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Effect of Multiple Exposure to Perfluorinated Chemicals on Thyroid Function among Adults in the US: The National Health and Nutrition Examination Survey 2007-2008 and 2011-2012

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Background and Objectives: Perfluoroalkyl substances (PFASs) are known to disrupt the thyroid hormone system. However, no study has assessed the association between multiple exposure to PFASs and the thyroid hormone system. This study aimed to identify the singular effects of each PFAS and the effects of multiple exposure to PFASs on the thyroid hormone profile in a representative sample of the US population. **Materials and Methods:** We used data from the US National Health and Nutrition Examination Survey (NHANES) 2007-2008 and 2011-2012. To assess the effect of simultaneous exposure to multiple PFASs on thyroid function, principal component (PC) analysis with varimax rotation was performed. Multivariate linear regression analysis was conducted to identify the effect of each PFAS and PC on thyroid function. **Results:** In this study, perfluorooctanoate (PFOA) was associated with a decrease in total T4 and Free T4 levels. Perfluorooctane sulfonate (PFOS) was associated with a decrease in total T4 level and perfluorononanoate (PFNA) and perfluorodecanoate (PFDeA) were associated with decreases in TSH levels. In PC analysis, two PCs were identified. PC1 included PFOA, PFOS, perfluorohexane sulfonate (PFHxS), PFNA and 2-(N-methyl-perfluorooctane sulfonamido) acetic acid with high loading. PC2 included PFNA, PFDeA, and perfluoroundecanoate (PFUA). In the multivariate linear regression analysis, PC1 showed negative correlations with total T4 and Free T4 levels, whereas PC2 showed a negative correlation with TSH level. **Conclusion:** We found that singular and multiple exposure to PFASs was associated with a disruption in thyroid hormone system.

Key Words: Endocrine disruptors, Perfluorinated chemicals, Thyroid hormones

Introduction

Perfluoroalkyl substances (PFASs) are used in various industrial products, including surfactants, lubricants, photographic emulsifiers, paints, fire-fighting foams, and food packaging materials.[1)] Humans may be exposed to PFASs by ingesting contaminated food and water or inhaling household dust, and PFASs are detected in over 95% of the general population.[1,2)] Animal studies have reported that PFASs are associated with tumors and neonatal deaths,[3,4)] while epidemiological studies have also reported that PFASs are associated with infertility and a decrease in birth weight.[5,6)] As a potential health hazard, PFASs are regulated in many countries, including the USA.[7)]

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Owing to the regulation of PFASs, the serum concentration of PFASs is decreasing in the USA, Australia, and Asia. Although PFASs may be present in the environment for long periods owing to their high stability and long half-lives,[8,9] they are still used extensively in some countries, including China.[10] Consequently, PFASs are detected in most of the population and some PFASs with fewer regulations are showing increasing concentrations inside the human body.[11-13] Therefore, PFASs remain key environmental pollutants that pose health hazards.[5,6]

Endocrine disrupting chemicals (EDCs) may disrupt the hypothalamic-pituitary-thyroid (HPT) axis and thyroid hormone action.[14] PFASs are also known to disrupt the thyroid hormone system. In animal experiments, PFASs were found to cause hypertrophy or hyperplasia of thyroid follicular cells in rats and showed an association with low total and free thyroxine (T4) concentrations.[15-17] Moreover, a recent meta-analysis of 12 observational studies reported that PFASs showed a negative correlation with total T4 concentrations among humans.[18] Most observational studies reported on the singular effect of only one chemical. Nevertheless, humans are exposed simultaneously to a variety of EDCs and the exposure level also varies depending on the chemical even among chemicals of the same class.[19] Therefore, the simultaneous effects of multiple exposure to various chemicals must be considered. Despite this, almost no study has assessed the association between PFASs and the thyroid hormone system with consideration of multiple exposure. It is also necessary to consider the iodine status and thyroid autoantibodies in addition to accurately identifying the effects of PFASs on the thyroid hormone system.

This study used the US National Health and Nutrition Examination Survey (NHANES) to assess the singular effects of each PFAS and the effects of multiple exposure to PFASs on the thyroid hormone profile in a representative sample of the US population.

