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Associations of serum perfluoroalkyl substance and vitamin D biomarker concentrations in NHANES, 2003–2010

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ABSTRACT

Perfluoroalkyl substances (PFAS) are persistent endocrine disrupting chemicals found in industrial and commercial products. Previous research has shown that other endocrine disrupting chemicals such as phthalates and bisphenol A may alter circulating levels of vitamin D; however, no research has examined associations between PFAS and vitamin D biomarkers. We conducted a cross-sectional analysis of 7040 individuals aged 12 years and older participating in the 2003–2010 cycles of the United States National Health and Nutrition Examination Survey (NHANES). Concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and total 25-hydroxyvitamin D [25(OH)D] were measured in serum samples. We used multivariable linear regression to estimate covariate-adjusted differences in total 25(OH)D or prevalence odds of vitamin D deficiency per $\log_2$ change in PFAS concentrations. We also assessed potential effect measure modification by gender, age, and race/ethnicity. PFAS were detected in over 98% of the samples. In adjusted models, each 2-fold increase in PFOS was associated with 0.9 nmol/L (95% CI: 0.2, 1.5) lower total 25(OH)D concentrations, with associations significantly stronger among whites (β: −1.7; 95% CI: −2.6, −0.7) and individuals older than 60 years of age (β: −1.7; 95% CI: −2.9, −0.5). Each 2-fold increase in PFHxS was associated with 0.8 nmol/L (95% CI: 0.3, 1.3) higher total 25(OH)D, and this association was not modified by age, gender, and race/ethnicity. PFOA and PFNA were not associated with total 25(OH)D. When assessing prevalence odds of vitamin D deficiency, we observed similar patterns of association with PFAS concentrations. Our results suggest that some PFAS may be associated with altered vitamin D levels in the United States population, and associations may vary by chemical, age, and race/ethnicity. Prospective epidemiological studies are needed to confirm our findings and determine their implications for vitamin D-associated health outcomes in children and adults.

1. Introduction

Perfluoroalkyl substances (PFAS) are persistent endocrine disrupting chemicals widely used in commercial products and industrial applications including some carpeting, textiles, paint, paper, food packaging, cleaning agents, and firefighting foams (Buck et al., 2011; Fromme et al., 2010). Four PFASs, perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA), were detected in 97–100% of serum samples in a representative survey of the United States population (United States Department of Health and Human Services Centers for Disease Control and Prevention, January 2017). PFAS biomarker concentrations have been associated with altered thyroid hormone levels, reduced immune function, and developmental problems (Ballesteros et al., 2017; Braun 2016; National Institute of Environmental Health Sciences; NTP (National Toxicology Program) 2016). In addition, emerging studies report associations of serum PFAS concentrations with lower bone density and increased prevalence of osteoporosis (Khalil et al. 2016, 2018; Lin et al., 2014).

Vitamin D is a steroid hormone that is acquired by sunlight exposure or ingestion and plays a major role in bone biology by promoting calcium absorption from the gut (Norman, 2008). In addition to its important contribution to musculoskeletal health, vitamin D status has been associated with cancer (Feldman et al., 2014), cardiovascular disease (Norman and Powell, 2014), autoimmune disease (Prietl et al., 2013), and pregnancy and birth outcomes (Weinert and Silveiro, 2015).

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Only two-thirds of the United States population have sufficient levels of vitamin D (Forrest and Stuhldreher, 2011) and vitamin D insufficiency is more prevalent among African Americans (Harris, 2006).

Many known factors influence circulating vitamin D levels, including variation in sun exposure, diet, age, and obesity (Tsiaras and Weinstock, 2011). Vitamin D homeostasis may also be influenced by endocrine disrupting chemicals because the active metabolite of vitamin D, 1,25-hydroxyvitamin D [1,25(OH)D] is similar in molecular structure to classic steroid hormones and its nuclear receptor is similar to steroid and thyroid hormone receptors (Norman and Hurwitz, 1993; Norman et al., 1993). Two previous studies of adult NHANES participants and pregnant women reported inverse associations of bisphenol A and phthalates with total 25-hydroxyvitamin D [25(OH)D] levels, with some differences in associations by gender and race/ethnicity (Johns et al. 2016, 2017). While PFAS are also endocrine disrupting chemicals that may affect estrogen (Benninghoff et al., 2011; Gao et al., 2013) or glucocorticoid (Goudarzi et al., 2017; Zhao et al., 2011) activity, no previous studies have examined whether PFAS exposures are associated with vitamin D levels.

