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Association between serum levels of TSH and free T4 and per- and polyfluoroalkyl compounds concentrations in pregnant women

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ABSTRACT

Many per- and polyfluoroalkyl substances (PFASs) may disrupt maternal thyroid hormone homeostasis in pregnancy. Concerns should be raised regarding the PFASs exposure in pregnant women because thyroid hormones are involved in the early development of the fetus. In this study, we measured the concentrations of 13 PFASs, including five novel short-chain PFASs, in serum from 123 pregnant women in Beijing, China. Linear regression models were used to investigate the association between thyroid-stimulating hormone (TSH) or free thyroxine (FT4) levels and PFASs concentrations under consideration of the impacts of pregnancy-induced physiological factors. We found that perfluorobutanoic acid (PFBA) (β=0.189, 95%CI=-0.039, 0.417, p=0.10) and perfluorodecanoic acid (PFDA) (β=-0.554, 95%CI=-1.16, 0.049, p=0.071) were suggestive of significant association with TSH in thyroid peroxidase antibody (TPOAb) negative women. No association was observed between all PFASs and FT4 levels after controlling for these confounding factors, such as BMI, gestational weight gain and maternal age. These findings suggest that it should pay more attention to the association between thyroid hormone levels and short-chain PFASs concentrations. Future studies could consider a greater sample and the inclusion of other clinical indicators of thyroid function, such as free T3 and total T3.

Introduction

Per- and polyfluoroalkyl substances (PFASs) are a group of synthetic organic compounds with hydrophobic and lipophobic properties. PFASs have been widely used in the metal-

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plating industry and in commercial products such as lubricants, carpets, and food packaging materials (Lindstrom et al., 2011; Xie et al., 2013; Ji et al., 2020). According to Organisation for Economic Co-operation and Development (OECD), perfluoroalkyl carboxylic acids (PFCAs) with eight carbons and greater (i.e., with 7 or more perfluorinated carbons) and perfluoroalkane sulfonates (PFSAs) with six carbons and greater (i.e., with 6 or more perfluorinated carbons) are defined as long-chain PFASs (OECD, 2011). The adverse health effects of exposure to some PFASs, especially perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are of great concern because of their persistence in the environment, bioaccumulation, and toxicity (Su et al., 2017; Xiao, 2017; Patlewicz et al., 2019; Peshoria et al., 2020).

It has raised concerns about the potential adverse effects of prenatal exposure to PFASs because a series of PFASs can across the placental barrier and accumulate in placental and fetal tissue (Gao et al., 2019; Mamsen et al., 2017). Previous studies showed that many PFASs can disrupt thyroid function and lead to hypothyroidism or hypothyroxinemia in toxicological studies (Zoeller, 2010; Boas et al., 2012). Serum thyroid stimulating hormone (TSH) concentrations are a sensitive indicator of thyroid function. If TSH is high and the T4 is normal, the diagnosis is subclinical hypothyroidism (Vanderpump and Tunbridge, 2002). If TSH is normal and the FT4 is low, the diagnosis may be central hypothyroidism (Van Veggel et al., 2018).

Normal thyroid hormone levels in pregnant women are critical for neonatal growth and development (Preston et al., 2018; Jansen et al., 2019; Zhang et al., 2019). For example, maternal thyroid hormone levels in early or late pregnancy were found to be relate to low birth weight of infants (Zhang et al., 2019). Maternal thyroid dysfunction has been associated with cognitive deficits (Lyall et al., 2017), preterm delivery and neurodevelopmental deficits (Escobar et al., 2004). However, the results of epidemiological studies on the association between PFASs exposure and thyroid hormone levels are often inconsistent (Wang et al., 2013, 2014; Preston et al., 2018; Andersson et al., 2019; Aimuzi et al., 2020; Silva et al., 2020; Li et al., 2021). Some studies have reported that maternal perfluorohexanesulfonic acid (PFHxS) and PFOS concentration were positively associated with thyroid stimulating hormone (TSH) levels (Wang et al., 2013, 2014). On the contrary, Aimuzi et al. (2020) found that PFHxS was negatively associated with TSH levels. Wang et al. (2014) showed that perfluorononanoic acid (PFNA), perfluoroundecanoic acid (PFUdA), and perfluorododecanoic acid (PFDoA) were negatively associated with free thyroxine (FT4) and total T4 levels. However, in Preston's study, PFOS, PFOA and PFNA were negatively associated with TSH levels only in TPOAb positive women, and PFAS concentrations were not associated with maternal T4 levels (Preston et al., 2018).

Some physiology and biochemistry indicators may be confounding factors in such studies. A study in euthyroid adults showed that TSH levels were positively associated with total cholesterol and triglyceride levels, while FT4 levels were negatively associated, and that thyroid hormones could be modulated by body mass index (BMI) (Wang et al., 2017). Previous studies suggested that higher TSH levels were positively associated with higher pre-pregnancy BMI and total gestational weight gain (GWG), and the association was inverse for FT4 levels (Collares et al., 2017; Pearce, 2017). Thyroid hormone levels also seem to be associated with gestational weeks and TPOAb status (Preston et al., 2018; Aimuzi et al., 2020; Inoue et al., 2020). Therefore, some clinical factors, especially pregnancy-induced physiological factors, should be considered in the study of the association between thyroid hormone levels and PFASs concentrations.