Materials and Methods

Study Participants

The NHANES, conducted biennially by the National Center for Health Statistics, is a cross-sectional survey of a representative sample of the American population. The survey comprises questionnaire-based personal interviews, physical examinations, and laboratory tests. We used data from the NHANES 2007-2008 and 2011-2012. Among 19,905 people who participated in the NHANES, we analyzed the data regarding 3070 participants aged ≥20 years who had not been diagnosed with thyroid cancer and were not taking any medication for thyroid disease (Fig. 1).

Measurement of PFAS Concentrations

In the NHANES, 12 types of PFASs were assessed using high performance liquid chromatography-turbo ion spray ionization-tandem mass spectrometry (online SPE-HPLC-TIS-MS/MS): perfluorooctane sulfonamide (PFOSA), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSA-AcOH), perfluorobutane sulfonate (PFBuS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDeA), perfluoroundecanoate (PFUA), and perfluorododecanoate (PFDoA).[20] Of these, Et-PFOSA-AcOH, PFBuS, PFOSA, PFHpA, and PFDoA were measured at levels below the limit of detection (LOD) in over 90% of the participants. Accordingly, these PFASs were excluded

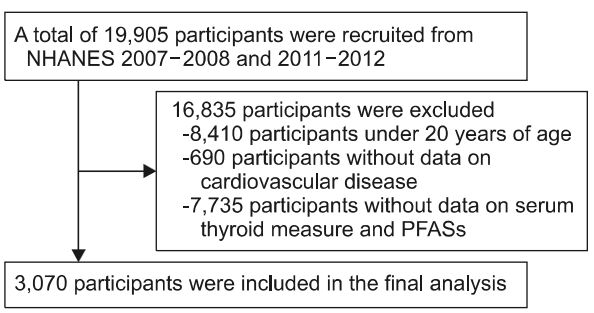

Fig. 1. Flowchart of participant selection.

Table 1. Serum thyroid measures and perfluoroalkyl substances based on demographic characteristics