We investigated relationships between serum concentrations of four PFAS with serum vitamin D biomarker levels in a population-based study of United States adults and children. Our second aim was to understand if relationships between PFAS and vitamin D levels were modified by gender, age, or race and ethnicity.

2. Materials and methods

2.1. Population and data collection

We used data from the National Health and Nutrition Examination Survey (NHANES), a series of nationally representative cross-sectional surveys on the health and nutrition of civilian, noninstitutionalized adults and children conducted by the Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention (CDC), 2003–2010). Study staff administered questionnaires at an in-home visit and collected biological specimens, including serum samples, at a mobile examination center (MEC).

2.2. PFAS exposure

Serum samples were collected at the MEC in standard collection containers. Samples were refrigerated after collection and transferred to storage at −20 °C in polypropylene or polyethylene containers (Berman et al., 2001). Samples were analyzed for PFAS biomarker concentrations using Solid Phase Extraction-High Performance Liquid Chromatography-Turbo Ion Spray-Tandem Mass Spectrometry (SPE-HPLC-TIS-MS/MS) as described in detail elsewhere (Kuklenyik et al., 2005). Concentrations below the limits of detection (LOD) were replaced with LOD divided by the square root of 2 (Hornung and Reed, 1990). We focused on four PFAS biomarkers that were detected in > 98% of participants: PFOA, PFOS, PFHxS, and PFNA Due to their right-skewed distribution, PFAS concentrations were log-2 transformed for analysis to reduce the influence of outliers.

2.3. Vitamin D measurement

The DiaSorin assay kit was used to measure serum total 25(OH)D in serum samples from the 2003–2004 and 2005–2006 cycles. Due to variation in 25(OH)D values from DiaSorin method variation, the CDC measured 25(OH)D using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) in serum samples from the 2007–2008 and 2009–2010 cycles (Centers for Disease Control and Prevention (CDC)). To account for assay differences, the CDC converted concentrations from the 2003–2004 and 2005–2006 cycles to allow for comparison with later cycles using methods described elsewhere (Centers for Disease Control and Prevention (CDC)). Vitamin D levels are reported in nmol/L (1 nmol/L = 0.4 ng/mL). In addition to examining 25(OH)D concentrations as a continuous variable, we also calculated a binary variable where individuals with a measured 25(OH)D level below 50 nmol/L (20 ng/mL) were considered vitamin D deficient based on the World Health Organizations recommendations (World Health Organization Scientific Group on the Prevention and Management of Osteoporosis, 2003).

2.4. Covariates

Using a directed acyclic graph (DAG), we identified potential confounders from previous research that were associated with both PFAS chemical exposure and vitamin D levels as well as strong predictors of vitamin D. Information about sociodemographic factors including age, gender, race/ethnicity, education level, and family income to poverty ratio was collected from a questionnaire administered during the in-home visit. A binary variable for six-month time period classified examinations performed during November 1st-April 30 or May 1st-October 31st and was used as a proxy for season because examination dates are not released by NHANES. Body mass index (BMI) and serum cotinine, a biomarker of tobacco smoke exposure, were assessed from examination and laboratory data, respectively. Individuals were considered a smoker if their serum cotinine concentrations were 3 ng/mL or higher (Benowitz et al., 2009). Among individuals 20 years or older, we used BMI to determine whether individuals were underweight (< 18.5), normal or healthy weight (18.5- < 25.0), overweight (25.0- < 30.0), or obese (≥30.0). For individuals less than 20 years of age, we calculated sex-specific BMI-for-age percentiles based on the 2000 CDC Growth charts (Centers for Disease Control and Prevention (CDC), 2017) and classified individuals as underweight (< 5th percentile), normal or healthy weight (5th- < 85th percentile), overweight (85th- < 95th percentile), or obese (≥95th percentile). Finally, we used data from dietary supplement questionnaires to assess vitamin D supplement use in the past 30 days as a binary variable.

2.5. Statistical methods

Of the 41,156 individuals from the 2003–2010 NHANES cycles, we excluded individuals who were not included in a one-third random subsample for measurement of serum PFAS (n = 32,609) or who lacked a 25(OH)D measurement (n = 271). Due to the potential effects on PFAS metabolism, we also excluded individuals who had liver or kidney disease (n = 352). Finally, we excluded individuals who were missing covariate information (n = 884), most of whom were missing BMI. These exclusions left 7040 individuals for our analysis.