With global restrictions on the use and production of PFOS and PFOA, their short-chain alternatives with similar physiochemical properties are widely used to meet the large demand for manufactured products (Glynn et al., 2012; Su et al., 2017). Nian et al. (2020) showed that prenatal exposure to perfluorobutanesulfonate (PFBS) and perfluoroheptanoic acid (PFHpA) could affect fetal thyroid function during pregnancy. However, to date, very few study has investigated the association between short-chain PFASs concentrations and thyroid hormone levels.

In this study, 123 serum samples of pregnant women were collected in Beijing, China. The serum concentrations of 13 PFASs (perfluorinated carbons C3–C11) were measured using HPLC-MS/MS, and TSH and FT4 levels were measured using chemiluminescence assays. The associations between PFASs concentrations and TSH and FT4 levels were analyzed using multiple linear regression models. In order to adjust the potential confounding factors caused by pregnancy, a generalized linear model was used to investigate the impacts of pregnancy-induced physiological factors.

1. Materials and methods

1.1. *Study design*

This study is a cross-sectional analysis of pregnant women from Beijing, China. Recruitment was carried out during the medical exam with the approval of the Ethics Review Board of the Medical Ethics Committee of Beijing Obstetrics and Gynecology Hospital (the ethic permit number: IEC-B-03-V01-FJ1). On the premise that the participants fully understand the purpose and content of this study, all participating mothers provided written informed consent. The inclusion criteria are >18 years old, without chronic metabolic disease and thyroid-related disease (including diabetes, gout, hypertension, hyperglycemia, hyperthyroidism, and hypothyroidism), residing locally (living and working in Beijing for more than two years), and intending to deliver at this hospital. Total 123 eligible pregnant women were recruited after screened through questionnaire and some clinical diagnosis. The pre-pregnancy anthropometric information and demographic information were obtained by questionnaire and from the hospital information system.

1.2. *PFAS, outcome indicators and covariates*

In this study, 13 PFASs (**Appendix A Table S1**), including five novel short-chain PFASs, were selected based on literature research. TSH and FT4 were detected as outcome indicators because they are mainly used as sensitive index for screening thyroid dysfunction in clinic (Talat et al., 2019). Some clinical and physiological factors incorporated into regression models

as covariates. These physiological factors included maternal age, gestational weight gain, parity (0, 1), gestational weeks at blood sampling, pre-pregnancy BMI, glomerular filtration rate (GFR), TPOAb status, and serum albumin levels. Pre-pregnancy BMI was calculated as weight divided by height squared. According to World Health Organization (WHO) recommended cut-off values for BMI in China, underweight was defined as BMI<18.5 kg/m^2, normal weight as BMI 18.5–23.9 kg/m^2, overweight as BMI 24–27.9 kg/m^2, and obesity as BMI ≥28 kg/m^2 (WHO, 2004). Fasting total cholesterol and fasting triglycerides are indicators related to body weight, and were also examined as covariates. GFR was calculated using the Cockcroft-Gault formula as (140 minus age) × pre-pregnancy weight × 1.04, and divided by the level of serum creatinine (Cockcroft and Gault, 1976). Serum creatinine, TPOAb status, and serum albumin levels were all quantified.

1.3. *PFASs, TSH, FT4 and TPOAb measurements*

Before sample collection, all tubes and blood sampling device were checked to ensure that they were free of perfluorinated compounds (Methods in Appendix A Supplementary data). Peripheral blood from pregnant women in the fasting state was collected into the BD Vacutainer serum separator tubes (BD SST, Becton-Dickinson, USA). The blood was centrifuged at 2000 ×*g* for 10 min, and the serum was collected. The level of Clinical indicators (including total cholesterol, triglycerides, creatinine, albumin and TPOAb) and thyroid hormones (TSH and FT4) were determined quickly in the hospital (Appendix A Methods and **Appendix A Table S2**). The calibration, quantification, and quality control were performed according to standard operating procedures. Based on historical data and sufficient sample size, the 95% confidence interval was selected to establish the reference range of pregnancy by measuring TSH and FT4 in the normal pregnant women. The 2.5th percentile is the lower limit and the 97.5th percentile is the upper limit for the reference range. The reference values are 0.55–4.78 mIU/L for TSH and 11.5–22.7 pmol/L for FT4. TPOAb are divided into negative (≤60 U/mL) and positive (>60 U/mL).

PFASs in serum were analyzed using HPLC-MS/MS coupled with an on-line Turboflow SPE method according to a previous study (Gao et al., 2016). Briefly, 25 µL serum sample was diluted to 200 µL with ultrapure water, containing 5 ng isotope internal standard. Then 25 µL serum diluent were injected directly into HPLC-MS/MS for analysis. Concentrations below the limit of detection were replaced by the limit of detection divided by square root of 2 (Gao et al., 2016). Twelve PFASs with detection rate > 60% (except PFHpA with detection rate < 40%) were included in statistical analysis.

1.4. *Statistical analysis*

Demographic and clinical characteristics of the pregnant women were analyzed with descriptive statistical analysis. The categorical and continuous variables were expressed as number (percent) and mean (standard deviation, SD). The differences of PFASs concentration among groups were examined using Kruskal-Wallis test or Mann-Whitney U test. The correlations among 12 PFASs were assessed using the Pearson test (**Appendix A Fig. S1**). Generalized linear models (GLMs) were used to assess independent associations between clinical factors and TSH or FT4 levels, and potential interaction effect on these associations were further assessed by including the main effects of two factors and their cross-terms.