Characteristic	N (%)	Thyroid measures				Perfluoroalkyl substances						
		TSH (μIU/mL)	Total T4 (ng/dL)	Total T3 (ng/dL)	FreeT4 (ng/dL)	PFOA (μg/L)	PFOS (μg/L)	PFHxS (μg/L)	Me-PFOSA-AcOH (μg/L)	PFNA (μg/L)	PFDeA (μg/L)	PFUA (μg/L)
		M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)
%>LOD	3070	-	-	-	-	99.6	99.7	98.6	54.6	99.3	71.0	41.5
Total	3070	1.55 [1.06;2.30]	7.74 [6.88;8.80]	112 [99;126]	0.80 [0.70;0.90]	3.00 [1.90;4.80]	10.40 [5.80;18.00]	1.60 [0.87;2.80]	0.20 [0.14;0.40]	1.07 [0.74;1.64]	0.25 [0.14;0.40]	0.14 [0.14;0.29]
Age												
20-50	1671 (54.4%)	1.44 [1.01;2.09]	7.66 [6.80;8.69]	116 [105;131]	0.80 [0.70;0.89]	2.64 [1.71;4.39]	8.40 [4.80;14.10]	1.28 [0.70;2.50]	0.14 [0.14;0.30]	0.98 [0.66;1.54]	0.22 [0.14;0.40]	0.14 [0.14;0.23]
51-65	738 (24.0%)	1.60*** [1.12;2.37]	7.75*** [6.86;8.80]	110*** [98;124]	0.80*** [0.70;0.90]	3.30*** [2.10;5.16]	11.65*** [7.02;20.50]	1.73*** [1.00;2.86]	0.20*** [0.14;0.40]	1.15*** [0.82;1.72]	0.28** [0.14;0.40]	0.14*** [0.14;0.30]
≥66	661 (21.5%)	1.77*** [1.18;2.71]	8.00*** [7.02;9.08]	104*** [90;114]	0.80*** [0.70;0.90]	3.50*** [2.38;5.40]	15.60*** [9.30;25.00]	2.15*** [1.30;3.51]	0.30*** [0.14;0.60]	1.15*** [0.81;1.80]	0.28** [0.14;0.48]	0.14*** [0.14;0.30]
Sex												
Male	1586 (51.7%)	1.52 [1.06;2.27]	7.60*** [6.70;8.60]	113.*** [102;127]	0.80 [0.70;0.90]	3.46*** [2.26;5.40]	12.70*** [7.75;21.30]	2.10*** [1.23;3.40]	0.20 [0.14;0.40]	1.16*** [0.82;1.72]	0.29*** [0.14;0.40]	0.14** [0.14;0.30]
Female	1484 (48.3%)	1.58 [1.07;2.32]	7.97 [7.08;9.00]	110.00 [98;124]	0.80 [0.70;0.90]	2.57 [1.56;4.20]	8.10 [4.44;13.75]	1.10 [0.61;2.02]	0.18 [0.14;0.36]	0.98 [0.66;1.48]	0.21 [0.14;0.40]	0.14 [0.14;0.26]
Race												
Mexican American	427 (13.9%)	1.55 [1.06;2.28]	8.06 [7.10;9.10]	118 [105;132]	0.80 [0.70;0.90]	9.10 [5.64;14.65]	1.40 [0.80;2.50]	0.14 [0.14;0.30]	0.95 [0.69;1.46]	0.20 [0.14;0.38]	0.14 [0.14;0.14]	9.10 [5.64;14.65]
Other Hispanic	349 (11.4%)	1.59*** [1.06;2.30]	7.90*** [7.10;9.00]	116*** [104;129]	0.80*** [0.70;0.90]	8.30*** [4.77;13.40]	1.40*** [0.66;2.40]	0.14*** [0.14;0.28]	1.04*** [0.68;1.51]	0.20*** [0.14;0.39]	0.14*** [0.14;0.27]	8.30*** [4.77;13.40]
Non-Hispanic White	1263 (41.1%)	1.72*** [1.19;2.55]	7.60*** [6.70;8.57]	110*** [97;125]	0.80*** [0.70;0.89]	11.00*** [6.46;18.40]	1.80*** [1.07;3.10]	0.30*** [0.14;0.50]	1.03*** [0.73;1.52]	0.21*** [0.14;0.40]	0.14*** [0.14;0.20]	11.00*** [6.46;18.40]
Non-Hispanic Black	675 (22.0%)	1.29*** [0.88;1.88]	7.75*** [6.83;8.80]	111*** [98;123]	0.80*** [0.70;0.89]	12.00*** [6.19;23.10]	1.78*** [0.90;3.06]	0.20*** [0.14;0.42]	1.23*** [0.82;1.81]	0.30*** [0.17;0.50]	0.18*** [0.14;0.36]	12.00*** [6.19;23.10]
Other race	356 (11.6%)	1.52*** [1.03;2.14]	7.80*** [6.96;8.89]	111*** [101;123]	0.85*** [0.77;0.94]	9.41*** [4.86;16.25]	1.12*** [0.69;1.90]	0.14*** [0.14;0.30]	1.19*** [0.70;1.98]	0.36*** [0.19;0.69]	0.30*** [0.14;0.80]	9.41*** [4.86;16.25]
BMI (kg/m^2)												
<25	1951 (64.4%)	1.49*** [1.02;2.20]	7.61*** [6.80;8.65]	111*** [99;125]	0.80 [0.70;0.90]	3.01 [1.98;4.90]	10.80* [6.11;18.20]	1.60* [0.90;2.90]	0.20 [0.14;0.40]	1.07 [0.74;1.64]	0.28*** [0.14;0.40]	0.14*** [0.14;0.30]
≥25	1079 (35.6%)	1.64 [1.15;2.43]	7.97 [7.00;9.04]	114 [101;128]	0.80 [0.70;0.90]	3.00 [1.82;4.78]	9.74 [5.54;17.85]	1.50 [0.80;2.68]	0.19 [0.14;0.35]	1.07 [0.74;1.58]	0.21 [0.14;0.40]	0.14 [0.14;0.20]