First, we conducted descriptive analyses to compare weighted mean 25(OH)D levels (nmol/L) by covariates, to calculate weighted geometric mean serum PFAS concentrations, and assess Spearman correlation coefficients between the four PFAS chemicals. To determine optimal parameterization of continuous covariates, we assessed whether bivariate relationships between serum PFAS concentrations and 25(OH)D with covariates were non-linear using restricted cubic splines. Because all four PFAS exhibited non-linear associations with age (all non-linearity p-values < 0.001), we modeled age using a restricted cubic spline with four knots.

Next, we modeled differences in serum 25(OH)D levels with increasing continuous PFAS biomarkers using linear regression. Then, we performed multivariable linear regression adjusting for gender, age, race/ethnicity, family income to poverty ratio category, BMI category, vitamin D supplement use, smoking status, and six-month examination period. We also used multivariable linear regression to estimate the predicted adjusted mean 25(OH)D level in each quintile of PFAS concentrations. Because previous research suggests that both PFAS concentrations and vitamin D levels vary by age, gender, and race/ethnicity (Tsiaras and Weinstock, 2011; United States Department of Health and Human Services Centers for Disease Control and Prevention,

January 2017), we examined effect measure modification (EMM) by age category (< 20, 20–60, 60+), gender, and race/ethnicity (Mexican American/Hispanic, Non-Hispanic White, Non-Hispanic Black, Other). We used an augmented product term approach to account for modifier-dependent confounding (Buckley et al., 2017) and considered modification to be present when the p-value was ≤0.10. Finally, we used logistic regression to calculate prevalence odds ratios (POR) for vitamin D deficiency (< 50 nmol/L) per 2-fold change in PFAS concentrations.

We analyzed all data using SAS version 9.4 (SAS Institute, Inc) and R Studio version 1.0.153 (RStudio: Integrated Development Environment for R). NHANES uses a complex sampling design and utilizes sampling weights in order to produce nationally representative data. Following recommended methods, we accounted for the NHANES survey design by using cluster and strata weights from demographic datasets (Centers for Disease Control and Prevention (CDC)). Additionally, PFAS concentrations are only measured in a one-third random sample of NHANES participants, so subsample weights were used to account for the individual probability of inclusion in the PFAS subsamples (Centers for Disease Control and Prevention (CDC)).

2.6. Sensitivity analysis

We performed several sensitivity analyses to evaluate the robustness of our results. First, due to moderate correlation between serum concentrations of individual PFAS (Supplemental Table 1), we estimated associations between PFAS biomarkers and total serum 25(OH)D levels or prevalence odds of vitamin D deficiency by simultaneously including all four PFAS biomarkers in adjusted models. Second, our main models used family income to poverty ratio to measure socioeconomic status because education status was not available for individuals < 20 years of age. Therefore, in addition to adjusting for family income to poverty ratio, we adjusted for education status among individuals ≥20 years of age to determine whether there was residual confounding by socioeconomic status. Third, we adjusted for continuous body size. Our primary models adjusted for BMI in categories that were assigned based on BMI (kg/m^3) in adults and BMI percentiles in children. To assess the possibility of residual confounding within categories, we compared our primary results to estimates adjusted for continuous BMI in models restricted to individuals ≥20 years of age, and continuous BMI percentiles in individuals < 20 years of age. Finally, because vitamin D supplement use is a source of vitamin D, we compared our primary results to the results from our final model but without adjustment for vitamin D supplement use.

3. Results

NHANES participants in our sample were predominantly 20–59 years of age (66.8%), Non-Hispanic White (71.3%), female (51.4%), had a family income to poverty ratio greater than 5 (24.1%), were normal weight (34.1%), had a urinary cotinine level below 3 ng/mL (73.1%), did not use vitamin D supplements (63.8%), and were not vitamin D deficient (71.7%) (Table 1). The mean 25(OH)D concentration in our weighted sample was 64.5 nmol/L (SE = 0.69). Vitamin D concentrations were lower among younger individuals, Non-Hispanic Blacks, individuals with lower family income to poverty ratio, and individuals who were overweight or obese (Table 1).

PFAS biomarkers were detected in serum of nearly all participants with a geometric mean PFOA, PFOS, PFHxS, and PFNA concentration of 3.8, 14.5, 1.8, and 1.1 ng/mL, respectively (Table 2). Serum PFAS concentrations were all moderately correlated (range: 0.26–0.64) (Supplemental Table 1).

