cycles (Fraser et al. 2011). A short cycle was defined as less than 21 d, whereas a long cycle was more than 35 d, based on the Federation Internationale of Gynecologie and Obstetrique (FIGO) classification (Fraser et al. 2011). Incomplete information on menstrual characteristics occurred in 2% of the women.

Statistical Analysis

The association between PFASs exposure and menstrual regularity was analyzed using a multiple logistic regression model. PFASs concentrations were treated as a continuous variable (log transformed) as well as categorized into quartiles, with the lowest quartile as the reference level. Risks of long and short cycles were evaluated by multinomial logistic regression model, using normal cycles (defined as cycle length between 21 and 35 d) as the reference. ORs for hypomenorrhea and menorrhagia were also calculated in multinomial logistic regression model using normal volume as the control. Potential confounders included current age, BMI, age at menarche, household income, and parity. Education, smoking, and alcohol consumption were also considered but not included in the final models because they had little impact on the estimated associations. The prevalence of smoking (1.6%) and alcohol consumption (3.7%) were very low in the study population. We categorized women missing a value on income as a new group (group = 0) and imputed age at menarche using the mean value. Current age, age at menarche, and BMI were entered as continuous variables in the adjusted regression model. All outcome estimates are presented as crude and adjusted ORs with 95% CIs. All analyses were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA).

Results

Characteristics of the 950 study participants are shown in Table 1. The median age at the time of recruitment was 30 y; 91% were nulliparous. Most of women had normal weight with the median BMI of 20.5 kg/m^2 . The median age at menarche was 13 y. The prevalence of irregular cycles (variation ≥ 7 d) was 20.1% (190/947). One and

Table 1. Characteristics of the preconception women in Shanghai, China ($n = 950$), 2013–2015.

Characteristics	Median (p25, p75) or n (%)
Age (years)	30 (28, 32)
Missing	10 (1.1)
Age at menarche (years)	13 (13, 14)
Missing	143 (15.1)
Parity	
Nulliparous	859 (91.0)
Parous	85 (9.0)
Missing	6 (0.6)
BMI(kg/m ²)	20.5 (19.1, 22.5)
Missing	13 (1.4)
Income (10 ³ CNY)	
<10	67 (7.5)
10–15	155 (17.3)
15–30	503 (56.2)
>30	170 (19.0)
Missing	55 (5.8)
Irregular cycle	190 (20.1)
Missing	3 (0.3)
Short cycle (<21 d)	14 (1.5)
Long cycle (>35 d)	189 (20.1)
Missing	12 (1.3)
Menorrhagia	63 (6.7)
Hypomenorrhea	76 (8.0)
Missing	3 (0.3)

one-half percent (14/938) of women had a menstrual cycle length of <21 d, whereas 20.1% (189/938) had lengths >35 d. The prevalence of menorrhagia and hypomenorrhea were 6.7%(63/947) and 8.0% (76/947), respectively. There was no significant difference in demographic characteristics between those with and without PFAS information (see Table S1).

The median serum concentrations of PFOA, PFOS, PFNA, and PFHxS were 13.8, 10.5, 1.4, and 0.69 ng/mL, respectively (Table 2).

We observed statistically significant associations between PFOA, PFOS, PFNA, and PFHxS exposure and menstrual cycle characteristics (Tables 3 and 4). On the other hand, no meaningful relationships were found between the other six PFASs (PFBS, PFHpA, PFDeA, PFUA, PFOSA, PFDoA) and menstrual cycle characteristics. Thus, we present detailed results only in the former four PFASs; the rest are shown in Tables S2–S6.

Irregular Menstrual Cycle

Table 3 presents that most PFASs exposures were positively associated with irregular menstrual cycle, and the associations with PFOA, PFNA, and PFHxS levels were statistically significant. In the adjusted model, a log-unit increase in PFOA, PFNA, and PFHxS was associated with significantly increased odds of self-reported history of irregular menstrual cycle by 52%, 50%, 80%, respectively [PFOA OR = 1.52 (95% CI: 1.08, 2.15);

PFNA OR = 1.50 (95% CI: 1.03, 2.07); PFHxS OR = 1.80 (95% CI: 1.17, 2.77)]. The strongest association was shown with PFHxS. After categorization of exposure levels into quartiles, elevated risk estimates for irregular cycles were observed at almost all levels of these three chemicals (Table 3).