Table 1. Continued

Characteristic	N (%)	Thyroid measures				Perfluoroalkyl substances						
		TSH (μIU/mL)	Total T4 (ng/dL)	Total T3 (ng/dL)	FreeT4 (ng/dL)	PFOA (μg/L)	PFOS (μg/L)	PFHxS (μg/L)	Me-PFOSA-AcOH (μg/L)	PFNA (μg/L)	PFDeA (μg/L)	PFUA (μg/L)
		M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)	M (IQR)
Smoking												
Never	1687 (55.0%)	1.60** [1.09;2.38]	7.75 [6.90;8.80]	112 [100;125]	0.80 [0.70;0.90]	2.81*** [1.80;4.60]	9.92** [5.60;17.25]	1.40*** [0.78;2.60]	0.16*** [0.14;0.30]	1.07 [0.73;1.64]	0.26* [0.14;0.40]	0.14*** [0.14;0.30]
Smoker	1380 (45.0%)	1.48 [1.03;2.22]	7.71 [6.80;8.76]	112 [98;127]	0.80 [0.70;0.90]	3.20 [2.08;5.10]	10.80 [6.20;18.90]	1.80 [1.00;3.00]	0.20 [0.14;0.41]	1.07 [0.74;1.64]	0.24 [0.14;0.40]	0.14 [0.14;0.23]
Alcohol												
Never	766 (27.3%)	1.60 [1.08;2.43]	8.06*** [7.11;9.10]	111 [98;125]	0.80* [0.70;0.90]	2.90** [1.71;4.70]	9.80** [5.59;17.70]	1.30*** [0.70;2.41]	0.20 [0.14;0.40]	1.07 [0.67;1.64]	0.23 [0.14;0.40]	0.14 [0.14;0.30]
Drinker	2042 (72.7%)	1.53 [1.06;2.26]	7.60 [6.72;8.68]	112 [100;126]	0.80 [0.70;0.90]	3.11 [2.05;4.93]	10.70 [6.15;18.40]	1.71 [0.98;3.00]	0.20 [0.14;0.40]	1.07 [0.74;1.64]	0.26 [0.14;0.40]	0.14 [0.14;0.26]
Thyroid autoantibody status												
Negative	2689 (88.1%)	1.50*** [1.05;2.18]	7.73 [6.87;8.77]	112 [99;126]	0.80** [0.70;0.90]	3.05 [1.93;4.85]	10.30 [5.91;18.10]	1.60 [0.88;2.80]	0.20 [0.14;0.40]	1.07 [0.74;1.64]	0.26 [0.14;0.40]	0.14 [0.14;0.29]
Positive	362 (11.9%)	2.01 [1.22;3.23]	7.80 [6.90;9.02]	112 [100;127]	0.80 [0.70;0.89]	2.80 [1.80;4.70]	10.85 [5.50;17.30]	1.50 [0.80;2.70]	0.16 [0.14;0.38]	1.07 [0.73;1.64]	0.21 [0.14;0.40]	0.14 [0.14;0.30]

*$p<0.05$, **$p<0.01$, ***$p<0.001$.

BMI: body mass index, IQR: interquartile range, LOD: limit of detection, M: median, Me-PFOSA-AcOH: 2-(N-methyl-perfluorooctane sulfonamido) acetic acid, PFDeA: perfluorodecanoate, PFHxS: perfluorohexane sulfonate, PFNA: perfluorononanoate, PFOA: perfluorooctanoate, PFOS: perfluorooctane sulfonate, PFUA: perfluoroundecanoate, TSH: thyroid-stimulating hormone

from the analysis and only PFOA, PFHxS, PFOS, PFNA, PFDeA Me-PFOSA-AcOH, and PFUA were included in the analysis. The LOD of PFOA and PFHxS was 0.1 ng/mL; that of PFOS, Me-PFOSA-AcOH, and PFUA was 0.2 ng/mL; and that of PFNA was 0.082 ng/mL.[21] For PFASs with levels below the LOD, such levels were substituted with values calculated by dividing the LOD by the square root of 2.

Serum Thyroid Measures

The thyroid stimulating hormone (TSH) level was measured using a 3rd generation, two-site immunoenzymatic assay (Beckman Coulter Inc., CA, USA). The reference range of the TSH level was 0.24-5.4 μIU/mL. Total T3 and total T4 levels were measured using a competitive binding immunoenzymatic assay (Beckman Coulter Inc.). The reference ranges of total T3 and total T4 levels were 80-200 ng/dL and 6.09-12.23 μg/dL, respectively. Free T4 (FT4) levels were measured using a two-step enzyme immunoassay (Beckman Coulter Inc.). The reference range of FT4 was 0.6-1.6 μg/dL.