The potential nonlinear relationships between PFASs and TSH or FT4 were examined using generalized additive models with restricted cubic spline. The serum PFASs concentration was natural logarithmic transformed to normalize the data in the models. The potential linear relationships between PFASs and TSH or FT4 were examined using the multiple liner regression. To improve the robustness of models, PFASs were screened as independent variables using the univariate and stepwise regression models. On the basis of the identified independent variables, covariables were then added into the models to investigate the impact of these factors on the association between PFASs and TSH and FT4 levels. In this study, samples were divided into two groups according to TPOAb status, and the relationship between PFASs and TSH or FT4 was further investigated in two groups, respectively. Preconditions for regression analyses were checked in terms of independence of residuals (Durbin-Watson test), normal distribution of residuals, and homoscedasticity. They all showed to be satisfying for TSH and FT4 models (**Appendix A Figs. S2 and S3**). The homogeneity of variance was represented by the scatter plot of normalized residuals and normalized predicted values. The fitting points are distributed around the 0 value on average. The residuals plot reflects the randomness and unpredictability. The multicollinearity of independent variables was evaluated based on variance inflation factor (VIF). Typical suggestions for VIF cutoff point are 5 or 10 (Craney and Surles, 2002).

Statistical analyses were performed in IBM SPSS version 23 (IBM SPSS, Armonk, NY, USA). The generalized additive models were used to evaluate non-linear associations of PFASs and TSH and FT4 levels using the "mgcv" package in R version 4.0.3. $p<0.05$ was considered statistically significant.

2. Results and discussion

2.1. *Participant characteristics*

Appendix A Table S3 lists the demographic characteristics of all participants. According to WHO recommended cut-off values for BMI in China, 18 (14.6%) were underweight, 80 (65%) had normal pre-pregnancy weight, and 25 (20.3%) were overweight or obese. The average GWG was 15 kg. Q1 (GWG ≤ 12 kg) was 35 (28.5%), Q2 (GWG 13–15 kg) was 36 (29.3%), Q3 (GWG 16–18 kg) was 24 (19.5%), and Q4 (GWG > 18 kg) was 28 (22.8%). 97 (78.9%) participants were the first pregnancy. The mean levels of TSH, FT4, albumin, creatinine, fasting total cholesterol, and fasting triglycerides, and GFR were also shown in **Appendix A Table S3.** Among 123 women, 102 (82.9%) were TPOAb-negative, and 21 (17.1%) were TPOAb-positive.

2.2. *PFASs levels*

As shown in Table 1, PFOA, PFNA, PFDA, PFUdA, and PFOS were the dominant PFASs, which were detected in over 90% of samples. The detection rate of PFHpA was only 33.4%, therefore

Table 1 – Detection rate (%) and concentration (ng/mL) of PFASs in serum samples from pregnant women (n=123).

Analyte	Detection rate	Median	25th-75th percentile	Range
PFBA	81.4	0.10	0.06-0.15	<LOD-1.13
PFPeA	61	0.05	<LOD-0.08	<LOD-0.20
PFHxA	75.7	0.17	0.13-0.22	<LOD-0.65
PFHpA	33.4	<LOD	<LOD-0.17	<LOD-0.58
PFOA	93.5	2.27	1.46-3.43	<LOD-25.4
PFNA	96.8	0.57	0.42-0.83	<LOD-3.98
PFDA	96	0.47	0.32-0.67	<LOD-3.15
PFUdA	96	0.47	0.32-0.76	<LOD-3.86
PFDoA	83.8	0.26	0.15-0.39	<LOD-1.24
ΣPFCAs	/	4.59	3.28-6.38	0.23-29.9
PFBS	86.2	0.14	0.10-0.18	<LOD-0.75
PFHxS	88.7	0.24	0.14-0.34	<LOD-1.15
PFOS	100	4.09	2.66-5.63	0.07-22.6
6:2 Cl-PFESA	80.5	0.09	0.09-0.10	<LOD-3.34
ΣPFSAs	/	4.84	3.13-6.25	0.12-23.2
ΣPFASs	/	9.97	6.98-13.5	0.35-36.4

LOD: limit of detection. "/"means no data.

it was excluded from further analysis. The highest concentration of PFASs was PFOS (median: 4.09 ng/mL), followed by PFOA (2.27 ng/mL) and PFNA (0.57 ng/mL), indicating that PFOS and PFOA remain abundant in the environment although their use has been reduced for years (Lindim et al., 2016). Logarithmic transformed Pearson correlation calculations suggested positive correlations among some PFASs (**Appendix A Fig. S1**). In this study, the median concentration of PFOS was lower than that reported in Massachusetts, US (24.0 ng/mL) (Preston et al., 2018), Denmark (29.5 ng/mL) (Inoue et al., 2020), and Spain (6.05 ng/mL) (Matilla-Santander et al., 2017), but higher than that in Colorado, US (2.4 ng/mL) (Starling et al., 2017). The chemical 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA) was an alternative to PFOS and has been used for decades in the Chinese metal plating industry (Pan et al., 2017). Its median concentration was 0.09 ng/mL in this study, lower than that of Wuhan, China (1.89 ng/mL) (Pan et al., 2017). The median concentration of PFBS (0.14 ng/mL) in this study was higher than that in a study from the Shanghai Birth Cohort (0.03 ng/mL) (Nian et al., 2020). The median concentration of PFBA (0.1 ng/mL) was lower than that in Guangdong, China (0.7 ng/mL), but the median concentration of PFHxA (0.17 ng/mL) was higher than that study (0.02 ng/mL) (Cai et al., 2020). Short-chain PFASs are increasingly used as alternatives to traditional long-chain PFASs. The frequent detection of short-chain PFASs in maternal serum suggests that concerns should be raised regarding the potential adverse effects of these short-chain PFASs during pregnancy.