Long and Short Menstrual Cycles

There were significant positive associations between PFOA, PFOS, PFNA, and PFHxS concentrations and self-reported long menstrual cycles according to the adjusted multivariable logistic regression models [PFOA OR = 1.50 (95% CI: 1.06, 2.10); PFOS OR = 1.34 (95% CI: 1.02, 1.75); PFNA OR = 1.49 (95% CI: 1.05, 2.11); PFHxS OR = 1.73 (95% CI: 1.13, 2.65)] (Table 3). PFHxS also showed the strongest association with long cycles. In the models including quartiles of chemicals, elevated risk estimates for long cycles were observed at all levels of these four substances. Owing to few women with short cycles ($n = 14$), no significant association was found between PFASs exposure and short cycles (see Table S4).

Menorrhagia and Hypomenorrhea

Table 4 shows that each log-unit increase in PFOA, PFOS, PFNA, and PFHxS exposure was associated with decreased odds of self-reported menorrhagia [PFOA OR = 0.37 (95% CI: 0.21, 0.65); PFOS OR = 0.57 (95% CI: 0.37, 0.90); PFNA OR = 0.47 (95% CI: 0.26, 0.86); PFHxS OR = 0.14 (95% CI: 0.06, 0.36)]. Conversely, increasing PFOA, PFNA, and PFHxS levels were associated with higher odds of self-reported history of hypomenorrhea in certain categories. No significant associations were found between PFOS exposure and hypomenorrhea.

Discussion

Our study found that increased exposure to PFOA, PFOS, PFNA, and PFHxS was associated with higher odds of irregular and long menstrual cycle and lower risks of menorrhagia in women who plan to be pregnant. In contrast, women with higher levels of PFOA, PFNA, and PFHxS were more likely to have hypomenorrhea.

Two previous studies have evaluated the association between PFOA and PFOS and menstrual irregularity and length. Our results are consistent with those reported in a subset of 1,240 pregnant women randomly selected from the Danish National Birth Cohort (Fei et al. 2009). They found that the risk of irregular menstrual cycle was higher in women exposed to PFOA (15.0% in the upper three quartiles vs. 9.0% in the lowest quartile) and PFOS (14.2% in the upper three quartiles vs. 11.6% in the lowest quartile) (Fei et al. 2009). The INUENDO cohort enrolled 1,623 pregnant women in three countries (Greenland, Poland, and Ukraine) (Lyngsø et al. 2014) and found that PFOA exposure levels were positively associated with long menstrual

Table 2. Plasma concentrations of PFASs (ng/mL) in preconception women in Shanghai, China, 2013–2015.

PFASs ($n = 950$)	LOD (ng/mL)	Percent >LOD (%)	5th Percentile	25th Percentile	Median	75th Percentile	95th Percentile
PFOA	0.09	100	6.58	10.08	13.84	18.83	31.85
PFOS	0.09	100	4.38	7.55	10.49	15.37	30.38
PFNA	0.02	100	0.72	1.04	1.36	1.85	3.17
PFHxS	0.02	100	0.45	0.56	0.69	0.88	1.46
PFDeA	0.02	100	0.52	0.91	1.31	1.92	3.95
PFUA	0.02	100	0.54	0.85	1.18	1.63	2.95
PFBS	0.009	98.9	0.21	0.23	0.24	0.27	0.32
PFDoA	0.05	99.9	0.12	0.16	0.20	0.27	0.41
PFHpA	0.03	99.4	0.15	0.18	0.22	0.27	0.49
PFOSA	0.12	98.2	0.20	0.21	0.21	0.22	0.23

Note: PFBS, perfluorobutane sulfonate; PFDeA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexanesulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFOSA, perfluorooctane sulfonamide; PFUA, perfluoroundecanoic acid.

Table 3. Associations between PFASs (ng/mL) and irregular and long cycles in preconception women in Shanghai, China, 2013–2015.