Study Covariates

Age, gender, race, body mass index (BMI), alcohol consumption, smoking status, and urinary iodine levels were considered as potential confounders of thyroid function. Sociodemographic information, such as age, gender, and race, were acquired via household interviews. The BMI was calculated by dividing the body weight (in kg) by the square of the height (in m). The height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, by a trained health technician. Alcohol intake and smoking status were assessed via a questionnaire. Alcohol intake was categorized based on ≥12 alcoholic drinks within one year. Smoking status was categorized based on smoking ≥100 cigarettes throughout one's life. Urine iodine levels were measured by inductively coupled plasma dynamic reaction cell mass spectroscopy (ICP-DRC-MS). Thyroperoxidase (TPO) antibody and thyroglobulin (Tg) antibody levels were measured using a sequential two-step immunoenzymatic "sandwich" assay (Beckman Coulter Inc.). TPO antibody levels ≥9.0 IU/mL and Tg antibody levels ≥4.0 IU/mL were considered to indicate positive results.

Statistical Analysis

Data regarding serum thyroid measures and PFASs are presented as the median and interquartile range (IQR) according to demographic characteristics. The levels of PFASs and TSH had a skewed distribution and were thus analyzed after natural log-transformation. To assess the effect of each PFAS on thyroid function, multivariate linear regression analysis was performed with adjustment for potential confounding variables (age, gender, race, smoking status, alcohol consumption, BMI, urinary iodine level, Tg antibody level, and TPO antibody level). The results were used to investigate the association with log transformed values of TSH, total T4, total T3, and FT4 levels. To assess the effect of simultaneous exposure to multiple PFASs on thyroid function, principal component (PC) analysis with varimax rotation was performed. Each PC could explain at least 5% of the variance in the data and had an eigenvalue ≥1. We considered factor loadings ≥ 0.4 as indicative of high loading. All statistical analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Analysis items with p-value <0.05 were considered statistically significant.

Results

A total of 3070 participants were included in the present study (Fig. 1). The basic demographics of the sample population and their serum thyroid measures and PFAS concentrations are summarized in Table 1. The mean age of the participants was 48.5 years, and there were 1586 (51.7%) males. PFOA, PFHxS, PFOS, and PFNA were detected in most participants (>90%), while PFDeA (71%), Me-PFOSA-AcOH (54.6%), and PFUA (41.5%) were detected at levels below the LOD. The distribution of each PFAS varied depending on the age group, sex, race, BMI, smoking status, and alcohol intake (Table 1). The correlation between each PFAS is summarized in Table 2. PFOA, PFHxS, and PFOS were correlated with one another, whereas PFNA, PFDeA, and PFUA were correlated with one another

Table 2. Spearman correlation between each perfluoroalkyl substances (n=3070)

PFASs	PFOA	PFOS	PFHxS	Me-PFOSA-AcOH	PFNA	PFDeA	PFUA
	correlation coefficients	correlation coefficients	correlation coefficients	correlation coefficients	correlation coefficients	correlation coefficients	correlation coefficients
PFOA	1	0.686**	0.636**	0.309**	0.634**	0.444**	0.134**
PFOS		1	0.689**	0.339**	0.670**	0.578**	0.337**
PFHxS			1	0.252**	0.449**	0.292**	0.118**
Me-PFOSA-AcOH				1	0.166**	0.100**	-0.035
PFNA					1	0.765**	0.546**
PFDeA						1	0.686**
PFUA							1

*p<0.05, **p<0.01.
Me-PFOSA-AcOH: 2-(N-methyl-perfluorooctane sulfonamido) acetic acid, PFDeA: perfluorodecanoate, PFHxS: perfluorohexane sulfonate, PFNA: perfluorononanoate, PFOA: perfluorooctanoate, PFOS: perfluorooctane sulfonate, PFUA: perfluoroundecanoate

(Spearman' correlation coefficients >0.5, p<0.01).

Multivariate linear regression analysis was performed on each PFAS. The results showed negative associations between PFOA and total T4 and FT4 levels, a negative association between PFOS and total T4 level, a positive association between PFHxS and total T3 level, and negative associations among TSH and PFNA and PFDeA (Table 3). Three hundred and sixty-two participants (11.9%) had positive results regarding thyroid autoantibody (TPO antibody or Tg antibody) levels. Multivariate linear regression analysis showed no association between PFASs and thyroid autoantibody levels (Table 3).