In our adjusted analysis, each 2-fold increase in PFOS was associated with 0.9 nmol/L (95% CI: −1.5, −0.2) lower total serum 25(OH)D levels and higher prevalence odds of being vitamin D deficient (POR = 1.05, 95% CI: 0.97, 1.13). In contrast, a 2-fold increase in PFHxS was associated with *higher* serum 25(OH)D levels (β = 0.8, 95% CI: 0.3, 1.3) and *lower* prevalence odds of being vitamin D deficient (POR = 0.94, 95% CI: 0.89, 0.99). PFOA and PFNA were not associated with total serum 25(OH)D concentrations (Table 3).

When examining dose-response relationships using quintiles of serum PFAS concentrations, we observed a monotonic decrease in total serum 25(OH)D across PFOS quintiles (Fig. 1, Table 4). For example, individuals in the fifth quintile of PFOS concentrations had 2.8 nmol/L lower total serum 25(OH) D levels (95% CI: −4.7, −0.8) than individuals in the first quintile. For PFHxS, quintiles two through five had significantly higher total serum 25(OH)D levels compared to individuals in the first quintile, but there was not a monotonic dose-response relationship. Similar patterns were observed in analyses of PORs for vitamin D deficiency by PFAS quintile (Table 4).

The association between PFOS and total 25(OH)D was modified by age and race/ethnicity (Table 5). Compared with individuals 20–60 years of age (β: −0.4, 95% CI: −1.1, 0.3) we observed stronger associations for PFOS among individuals older than 60 years of age (β: −1.7; 95% CI: −2.9, −0.5; p-value = 0.06). We also observed stronger PFOS associations among non-Hispanic Whites (β: −1.7; 95% CI: −2.6, −0.7) compared to Non-Hispanic Blacks (β: −0.2; 95% CI: −1.3, 0.9; p-value = 0.05), Hispanics (β: 0.3; 95% CI: −0.7, 1.4; p-

Table 1
Total 25-hydroxyvitamin D [25(OH)D] levels by covariates among participants in the National Health and Nutrition Examination Survey (NHANES), 2003–2010.

Characteristics	N (weighted %)	25(OH)D nmol/L Mean (SE)
Overall	7040	64.5 (0.69)
Age		
12-19	1771 (13.6)	64.0 (1.03)
20-59	3493 (66.8)	64.0 (0.90)
60+	1776 (19.6)	66.9 (0.74)
Race/Ethnicity		
Mexican American/Hispanic	1951 (12.6)	54.3 (0.8)
Non-Hispanic White	3251 (71.3)	70.5 (0.6)
Non-Hispanic Black	1557 (11.0)	42.4 (1.0)
Other	281 (5.2)	54.1 (1.6)
Gender		
Male	3478 (48.6)	63.9 (0.7)
Female	3562 (51.4)	65.2 (0.8)
Family Poverty Income Ratio[a]		
0 - < 1	1540 (13.5)	57.3 (1.2)
1 - < 2	1933 (20.5)	59.7 (0.9)
2 - < 3	1096 (16.0)	65.1 (1.0)
3 - < 4	794 (14.5)	65.0 (1.2)
4 - < 5	565 (11.2)	69.4 (1.6)
5+	1112 (24.1)	69.8 (1.1)
Body Mass Index (BMI)[b]		
Underweight	143 (2.2)	71.7 (3.2)
Normal or Healthy Weight	2506 (34.1)	69.0 (0.9)
Overweight	2133 (32.3)	66.6 (0.9)
Obese	2258 (31.4)	57.1 (0.9)
Cotinine Concentrations		
< 3 ng/mL	5346 (73.1)	65.3 (0.7)
≥3 ng/mL	1694 (26.9)	62.6 (1.0)
Vitamin D Supplement Use		
No	4861 (63.8)	60.2 (0.8)
Yes	2179 (36.2)	72.2 (0.8)
Six Month Cycle		
November 1st - April 30th	3265 (39.1)	58.1 (0.8)
May 1st – October 31st	3775 (60.9)	68.7 (0.7)
Vitamin D Deficiency		
Deficient (< 50 nmol/L)	2648 (28.3)	37.2 (0.3)
Not Deficient (> = 50 nmol/L)	4392 (71.7)	75.3 (0.5)

* 1 nmol/L = 0.4 ng/mL.
[a] A higher family poverty income ratio indicates higher socioeconomic status.
[b] For individuals 20 or older BMI categories were based on kg/m^3 and individuals that were 12–19 were assigned a BMI category based on BMI percentiles.