PFASs	Irregular cycle			Long cycle		
	<i>n</i>	Crude OR (95%CI)	Adjusted ^a OR (95%CI)	<i>n</i>	Crude OR (95%CI)	Adjusted ^a OR (95%CI)
PFOA						
Continuous ^b	947	1.34 (0.97, 1.85)	1.52 (1.08, 2.15)	924	1.38 (1.00, 1.92)	1.50 (1.06, 2.10)
Q1 (≤10.08)	238	ref	ref	231	ref	ref
Q2 (10.08–13.84)	236	1.67 (1.04, 2.66)	1.71 (1.05, 2.78)	231	1.49 (0.93, 2.38)	1.50 (0.92, 2.42)
Q3 (13.84–18.83)	236	1.32 (0.82, 2.14)	1.34 (0.81, 2.22)	231	1.20 (0.74, 1.94)	1.21 (0.73, 1.99)
Q4 (>18.83)	237	1.70 (1.07, 2.70)	1.99 (1.22, 3.24)	231	1.76 (1.11, 2.79)	1.95 (1.21, 3.14)
PFOS						
Continuous ^b	947	1.26 (0.97, 1.63)	1.29 (0.98, 1.70)	924	1.32 (1.01, 1.71)	1.34 (1.02, 1.75)
Q1 (≤7.55)	238	ref	ref	230	ref	ref
Q2 (7.55–10.49)	237	0.85 (0.53, 1.36)	0.93 (0.57, 1.51)	235	1.03 (0.65, 1.65)	1.11 (0.69, 1.80)
Q3 (10.49–15.37)	236	1.24 (0.80, 1.94)	1.27 (0.80, 2.03)	230	1.21 (0.77, 1.92)	1.30 (0.81, 2.08)
Q4 (>15.37)	236	1.24 (0.80, 1.94)	1.32 (0.83, 2.09)	229	1.38 (0.88, 2.17)	1.44 (0.90, 2.30)
PFNA						
Continuous ^b	947	1.37 (0.98, 1.91)	1.50 (1.03, 2.07)	924	1.42 (1.01, 1.99)	1.49 (1.05, 2.11)
Q1 (≤1.04)	234	ref	ref	232	ref	ref
Q2 (1.04–1.36)	242	1.27 (0.79, 2.03)	1.24 (0.76, 2.02)	230	1.31 (0.82, 2.10)	1.36 (0.84, 2.22)
Q3 (1.36–1.85)	233	1.34 (0.84, 2.12)	1.38 (0.85, 2.23)	235	1.38 (0.86, 2.20)	1.48 (0.92, 2.40)
Q4 (>1.85)	238	1.52 (0.96, 2.40)	1.60 (0.99, 2.58)	227	1.59 (1.00, 2.53)	1.67 (1.03, 2.70)
PFHxS						
Continuous ^b	947	1.66 (1.10, 2.51)	1.80 (1.17, 2.77)	924	1.65 (1.08, 2.50)	1.73 (1.13, 2.65)
Q1 (≤0.56)	235	ref	ref	230	ref	ref
Q2 (0.56–0.69)	240	1.50 (0.91, 2.46)	1.41 (0.84, 2.35)	235	1.44 (0.87, 2.38)	1.35 (0.81, 2.25)
Q3 (0.69–0.88)	232	2.12 (1.31, 3.43)	2.16 (1.31, 3.57)	228	2.24 (1.39, 3.62)	2.24 (1.37, 3.66)
Q4 (>0.88)	240	2.06 (1.28, 3.34)	2.14 (1.30, 3.51)	231	2.05 (1.27, 3.33)	2.08 (1.27, 3.40)

Note: Irregular cycle is defined as ≥7 d of variation. Long cycle is defined as >35 d and is compared with a normal cycle (21–35 d) as reference (ref).

^aAdjusted for age (continuous), BMI (continuous), income (categorical), age at menarche (continuous) and parity (categorical).

^bLog-transformed PFASs as continuous variables.

cycles. The OR of long periods was 1.8 (95% CI: 1.0, 3.3) when comparing the highest tertile of exposure level with the lowest. Although no significant associations were found between PFOS exposure levels and cycle irregularity and length, there appeared to be a tendency for more irregular cycles with higher PFOS exposure [OR = 1.7 (95% CI: 0.8, 3.5)].

Although still scarce, experimental animal studies have consistently observed estrous cyclicity disruption and prolongation with

increased PFASs exposure. Austin et al. found that the administration of high-dose PFOS (10 mg/kg) for 2 wk could cause a persistent diestrus in rats, whereas the doses of 1 mg/kg did not (Austin et al. 2003). Another animal study examined the influence of chronic exposure to a low-dose PFOS (0.1 mg/kg/d) on female reproductive function and showed that adult female mice exposed to PFOS for 4 mo had a prolongation of diestrus without signs of toxic effects. Serum estrogen (E₂) and progesterone levels at

Table 4. Associations between PFASs (ng/mL) and menorrhagia and hypomenorrhea in preconception women in Shanghai, China, 2013–2015.