Studies have shown that PFASs concentrations might be influenced by maternal age, pre-pregnancy BMI and parity (Sagiv et al., 2015; Collares et al., 2017; Pearce, 2017). As shown in **Appendix A Table S4**, we found that the concentration of PFDA, PFDoA, and PFHxS was influenced by maternal age, gestational weight gain, and parity, respectively. It has been showed women with older age had higher PFASs levels (Berg et al., 2014). Here the median PFDA concentration in high age group (>35 years) (0.73 ng/mL) was significantly higher than that of women in the other three age groups. In general, age trends of PFASs may reflect their persistence and metabolic rate in human. The PFHxS concentration in nulliparous women (Parity = 0) were significantly higher than that in parous women (Parity = 1) in our study, which was consistent with previous studies (Sagiv et al., 2015). One of the reasons may be that PFASs can be transferred to fetuses and infants through the way of trans-placental and breastfeeding (Mondal et al., 2014; Cai et al., 2020).

2.3. Association of physiological and biochemical indicators and maternal TSH and FT4 levels

Studies have suggested that TSH and FT4 levels may be associated with pre-pregnancy BMI and GWG (Pop et al., 2013; Collares et al., 2017). Here a generalized linear model was used to examine the association between detected clinical indicators and TSH and FT4 levels. These clinical indicators, including pre-pregnancy BMI, gestational weight gain, total cholesterol, triglyceride, GFR, albumin, and TPOAb status, were considered as independent variables in this model. As shown in **Appendix A Table S5**, the models including the main effect of paired independent variables and their interaction term, showed that multiplicative interactions effect on TSH were found between TPOAb and total cholesterol, GFR or GWG (all $p<0.05$), while only significant multiplicative interaction on FT4 was found between TPOAb and GFR. The pregnant women with GWG-Q1, Q2 and Q3 were related to a 2.23, 2.15 and 2.32 decreased in TSHs values compared the ones with GWG-Q4 pregnant women (**Appendix A Table S5**), indicating that GWG was positively associated with TSH levels.

No association was found between pre-pregnancy BMI and TSH or FT4 levels in our study. However, we found a positive association between triglyceride and TSH levels, which was consistent with a cross-sectional study from a large Spanish population (Santos-Palacios et al., 2013). Interestingly, we found that TPOAb status played an important role in the associations between clinical indicators and TSH or FT4 levels. As shown in **Appendix A Table S6**, TSH level in TPOAb-positive group was significantly higher than that in TPOAb-negative group in overweight and GWG-Q4 group pregnant women. FT4 level in TPOAb-negative group was significantly higher than that in TPOAb-positive group for normal weight pregnant women. In TPOAb-negative pregnant women, FT4 level in overweight pregnant was significantly higher than that in underweight pregnant women. FT4 level in TPOAb-positive group was significantly lower than that in TPOAb-negative group in GWG-Q1.

2.4. Association of PFASs concentrations and maternal TSH or FT4 levels

The potential nonlinear relationship between PFASs and TSH or FT4 was firstly investigated using generalized additive models with smoothing cubic splines. No evidence was found for nonlinearity between natural-log PFASs values and TSH or FT4 (p nonlinear > 0.05, **Appendix A Fig. S4**). Then the linear association between PFASs and TSH or FT4 levels was further investigated by multiple linear regression models. To improve the robustness of the models, PFASs as independent

Table 2 – Univariate and multivariate regression analysis between PFASs and TSH stratified by TPOAb status.

TPOAb		Univariate analysis			Multivariate analysis		
		β	95%CI	p	β	95%CI	p
Negative	PFBA	0.181	-0.042, 0.404	0.111	0.189	-0.039, 0.417	**0.10**
n=102	PFOS	-0.151	-0.425, 0.123	0.278	0.242	-0.217, 0.702	0.298
	PFPeA	0.089	-0.188, 0.366	0.527			
	PFHxA	-0.102	-0.268, 0.064	0.227	-0.098	-0.272, 0.076	0.265
	PFNA	-0.197	-0.483, 0.09	0.176	0.247	-0.41, 0.905	0.457
	PFOA	-0.067	-0.219, 0.084	0.381			
	PFDA	-0.275	-0.525, -0.025	**0.032**	-0.554	-1.16, 0.049	**0.071**
	PFUdA	-0.2	-0.46, 0.06	0.13	-0.052	-0.427, 0.323	0.784
	PFDoA	-0.039	-0.236, 0.158	0.696			
	PFBS	-0.099	-0.294, 0.096	0.318			
	PFHxS	-0.025	-0.241, 0.191	0.82			
	PFESA	-0.07	-0.264, 0.125	0.479			
Positive	PFBA	-0.225	-0.584, 0.135	0.206	-0.008	-0.517, 0.502	0.974
n=21	PFOS	0.106	-0.371, 0.583	0.647			
	PFPeA	-0.001	-0.419, 0.416	0.995			
	PFHxA	0.168	-0.216, 0.552	0.372			
	PFNA	-0.006	-0.37, 0.358	0.972			
	PFOA	-0.014	-0.267, 0.239	0.907			
	PFDA	0.012	-0.301, 0.325	0.936			
	PFUdA	-0.091	-0.397, 0.215	0.539			
	PFDoA	-0.135	-0.405, 0.136	0.311			
	PFBS	0.27	-0.13, 0.671	0.174	0.24	-0.301, 0.782	0.346
	PFHxS	-0.019	-0.319, 0.281	0.895			
	PFESA	-0.05	-0.341, 0.241	0.722			