PC analysis was performed to assess the effects of multiple exposure to PFASs, as a result of which two PCs were extracted (Kaiser-Meyer-Olkin measure, 0.791). PC1 included PFOA, PFOS, PFHxS, PFNA and Me-PFOSA-AcOH with high loading and could explain 53.5% of the variance. PC2 included PFNA, PFDeA, and PFUA with high loading, and could explain 20.4% of the variance (Table 4). In the multivariate linear regression analysis, PC1 showed negative correlations with total T4 and FT4 levels, whereas PC2 showed a negative correlation with TSH level. Similar results were found in the analyses with adjustment for PC1 or PC2 (Table 5). In the sub-analysis according to the presence of thyroid autoantibodies, PC1 showed a negative correlation with total T4 level regardless of the presence or absence of thyroid autoantibodies, whereas PC2 showed a significantly negative correlation among participants who tested positive for thyroid autoantibodies.

Discussion

The present study was the largest epidemiological study on the associations between PFASs and thyroid function and the first to identify the effects of multiple exposure to PFASs on thyroid function. In this study, PFOA was associated with a decrease in total T4 and FT4 levels. PFOS was associated with a decrease in total T4 level and PFNA and PFDeA were associated with decreases in TSH levels. Considering multiple exposure to PFASs, PC analysis was performed on seven PFASs; as a result, two PCs were identified. PC1 with 53.5% explanation for variance was associated with decreases in total T4 and FT4 levels, whereas PC2 with 20.4% explanation for variance was significantly associated with a decrease in TSH level.

Since 2002, manufacturers have changed the practice of manufacturing perfluorinated chemicals to eliminate long-chain PFASs under the supervision of the Unites States Environmental Protection Agency. Consequently, the NHANES showed that the concentrations of PFOA, PFOS, PFHxS, PFNA, and PFDeA had decreased by 50%, 75%, 27%, 30%, and 32%, respectively, between 2003 and 2014.[22] However, since PFASs are still being detected in the human body, it

Table 3. Linear regression analysis with perfluoroalkyl substances and thyroid hormone

PFASs	TSH	Total T4	Total T3	Free T4	TPO antibodies*	Tg antibodies*
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
PFOA	0.012 (−0.023, 0.047)	**−0.158 (−0.244, −0.072)**	0.122 (−1.049, 1.294)	**−0.014 (−0.022, −0.007)**	−0.073 (−4.525, 4.379)	2.438 (−1.792, 6.667)
PFOS	−0.010 (−0.04, 0.019)	**−0.083 (−0.155, −0.012)**	−0.812 (−1.785, 0.161)	−0.005 (−0.012, 0.001)	2.070 (−1.624, 5.764)	3.036 (−0.477, 6.549)
PFHxS	−0.003 (−0.03, 0.024)	−0.015 (−0.081, 0.051)	**0.913 (0.015, 1.812)**	−0.004 (−0.01, 0.002)	−0.011 (−3.423, 3.4)	2.732 (−0.514, 5.978)
Me-PFOSA-AcOH	0.019 (−0.014, 0.053)	−0.081 (−0.164, 0.001)	−0.462 (−1.584, 0.66)	−0.007 (−0.014, 0.001)	−0.120 (−4.387, 4.147)	1.829 (−2.219, 5.877)
PFNA	**−0.046 (−0.083, −0.009)**	−0.048 (−0.139, 0.043)	−0.316 (−1.559, 0.927)	−0.002 (−0.011, 0.006)	−0.603 (−5.331, 4.124)	3.828 (−0.669, 8.324)
PFDeA	**−0.04 (−0.078, −0.002)**	0.003 (−0.09, 0.096)	−1.162 (−2.425, 0.101)	−0.001 (−0.01, 0.007)	−0.032 (−4.84, 4.776)	1.456 (−3.114, 6.026)
PFUA	−0.033 (−0.074, 0.007)	0.055 (−0.044, 0.154)	−0.986 (−2.33, 0.358)	0.007 (−0.002, 0.016)	0.126 (−4.989, 5.241)	−0.692 (−5.555, 4.172)