PFASs	Menorrhagia			Hypomenorrhea		
	<i>n</i>	Crude OR (95% CI)	Adjusted OR ^a (95% CI)	<i>n</i>	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
PFOA						
Continuous ^b	871	0.41 (0.23, 0.70)	0.37 (0.21, 0.65)	884	1.45 (0.91, 2.32)	1.40 (0.85, 2.28)
Q1 (≤10.08)	227	ref	ref	213	ref	ref
Q2 (10.08–13.84)	216	0.65 (0.34, 1.25)	0.61 (0.31, 1.19)	219	1.75 (0.81, 3.76)	1.84 (0.82, 4.11)
Q3 (13.84–18.83)	211	0.62 (0.32, 1.21)	0.61 (0.31, 1.19)	222	2.44 (1.17, 5.06)	2.68 (1.24, 5.78)
Q4 (>18.83)	217	0.27 (0.11, 0.64)	0.21 (0.08, 0.52)	230	1.75 (0.82, 3.74)	1.73 (0.77, 3.86)
PFOS						
Continuous ^b	871	0.56 (0.36, 0.86)	0.57 (0.37, 0.90)	884	1.05 (0.71, 1.54)	1.05 (0.70, 1.57)
Q1 (≤7.55)	223	ref	ref	208	ref	ref
Q2 (7.55–10.49)	218	0.38 (0.19, 0.75)	0.38 (0.19, 0.77)	225	1.19 (0.59, 2.40)	1.15 (0.56, 2.36)
Q3 (10.49–15.37)	214	0.38 (0.19, 0.77)	0.39 (0.19, 0.78)	224	1.40 (0.71, 2.78)	1.33 (0.66, 2.68)
Q4 (>15.37)	216	0.28 (0.13, 0.60)	0.29 (0.13, 0.62)	227	1.24 (0.62, 2.50)	1.21 (0.59, 2.48)
PFNA						
Continuous ^b	871	0.50 (0.28, 0.89)	0.47 (0.26, 0.86)	884	1.57 (0.97, 2.54)	1.58 (0.95, 2.63)
Q1 (≤1.04)	228	ref	ref	214	ref	ref
Q2 (1.04–1.36)	214	0.77 (0.40, 1.48)	0.74 (0.38, 1.44)	218	2.43 (1.09, 5.43)	2.38 (1.05, 5.40)
Q3 (1.36–1.85)	213	0.58 (0.29, 1.18)	0.53 (0.25, 1.10)	227	3.07 (1.41, 6.70)	2.90 (1.31, 6.41)
Q4 (>1.85)	216	0.43 (0.20, 0.93)	0.41 (0.19, 0.90)	225	2.10 (0.93, 4.75)	2.05 (0.89, 4.73)
PFHxS						
Continuous ^b	871	0.18 (0.07, 0.43)	0.14 (0.06, 0.36)	884	1.78 (0.99, 3.21)	1.72 (0.93, 3.19)
Q1 (≤0.56)	226	ref	ref	207	ref	ref
Q2 (0.56–0.69)	220	0.69 (0.36, 1.29)	0.65 (0.34, 1.23)	225	3.25 (1.37, 7.75)	3.53 (1.46, 8.53)
Q3 (0.69–0.88)	213	0.42 (0.20, 0.87)	0.42 (0.20, 0.87)	222	2.83 (1.17, 6.84)	2.44 (0.99, 6.04)
Q4 (>0.88)	212	0.30 (0.13, 0.68)	0.26 (0.11, 0.61)	230	3.64 (1.55, 8.58)	3.59 (1.50, 8.60)

Note: ref, reference.

^aAdjusted for age (continuous), BMI (continuous), income (categorical), age at menarche (continuous) and parity (categorical).

^bLog-transformed PFASs as continuous variables.

proestrus and diestrus were reduced, and a luteinizing hormone (LH) surge did not emerge at proestrus (Feng et al. 2015). Previous *in vivo* and *in vitro* studies on rats also found a negative relationship between PFOA and testosterone level (Biegel et al. 1995; Liu et al. 1996). This may lead to deficits in the follicular development and ovulation, and, consequently, irregularity and prolongation of diestrus. To our knowledge, no experimental animal studies were conducted on other PFASs in this area.