Multivariate analysis was adjusted for pre-pregnancy BMI, Parity, GWG, Total cholesterol, Triglycerides, Albumin, GFR, Maternal age. Bold numbers indicate statistically significant ($p < 0.05$).

variables were screened by univariate regression and stepwise regression analysis. Here 12 PFASs with p value less than 0.3 were permitted to enter the multiple linear regression models. PFBA, PFNA, PFDA, PFUdA were selected by univariate regression model (**Appendix A Table S7**), and PFBA and PFDA were selected by stepwise regression model (**Appendix A Table S8**) as the candidate independent variables for TSH. PFNA, PFUdA, and PFHxS were selected by univariate regression model (**Appendix A Table S9**), and PFHxS was selected by stepwise regression model (**Appendix A Table S8**) as the candidate independent variables for FT4. No significant association was observed between PFASs and TSH or FT4 in multivariate regression analysis (**Appendix A Tables S7 and S9**).

The associations of some PFASs with thyroid hormone levels has been shown to be relate to TPOAb status (Preston et al., 2018; Aimuzi et al., 2020). In this study, all samples were stratified into two groups according to TPOAb status, and the associations of PFASs with TSH and FT4 were investigated. PFDA was negatively associated with TSH in TPOAb-negative group both in univariate regression model (β=-0.275, 95%CI=-0.525, -0.025, p=0.032) (Table 2) and in stepwise regression model (β=-0.303, 95%CI=-0.552, -0.055, p=0.017) (**Appendix A Table S10**). PFDA (β=-0.554, 95%CI=-1.16, 0.049, p=0.071) and PFBA (β=0.189; 95%CI=-0.039, 0.417; p=0.10) was negatively associated with TSH in TPOAb-negative group in the multivariate regression model (Table 2). Increased serum concentration of PFOA was significantly associated with elevated serum FT4 level in TPOAb-positive group in univariate regression model (β=0.057, 95%CI=0.001, 0.113, p=0.046) (Table 3) and in stepwise regression model (β=0.058, 95%CI=0.004, 0.112, p=0.038) (**Appendix A Table S11**). But no association was observed between PFOA and FT4 in TPOAb-positive groups in multivariate regression model (Table 3). The univariate regression analysis showed that the association among ΣPFCAs, ΣPFSAs, ΣPFASs concentration and TSH or FT4 level were not significant, and the model was not significant in all samples or sample stratified by TPOAb status (**Appendix A Tables S12** and **Table S13**).

PFAS-associated thyroid disruption has been found in adults with high TPOAb and low iodine status (Webster et al., 2016). A recent study has tested the urine iodine level of pregnant women according to the 2015 China Adult Chronic Diseases and Nutrition Surveillance (CACDNS), it showed that the urine iodine level of Chinese pregnant women (median: 146 μg/L) was close to the cutoff value (150 μg/L) recommended by international organizations, such as World Health Organization (WHO) (Yang et al., 2020). Given that the content of iodine in human body has obvious diurnal differences because it is affected by diet and urine volume, it cannot well reflect whether the human body is in a state of iodine deficiency by a single measurement. TPOAb is a marker of autoimmune hypothyroidism, and women with autoimmune thyroid impairment would reduce the regulation of thyroid hormones (Webster et al., 2016; Eick et al., 2020). The association between PFASs and thyroid hormones were mostly found in TPOAb-positive women. Preston et al. (2018) showed that increased IQR in PFOS, PFOA, and PFNA concentrations was significantly associated with decreased TSH levels in TPOAb-positive women. Aimuzi et al. (2020) showed a positive associ-

Table 3 – Univariate and multivariate regression analysis between PFASs and FT4 stratified by TPOAb status.