*Adjusted for sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, and urinary iodine concentration.
Adjusted for sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, urinary iodine concentration, thyroglobulin antibody and thyroperoxidase antibody.
CI: confidence interval, Me-PFOSA-AcOH: 2-(N-methyl-perfluorooctane sulfonamido) acetic acid, PFASs: perfluoroalkyl substances, PFDeA: perfluorodecanoate, PFHxS: perfluorohexane sulfonate, PFNA: perfluorononanoate, PFOA: perfluorooctanoate, PFOS:perfluorooctane sulfonate, PFUA: perfluoroundecanoate, TPO: thyroperoxidase, TSH: thyroid-stimulating hormone

Table 4. Summary of the rotated factor pattern in principal component analysis

	PC 1	PC 2
PFOA	0.831*	0.277
PFOS	0.776*	0.481
PFHxS	0.834*	0.157
Me-PFOSA-AcOH	0.569*	−0.117
PFNA	0.521*	0.723*
PFDeA	0.199	0.910*
PFUA	−0.076	0.919*

*High loading of ≥0.4.
Me-PFOSA-AcOH: 2-(N-methyl-perfluorooctane sulfonamido) acetic acid, PC: principal component, PFDeA: perfluorodecanoate, PFHxS: perfluorohexane sulfonate, PFNA: perfluorononanoate, PFOA: perfluorooctanoate, PFOS: perfluorooctane sulfonate, PFUA: perfluoroundecanoate

is necessary to assess their adverse effects on human health.

Most studies on PFASs have focused on PFOS and PFOA.[23] In our study, PFOS and PFOA showed negative correlations with total T4 level. There is evidence that PFASs reduce the total T4 level. Experimental studies on rats and monkeys have reported that exposure to PFOS and PFOA resulted in decreased levels of total T4 and FT4.[16,17,24,25] In epidemiological studies, varying results on the association between PFASs and human thyroid function have been reported.[18] Although a recent meta-analysis reported that PFOS and PFOA are negatively correlated with total T4 level, there was heterogeneity among the studies. The mechanism by which PFASs affect the thyroid function is not clearly understood. Possible underlying mechanisms have been suggested including the competitive binding of PFASs to human thyroid hormone transport proteins, thereby resulting in a decrease in thyroid hormone levels. A previous study reported that PFOS could compete with T4 for transthyretin,[26] and as a result, the total thyroid hormone level in the blood may decrease. Increased hepatic degradation of thyroid hormones due to PFOS-induced UDP-glucuronosyltransferase (UGT) could be another possible underlying mechanism.[17,27] However, the metabolic rates for xenobiotics are much faster in rodents than in humans, and thus, it is difficult to apply the results from animal studies to humans.[28] It takes several years for PFASs to be metabolized in humans, whereas their half-life is only a few days in rodents. Another difference is that the mechanism of peroxisome proliferation plays virtually no role in the metabolism of xenobiotics in humans, but plays an important role in rodents.[29]

In the present study, PFDeA showed an association with a decrease in TSH level. Epidemiological studies on the association between PFDeA and thyroid hormone level did not yield significant results.[30-32] Bloom et al.[30] failed to find an association between PFDeA and thyroid function in the New York State Angler Cohort Study (NYSACS). Ji et al.[31] also reported no association between PFDeA and thyroid function among Koreans. Wang et al.[32] reported that PFDeA was not associated with TSH level among pregnant women. Unlike epidemiological studies, few experimental studies have reported a negative association between PFDeA and thyroid hormone levels in rats. As a potential mechanism, it was suggested that changes in circulating thyroid hormone levels may have occurred because of decreased responsiveness of the HPT axis, and such results were consistent with the findings of the present study.[33,34]

The results of the present study showed that PFNA is associated with a decrease in TSH level. Epidemiological studies did not find a significant association between PFNA and thyroid hormone levels.[30-32] Although there are no experimental studies on the association between PFNA and thyroid hormone in mammals, one experimental study with zebrafish reported that long-term PFNA exposure caused a significant increase in total T3 levels.[35] Additional studies are needed to assess whether TSH levels decreased as a result of a compensatory mechanism of the HPT axis due to an increase in thyroid hormone levels, unlike PFDeA.