Table 2
Distribution of perfluoroalkyl substances serum concentrations (ng/mL) among participants in the National Health and Nutrition Examination Survey (NHANES), 2003–2010.

	Percent Detected	Geometric Mean[a]	Min.	25th Percentile	Median	75th Percentile	Max.
Perfluorooctanoic acid (PFOA)[b]	99.9%	3.8	< LOD	2.6	3.9	5.5	104.0
Perfluorooctane sulfonic acid (PFOS)[c]	99.9%	14.5	< LOD	9.1	15.1	23.8	435.0
Perfluorohexane sulfonic acid (PFHxS)[d]	98.6%	1.8	< LOD	1.0	1.8	3.2	82.0
Perfluorononanoic acid (PFNA)[e]	99.4%	1.1	< LOD	0.7	1.1	1.6	25.7

[a] To compute the geometric mean, biomarker concentrations < LOD were replaced by LOD/√2.
[b] Limit of detection (LOD) is 0.1.
[c] LOD for Survey cycle 03–04 and cycles 05–06, 07–08, and 09–10 are 0.4 and 0.2, respectively.
[d] LOD for Survey cycle 03–04 and cycles 05–06, 07–08, and 09–10 are 0.3 and 0.1, respectively.
[e] LOD for Survey cycles 03–04 and 05–06, and cycles 07–08 and 09–10 are 0.1 and 0.082, respectively.

Table 3
Difference in total serum 25-hydroxyvitamin D (nmol/L) and prevalence odds ratios (POR) for vitamin D deficiency[a] per 2-fold increase in serum perfluoroalkyl substance concentrations in National Health and Nutrition Examination Survey (NHANES) participants, 2003–2010.

	Total serum 25(OH) D (nmol/L)		Vitamin D deficiency	
	Unadjusted (95% CI)	Adjusted (95% CI)[b]	Unadjusted POR (95% CI)	Adjusted POR (95% CI)[b]
Perfluorooctanoic acid (PFOA)	1.0 (0.0, 2.0)	−0.3 (−1.0, 0.4)	0.88 (0.81, 0.66)	1.02 (0.93, 1.11)
Perfluorooctane sulfonic acid (PFOS)	−0.5 (−1.2, 0.3)	−0.9 (−1.5, −0.2)	0.99 (0.92, 1.05)	1.05 (0.97, 1.13)
Perfluorohexane sulfonic acid (PFHxS)	1.4 (0.9, 1.9)	0.8 (0.3, 1.3)	0.89 (0.85, 0.93)	0.94 (0.89, 0.99)
Perfluorononanoic acid (PFNA)	−0.4 (−1.5, 0.6)	0.4 (−0.5, 1.2)	1.01 (0.93, 1.11)	0.96 (0.87, 1.06)

*1 nmol/L = 0.4 ng/mL.
[a] 2648 individuals (37.6%) were classified as vitamin D deficient (< 50 ng/mL).
[b] Adjusted for gender, race/ethnicity, age, body mass index category, vitamin D supplement use, poverty to income ratio, smoking status, and 6-month examination time period.