		Univariate analysis			Multivariate analysis		
		β	95%CI	p	β	95%CI	p
Negative	PFBA	-0.007	-0.043, 0.03	0.717			
n=102	PFOS	0.018	-0.027, 0.062	0.438			
	PFPeA	0.009	-0.036, 0.054	0.685			
	PFHxA	0	-0.028, 0.027	0.977			
	PFNA	0.02	-0.027, 0.067	0.403			
	PFOA	-0.008	-0.033, 0.017	0.527			
	PFDA	0.006	-0.036, 0.047	0.791			
	PFUdA	0.017	-0.025, 0.06	0.427			
	PFDoA	-0.005	-0.037, 0.027	0.765			
	PFBS	0.002	-0.03, 0.034	0.896			
	PFHxS	0.029	-0.006, 0.064	0.103	0.012	-0.023, 0.046	0.492
	PFESA	-0.004	-0.036, 0.028	0.794			
Positive	PFBA	-0.014	-0.106, 0.078	0.751			
n=21	PFOS	0.031	-0.086, 0.148	0.586			
	PFPeA	-0.068	-0.165, 0.03	0.161	-0.032	-0.171, 0.106	0.613
	PFHxA	0.027	-0.069, 0.123	0.565			
	PFNA	0.02	-0.069, 0.109	0.643			
	PFOA	0.057	0.001, 0.113	**0.046**	0.015	-0.188, 0.217	0.871
	PFDA	0.007	-0.07, 0.084	0.844			
	PFUdA	0.034	-0.04, 0.109	0.345			
	PFDoA	0.019	-0.048, 0.087	0.556			
	PFBS	0.039	-0.063, 0.141	0.43			
	PFHxS	0.026	-0.047, 0.098	0.471			
	PFESA	-0.046	-0.114, 0.023	0.178	-0.037	-0.151, 0.077	0.483

Multivariate analysis was adjusted for pre-pregnancy BMI, Parity, GWG, Total cholesterol, Triglycerides, Albumin, GFR, Maternal age. Bold numbers indicate statistically significant ($p < 0.05$).

ation between PFNA concentrations and FT4 levels and a negative association between perfluoroundecanoic acid (PFUdA) concentrations and TSH levels in TPOAb-positive women. Webster et al. (2014) reported a positive association between PFASs (PFNA, PFOA and PFOS) concentrations and TSH levels and a negative association between PFASs (PFNA, PFOA, PFOS and PFHxS) concentrations and FT4 levels in TPOAb-positive women.

PFASs have been reported to disrupt maternal thyroid hormone homeostasis in pregnancy, but little is known about the associations between novel short-chain PFASs and thyroid hormones levels. Nian et al. (2020) reported a negative association between PFBS or PFHpA and several reproductive hormones (luteinizing hormone, follicle-stimulating hormone, total testosterone, sex hormone-binding globulin). They found that these two short chain PFASs may disturb reproductive hormones of fetal and lead to reproductive toxicity. The negative association between PFDA and TSH in our study was consistent with a study of cord blood (Aimuzi et al., 2019), but inconsistent with a study of Shanghai birth cohort (Aimuzi et al., 2020). The negative association between PFOA and free T4 index (FT4I) was reported in all pregnant women (Preston et al., 2018), however, the negative association between PFOA and FT4 was found only in TPOAb-positive women in another study (Webster et al., 2014). Moreover, positive association and no association between PFOA and FT4 were also observed in some studies (Aimuzi et al., 2020; Wang et al., 2014; Kato et al., 2016).

3. Conclusions

In this study, we measured the serum concentration of 13 PFASs, including 5 novel short-chain PFASs, in pregnant women. We found that PFBA (p=0.1) and PFDA (p=0.071) were suggestive significant association with TSH in TPOAb-negative women. To the best of our knowledge, this is the first study to examine the effect of PFBA on TSH in pregnant women. PFBA can cross the placental barrier to the fetuses, suggesting that it may have more adverse effects on birth length and weight than long chain PFASs (Gao et al., 2019). Therefore, more attention should be paid to the health effects of PFASs, especially short-chain PFASs. Future studies could consider a greater sample and the inclusion of other clinical indicators of thyroid function, such as free T3 and total T3.

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.10.026.

REFERENCES

Aimuzi, R., Luo, K., Chen, Q., Wang, H., Feng, L., Ouyang, F., et al., 2019. Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. Environ. Int. 130, 104929.

Aimuzi, R., Luo, K., Huang, R., Huo, X., Nian, M., Ouyang, F., et al., 2020. Perfluoroalkyl and polyfluroalkyl substances and maternal thyroid hormones in early pregnancy. Environ. Pollut. 264, 114557.

Andersson, E.M., Scott, K., Xu, Y., Li, Y., Olsson, D.S., Fletcher, T., et al., 2019. High exposure to perfluorinated compounds in drinking water and thyroid disease. A cohort study from Ronneby, Sweden. Environ. Res. 176, 108540.

Berg, V., Nøst, T.H., Huber, S., Rylander, C., Hansen, S., Veyhe, A.S., et al., 2014. Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use. Environ. Int. 69, 58–66.

Boas, M., Feldt-Rasmussen, U., Main, K.M., 2012. Thyroid effects of endocrine disrupting chemicals. Mol. Cell. Endocrinol. 355 (2), 240–248.

Cai, D., Li, Q.Q., Chu, C., Wang, S.Z., Tang, Y.T., Appleton, A.A., et al., 2020. High trans-placental transfer of perfluoroalkyl substances alternatives in the matched maternal-cord blood serum: evidence from a birth cohort study. Sci. Total Environ. 705, 135885.

Cockcroft, D.W., Gault, M.H., 1976. Prediction of creatinine clearance from serum creatinine. Nephron 16 (1), 31–41.

Collares, F.M., Korevaar, T.I.M., Hofman, A., Steegers, E.A.P., Peeters, R.P., Jaddoe, V.W.V., et al., 2017. Maternal thyroid function, prepregnancy obesity and gestational weight gain—the Generation R Study: a prospective cohort study. Clin. Endocrinol. 87 (6), 799–806.