Thyroid dysfunction could be another endocrine target for PFASs. Mounting evidence from animal (Chang et al. 2008; Lau et al. 2003; Luebker et al. 2005; Thibodeaux et al. 2003) and human studies (Dallaire et al. 2009; Wang et al. 2014) showed decreased levels of thyroid hormones at higher PFASs concentrations. For example, rats treated with PFOS were found to have decreased thyroxine (T₄) and triiodothyronine (T₃), without an expected increase in thyroid stimulating hormone (TSH) (Chang et al. 2008). A prospective cohort study demonstrated that pregnant women with higher PFHxS levels had elevated TSH levels (Wang et al. 2014). Exposure to PFNA, perfluorododecanoic acid (PFDoDA) and perfluoroundecanoic acid (PFUnDA) were also associated with lower free T₄ and total T₄ levels (Wang et al. 2014). These findings suggest that PFASs are associated with hypothyroidism. Because the hypothalamic–pituitary–ovarian axis (HPO) and the hypothalamic–pituitary–thyroid axis (HPT) are physiologically related and act together as a unified system, both hyper- and hypothyroidism may result in menstrual disturbances (Doufas and Mastorakos 2000). In women, hypothyroidism usually is associated with abnormal menstrual cycles characterized mainly by altered ovulatory function, menstrual irregularities, and subfertility (Cho 2015).

Compared with previous epidemiologic studies, the main strength of the present study was that the study population was restricted to pre-pregnant women planning to conceive and that their blood samples were collected before pregnancy. Because the concentration of PFASs may decline in pregnancy due to blood volume expansion, decreased serum albumin concentration, changes in PFAS pharmacokinetics during pregnancy, and placental transfer of PFASs to the fetus (Apelberg et al. 2007; Frederiksen 2001; Thibodeaux et al. 2003), prepregnancy PFAS concentrations reflect true exposure levels.

In addition, a recent study reported that the prevalence of irregular cycle, menorrhagia, and hupomenorrhea in Chinese nurses with nonshift work was 14.9%, 9.6% and 7.2%, respectively (Wang et al. 2016). The corresponding prevalence in our study was 20.1%, 6.7%, and 8.0%, respectively. Thus, the various cycle characteristics in our study are similar to those reported in other studies in China.

Our studies also have deficiencies. The amount of menstrual bleeding reported by the women (less than average, average, more than average, and excessive) is rather subjective. Misclassifications of that variable are likely. However, it is unlikely that the misclassifications are related to PFAS levels. Thus, the nondifferential misclassification may have drawn the results toward the null. Second, we excluded women who had difficulties in conception. Our study was actually a prospective cohort study, though the current analysis was cross-sectional in nature. After a 1-y follow-up, approximately 20% of women remained nonpregnant (J. Zhang, unpublished data, 2017), indicating that our study population was similar to the general population. Third, considering the low prevalence of short cycles (1.5%, 14/938), we did not have enough statistical power to examine the association between PFAS level and short cycles. Fourth, information on menstrual cycle characteristics in the past 12 mo was collected retrospectively. Several studies have compared the retrospectively with prospectively collected information on menstrual cycle and observed a moderate agreement on

menstrual cycle length with correlation coefficients ranging from 0.45 to 0.50 (Jukic et al. 2008; Small et al. 2007). Small et al. (2007) found that women who were married and trying to become pregnant were more likely to have accurate self-reported menstrual cycle information (Small et al. 2007). Thus, it is possible that self-reported information in our study is more accurate given that all of them were married and planning to be pregnant. Furthermore, this misclassification due to recall errors was likely to be nondifferential because all women were unaware of their level of PFAS burden.

Finally, this was a cross-sectional analysis. The causality remains uncertain. Literature shows that men have a higher level of PFASs than women. Physiologically based pharmacokinetic modeling estimated that up to 30% of the difference between men and women may be attributable to menstruation, indicating that menstruation might be an important elimination pathway for PFASs in women (Wong et al. 2014). Thus, it is possible that the association between higher PFAS levels and lighter menstrual flow and irregular or longer cycles might be a reverse causality due to less menstruation. On the other hand if this is true, we would expect that most PFAS levels would be affected. Moreover, animal studies have demonstrated disrupted estrous cycles after PFOS exposure (Austin et al. 2003; Feng et al. 2015). Further prospective studies are needed to verify the association between PFASs concentration and menstrual cycle in women.

Conclusions

Certain PFASs exposure at the environment-relevant dose is associated with self-reported menstrual cycle irregularity, longer length, and less bleeding volume. Due to the ubiquity of PFASs and the critical role of menstrual cycle in fecundity, our findings may have important public health implications.

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