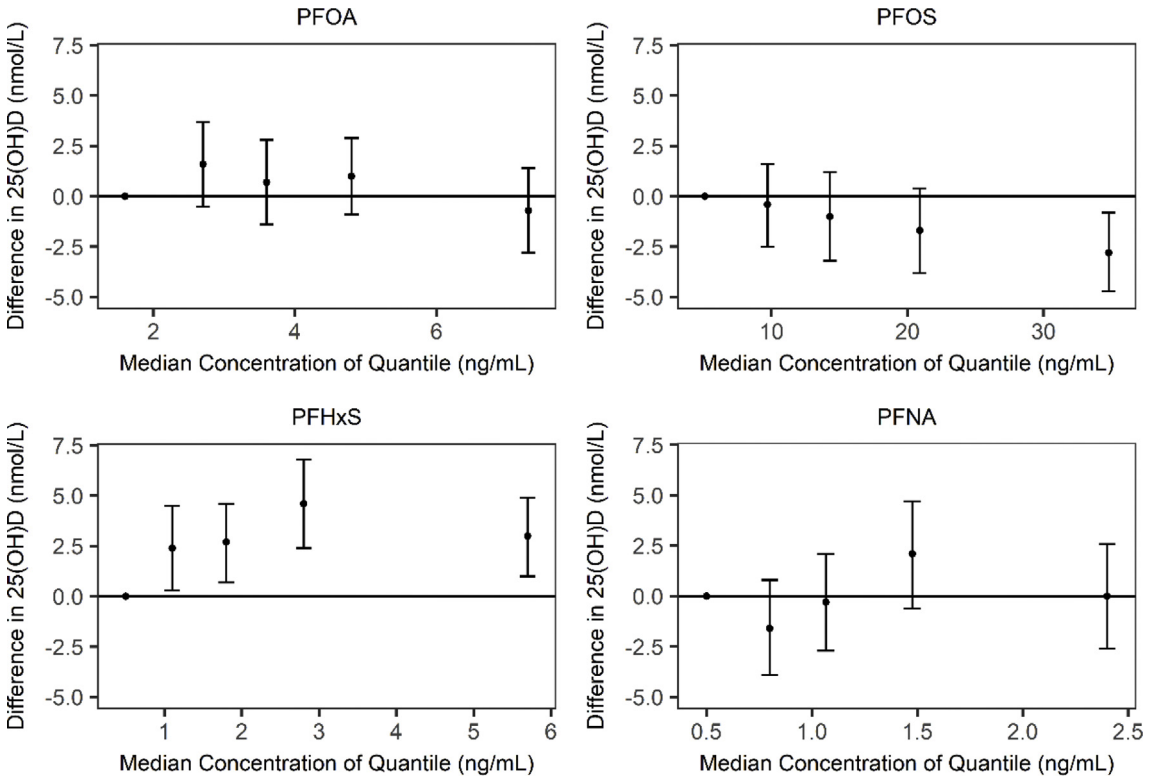

Fig. 1. Adjusted total serum 25-hydroxyvitamin D (nmol/L) by perfluoroalkyl substance concentration quintiles among participants in the National Health and Nutrition Examination Survey, 2003–2010.
*All models were adjusted for gender, race/ethnicity, age, body mass index category, vitamin D supplement use, poverty to income ratio, smoking status, and 6-month examination time period.
* 1 nmol/L = 0.4 ng/mL.

Table 4
Adjusted total serum 25-hydroxyvitamin D by perfluoroalkyl substance concentration quintiles in NHANES participants, 2003–2010.

Perfluoroalkyl Substance Quintile (Range, ng/mL)	N	Unadjusted Mean Total Serum 25(OH) D nmol/L	Difference in 25(OH)D nmol/L (95% CI)*	Prevalence Odds Ratio of Vitamin D Deficiency (95% CI)*
Perfluorooctanoic acid (PFOA)				
1st (< LOD-2.2)	1438	61.4	Ref.	Ref.
2nd (2.3–3.1)	1384	63.0	1.6 (−0.5, 3.7)	0.82 (0.61, 1.10)
3rd (3.2–4.1)	1350	62.1	0.7 (−1.4, 2.8)	0.89 (0.68, 1.15)
4th (4.2–5.6)	1448	62.4	1.0 (−0.9, 2.9)	0.94 (0.76, 1.16)
5th (5.7–104.0)	1420	60.7	−0.7 (−2.8, 1.4)	1.03 (0.80, 1.33)
Perfluorooctane sulfonic acid (PFOS)				
1st (< LOD-7.4)	1410	63.0	Ref.	Ref.
2nd (7.5–11.8)	1397	62.6	−0.4 (−2.5, 1.6)	0.93 (0.75, 1.17)
3rd (11.9–17.1)	1418	62.1	−1.0 (−3.2, 1.2)	1.01 (0.82, 1.24)
4th (17.2–25.8)	1403	61.4	−1.7 (−3.8, 0.4)	1.22 (0.96, 1.54)
5th (25.9–435.0)	1412	60.3	−2.8 (−4.7, −0.8)	1.12 (0.88, 1.40)
Perfluorohexane sulfonic acid (PFHxS)				
1st (< LOD-0.8)	1387	59.3	Ref.	Ref.
2nd (0.9–1.4)	1439	61.7	2.4 (0.3, 4.5)	0.85 (0.67, 1.08)
3rd (1.5–2.2)	1436	61.9	2.7 (0.7, 4.6)	0.85 (0.69, 1.07)
4th (2.3–3.7)	1381	63.8	4.6 (2.4, 6.8)	0.78 (0.60, 0.99)
5th (3.8–82.0)	1397	62.2	3.0 (1.0, 4.9)	0.75 (0.59, 0.96)
Perfluorononanoic acid (PFNA)				
1st (< LOD-0.65)	1480	61.7	Ref.	Ref.
2nd (0.7–0.9)	1289	60.2	−1.6 (−3.9, 0.8)	1.07 (0.86, 1.33)
3rd (0.902–1.2)	1410	61.5	−0.3 (−2.7, 2.1)	0.89 (0.70, 1.14)
4th (1.23–1.72)	1441	63.8	2.1 (−0.6, 4.7)	0.81 (0.62, 1.05)
5th (1.8–25.748)	1420	61.8	0.0 (−2.6, 2.6)	0.94 (0.73, 1.22)