Craney, T.A., Surles, J.G., 2002. Model-dependent variance inflation factor cutoff values. Qual. Eng. 14 (3), 391–403.

Eick, G.N., Cepon-Robins, T.J., Devlin, M.J., Kowal, P., Sugiyama, L.S., Snodgrass, J.J., 2020. Development and validation of an ELISA for a biomarker of thyroid dysfunction, thyroid peroxidase autoantibodies (TPO-Ab), in dried blood spots. J. Physiol. Anthropol. 39 (1), 16.

Escobar, G.M.D., Obregón, M.A.J., Rey, F.E.D., 2004. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract. Res. Clin. Endoc. Metab. 18 (2), 225–248.

Gao, K., Gao, Y., Li, Y., Fu, J., Zhang, A., 2016. A rapid and fully automatic method for the accurate determination of a wide carbon-chain range of per- and polyfluoroalkyl substances (C4–C18) in human serum. J. Chromatogr. A 1471, 1–10.

Gao, K., Zhuang, T., Liu, X., Fu, J., Zhang, J., Fu, J., et al., 2019. Prenatal exposure to per- and polyfluoroalkyl Substances (PFASs) and association between the placental transfer efficiencies and dissociation constant of serum proteins–PFAS complexes. Environ. Sci. Technol. 53 (11), 6529–6538.

Glynn, A., Berger, U., Bignert, A., Ullah, S., Aune, M., Lignell, S., et al., 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. Environ. Sci. Technol. 46 (16), 9071–9079.

Inoue, K., Ritz, B., Andersen, S.L., Ramlau-Hansen, C.H., Høyer, B.B., Bech, B.H., et al., 2020. Perfluoroalkyl substances and maternal thyroid hormones in early pregnancy; findings in the Danish National Birth Cohort. Environ. Health Perspect. 127 (11), 117002.

Jansen, T.A., Korevaar, T.I.M., Mulder, T.A., White, T., Muetzel, R.L., Peeters, R.P., et al., 2019. Maternal thyroid function during pregnancy and child brain morphology: a time window-specific analysis of a prospective cohort. Lancet Diabetes Endo. 7 (8), 629–637.

Ji, B., Kang, P., Wei, T., Zhao, Y., 2020. Challenges of aqueous per- and polyfluoroalkyl substances (PFASs) and their foreseeable removal strategies. Chemosphere 250, 126316.

Kato, S., Itoh, S., Yuasa, M., Baba, T., Miyashita, C., Sasaki, S., et al., 2016. Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environ. Health Prev. 21 (5), 334–344.

Li, Y., Xu, Y., Fletcher, T., Scott, K., Nielsen, C., Pineda, D., et al., 2021. Associations between perfluoroalkyl substances and thyroid hormones after high exposure through drinking water. Environ. Res. 194, 110647.

Lindim, C., Gils, J.V., Cousins, I.T., 2016. Europe-wide estuarine export and surface water concentrations of PFOS and PFOA. Water Res 103, 124–132.

Lindstrom, A.B., Strynar, M.J., Libelo, E.L., 2011. Polyfluorinated compounds: past, present, and future. Environ. Sci. Technol. 45 (19), 7954–7961.

Lyall, K., Anderson, M., Kharrazi, M., Windham, G.C., 2017. Neonatal thyroid hormone levels in association with autism spectrum disorder. Autism Res. 10 (4), 585–592.

Mamsen, L.S., Jönsson, B.A.G., Lindh, C.H., Olesen, R.H., Larsen, A., Ernst, E., et al., 2017. Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. Sci. Total Environ. 596-597, 97–105.

Matilla-Santander, N., Valvi, D., Lopez-Espinosa, M.J., Manzano-Salgado, C.B., Ballester, F., Ibarluzea, J., et al., 2017. Exposure to perfluoroalkyl substances and metabolic outcomes in pregnant women: evidence from the Spanish INMA Birth Cohorts. Environ. Health Perspect. 125 (11), 117004.

Mondal, D., Weldon, R.H., Armstrong, B.G., Gibson, L.J., Lopez-Espinosa, M.J., Shin, H.M., et al., 2014. Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environ. Health Perspect. 122 (2), 187–192.

Nian, M., Luo, K., Luo, F., Aimuzi, R., Huo, X., Chen, Q., et al., 2020. Association between prenatal exposure to PFAS and fetal sex hormones: are the short-chain PFAS safer? Environ. Sci. Technol. 54 (13), 8291–8299.

OECD (Organisation for Economic Co-operation and Development), 2011. OECD portal on per and poly fluorinated chemicals. [cited 2021 January 15]. Available: http://www.oecd.org/site/0,3407,en_21571361_44787844_1_1_1_1_1,00.html

Pan, Y., Zhu, Y., Zheng, T., Cui, Q., Buka, S.L., Zhang, B., et al., 2017. Novel chlorinated polyfluorinated ether sulfonates and legacy per-/polyfluoroalkyl substances: placental transfer and relationship with serum albumin and glomerular filtration rate. Environ. Sci. Technol. 51 (1), 634–644.

Patlewicz, G., Richard, A.M., Williams, A.J., Grulke, C.M., Sams, R., Lambert, J., et al., 2019. A chemical category-based prioritization approach for selecting 75 per- and polyfluoroalkyl substances (PFAS) for tiered toxicity and toxicokinetic testing. Environ. Health Perspect. 127 (1), 014501.