In the correlation analysis of PFASs, some substances showed strong associations with each other, including PFOA, PFOS, PFHxS, and PFNA. Therefore, a simple analysis of data regarding one substance presents the risk of false positive or false negative results,[36] necessitating the consideration of mixed exposure to various PFASs.[15] PC analysis was performed

Table 5. Association between PC of perfluoroalkyl substances and thyroid hormone

	TSH		Total T4		Total T3		Free T4	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Total								
PC 1	0.009 (−0.017, 0.036)	0.009 (−0.018, 0.035)	−0.099 (−0.164, −0.034)	−0.099 (−0.164, −0.033)	0.273 (−0.617, 1.163)	0.259 (−0.632, 1.149)	−0.009 (−0.015, −0.003)	−0.009 (−0.015, −0.003)
PC 2	−0.032 (−0.058, −0.006)	−0.032 (−0.058, −0.006)	0.022 (−0.041, 0.085)	0.020 (−0.043, 0.083)	−0.736 (−1.593, 0.121)	−0.731 (−1.589, 0.126)	0.003 (−0.003, 0.008)	0.003 (−0.003, 0.008)
Thyroid autoantibody status								
Negative								
PC1	0.021 (−0.006, 0.047)	0.02 (−0.007, 0.046)	−0.076 (−0.144, −0.009)	−0.076 (−0.143, −0.008)	0.187 (−0.765, 1.139)	0.165 (−0.788, 1.118)	−0.009 (−0.015, −0.003)	−0.009 (−0.015, −0.003)
PC2	−0.022 (−0.047, 0.003)	−0.021 (−0.046, 0.004)	0.023 (−0.041, 0.088)	0.021 (−0.043, 0.085)	−0.659 (−1.563, 0.245)	−0.654 (−1.559, 0.25)	0.002 (−0.004, 0.008)	0.001 (−0.004, 0.007)
Positive								
PC 1	−0.012 (−0.124, 0.099)	−0.003 (−0.114, 0.109)	−0.288 (−0.51, −0.066)	−0.292 (−0.515, −0.069)	1.081 (−1.452, 3.614)	1.204 (−1.335, 3.743)	−0.018 (−0.038, 0.003)	−0.019 (−0.04, 0.002)
PC 2	−0.133 (−0.251, −0.015)	−0.132 (−0.251, −0.014)	0.026 (−0.213, 0.265)	0.051 (−0.187, 0.289)	−1.545 (−4.243, 1.153)	−1.648 (−4.355, 1.06)	0.013 (−0.009, 0.036)	0.015 (−0.008, 0.037)

Negative means those with neither TPO Ab nor TgAb.
Positive means those with TPO Ab or TgAb.
Model 1: Adjusted for sex, age, race/ethnicity, body mass index, smoking status, alcohol consumption, and urinary iodine concentration.
Model 2: Adjusted for the variables in Model 1 plus PC1 and PC2.
CI: confidence interval, PC: principal component, TSH: thyroid-stimulating hormone

to investigate the effect of thyroid hormone disruption in mixed exposure to such PFASs; as a result, two PCs were extracted. PC1 was associated with decreased levels of total T3 or total T4, and PC2 was associated with decreased TSH level.

The significance of the present study lies in the fact that we used large-scale data to assess the effect of each PFAS on thyroid function, based on analyses adjusted for various confounding variables. In addition, the present study is the first to identify the effects of multiple exposure to PFASs. However, the present study also had several potential limitations. First, because this study had a cross-sectional design, it was unable to identify causality between PFASs and thyroid function. Second, the concentration of thyroid hormone was assessed at a single time point. Therefore, the present study could not consider the transient changes in thyroid function.

In conclusion, we found that a higher serum concentration of PFASs was associated with a disruption in thyroid hormone levels. Furthermore, multiple exposure to PFOA, PFOS, PFHxS, PFNA and Me-PFOSA-AcOH was negatively associated with total T4 and FT4 levels, whereas multiple exposure to PFNA, PFDeA, and PFUA was negatively associated with TSH concentration. Our study could provide clues about the effect of PFASs on thyroid function in the real world with simultaneous exposure to multiple environmental contaminants.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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