Pearce, E.N., 2017. Higher TSH and lower FT4 levels in pregnant women are associated with higher pregestational BMI and greater gestational weight gain. Clin. Thyroidol. 29 (8), 310–311.

Peshoria, S., Nandini, D., Tanwar, R.K., Narang, R., 2020. Short-chain and long-chain fluorosurfactants in firefighting foam: a review. Environ. Chem. Lett. 18, 1277–1300.

Pop, V.J., Biondi, B., Wijnen, H.A., Kuppens, S.M., Lvader, H., 2013. Maternal thyroid parameters, body mass index and subsequent weight gain during pregnancy in healthy euthyroid women. Clin. Endocrinol. 79 (4), 577–583.

Preston, E.V., Webster, T.F., Oken, E., Claus, H.B., McClean, M.D., Rifas-Shiman, S.L., et al., 2018. Maternal plasma per- and polyfluoroalkyl substance concentrations in early pregnancy

and maternal and neonatal thyroid function in a prospective birth cohort: Project Viva (USA). Environ. Health Perspect. 126 (2), 027013.
Sagiv, S.K., Rifas-Shiman, S.L., Webster, T.F., Mora, A.M., Harris, M.H., Calafat, A.M., et al., 2015. Sociodemographic and perinatal predictors of early pregnancy per- and polyfluoroalkyl substance (PFAS) concentrations. Environ. Sci. Technol. 49 (19), 11849–11858.
Santos-Palacios, S., Brugos-Larumbe, A., Guillén-Grima, F., Galofré, J.C., 2013. A cross-sectional study of the association between circulating TSH level and lipid profile in a large Spanish population. Clin. Endocrinol. 79 (6), 874–881.
Silva, A.V., Ringblom, J., Lindh, C., Scott, K., Jakobsson, K., Öberg, M., 2020. A probabilistic approach to evaluate the risk of decreased total triiodothyronine hormone levels following chronic exposure to PFOS and PFHxS via contaminated drinking water. Environ. Health Perspect. 128 (7), 076001.
Starling, A.P., Adgate, J.L., Hamman, R.F., Kechris, K., Calafat, A.M., Ye, X., et al., 2017. Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at birth: examining mediation by maternal fasting glucose in the healthy start study. Environ. Health Perspect. 125 (6), 067016.
Su, H., Shi, Y., Lu, Y., Wang, P., Zhang, M., Sweetman, A., et al., 2017. Home produced eggs: An important pathway of human exposure to perfluorobutanoic acid (PFBA) and perfluorooctanoic acid (PFOA) around a fluorochemical industrial park in China. Environ. Int. 101, 1–6.
Talat, A., Khan, A.A., Nasreen, S., Wass, J.A., 2019. Thyroid screening during early pregnancy and the need for trimester specific reference ranges: A cross-sectional study in Lahore, Pakistan. Cureus 11 (9), e5661.
Van Veggel, K.M., Rondeel, J.M., Anten, S., 2018. Occurrence and management of an aberrant free T4 in combination with a normal TSH. Neth. J. Med. 76, 314–321.
Vanderpump, M.P., Tunbridge, W.M., 2002. Epidemiology and prevention of clinical and subclinical hypothyroidism. Thyroid 12 (10), 839–847.
Wang, Y., Starling, A.P., Haug, L.S., Eggesbo, M., Becher, G., Thomsen, C., et al., 2013. Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. Environ. Health 12 (1), 76.
Wang, Y., Rogan, W.J., Chen, P.C., Lien, G.W., Chen, H.Y., Tseng, Y.C., et al., 2014. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ. Health Perspect. 122 (5), 529–534.
Wang, Y., Yin, Q., Xu, M., Ni, Q., Wang, W., Wang, Q., 2017. BMI modulates the effect of thyroid hormone on lipid profile in euthyroid adults. Int. J. Endocrinol. 2017, 8591986.
Webster, G.M., Rauch, S.A., Marie, N.S., Mattman, A., Lanphear, B.P., Venners, S.A., 2016. Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007–2008). Environ. Health Perspect. 124 (7), 935–942.
Webster, G.M., Venners, S.A., Mattman, A., Martin, J.W., 2014. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ. Res. 133, 338–347.
WHO (World Health Organization), 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 363 (9403), 157–163.
Xiao, F., 2017. Emerging poly- and perfluoroalkyl substances in the aquatic environment: A review of current literature. Water Res. 124, 482–495.
Xie, S., Lu, Y., Wang, T., Liu, S., Jones, K., Sweetman, A., 2013. Estimation of PFOS emission from domestic sources in the eastern coastal region of China. Environ. Int. 59, 336–343.
Yang, L., Li, M., Liu, X., Wu, M., Zhang, J., Zhao, L., et al., 2020. Evaluation of iodine nutritional status among pregnant women in China. Thyroid 30 (3), 443–450.
Zhang, C., Yang, X., Zhang, Y., Guo, F., Yang, S., Peeters, R.P., et al., 2019. Association between maternal thyroid hormones and birth weight at early and late pregnancy. J. Clin. Endocr. Metab. 104 (12), 5853–5863.
Zoeller, T.R., 2010. Environmental chemicals targeting thyroid. Hormones 9 (1), 28–40.