*1 nmol/L = 0.4 ng/mL.
*Adjusted for gender, race/ethnicity, age, body mass index category, vitamin D supplement use, poverty to income ratio, smoking status, and 6-month examination time period.

Table 5
Adjusted difference in total serum 25-hydroxyvitamin D (nmol/L) per 2-fold increase in serum perfluoroalkyl substance concentrations among NHANES participants, 2003–2010: Effect measure modification (EMM) by gender, age, and race/ethnicity.[a]

Gender[a]	Males (N = 3478)	Females (N = 3562)	EMM p-value				
PFOA	0.2 (−0.9, 1.2)	−0.6 (−1.5, 0.3)	0.26				
PFOS	−0.3 (−1.1, 0.5)	−1.3 (−2.4, −0.3)	0.10				
PFHxS	0.9 (0.2, 1.6)	0.8 (0.2, 1.5)	0.84				
PFNA	1.0 (−0.3, 2.3)	−0.2 (−1.4, 0.9)	0.14				
Age[b]	< 20 Years: Beta (95% CI) (N = 1771)	20–60 Years: Beta (95% CI) (N = 3493)	EMM p-value (< 20 vs. 20–60)	60 + Years: Beta (95% CI) (N = 1776)	EMM p-value (60 + vs. 20–60)		
PFOA	−0.9 (−3.4, 1.7)	0.1 (−0.8, 0.9)	0.50	−1.1 (−2.4, 0.1)	0.12		
PFOS	−1.8 (−3.9, 0.4)	−0.4 (−1.1, 0.3)	0.23	−1.7 (−2.9, −0.5)	0.06		
PFHxS	0.4 (−0.7, 1.4)	0.8 (0.2, 1.4)	0.49	1.1 (−0.1, 2.3)	0.64		
PFNA	−0.1 (−2.0, 1.7)	0.4 (−0.6, 1.5)	0.59	−0.0 (−1.3, 1.3)	0.59		
Race/Ethnicity[c]	Non-Hispanic White: Beta (95% CI) (N = 3251)	Non-Hispanic Black (N = 1557) Beta (95% CI)	EMM p-value (Black vs. White)	Hispanic: Beta (95% CI) (N = 1951)	EMM p-value (Hispanic vs. White)	Other: Beta (95% CI) (N = 281)	EMM p-value (White vs. Other)
PFOA	−0.8 (−1.7, 0.2)	0.1 (−0.9, 1.1)	0.22	0.5 (−0.8, 1.7)	0.12	0.7 (−2.0, 3.3)	0.31
PFOS	−1.7 (−2.6, −0.7)	−0.2 (−1.3, 0.9)	0.05	0.3 (−0.7, 1.4)	0.005	1.3 (−0.3, 2.8)	0.001
PFHxS	0.9 (0.1, 1.6)	0.4 (−0.4, 1.3)	0.43	0.2 (−0.5, 0.9)	0.19	0.0 (−1.8, 1.8)	0.39
PFNA	0.1 (−1.2, 1.3)	0.8 (−0.8, 2.3)	0.48	0.2 (−0.9, 1.2)	0.91	1.8 (0.0, 3.6)	0.10

*PFOA = Perfluorooctanoic acid.
*PFOS = Perfluorooctane sulfonic acid.
*PFHxS = Perfluorohexane sulfonic acid.
*PFNA = Perfluorononanoic acid.
*1 nmol/L = 0.4 ng/mL.
[a] Models were adjusted for race/ethnicity, age, body mass index category, vitamin D supplement use, poverty-to-income ratio, smoking status, and 6-month examination time period.
[b] Models were adjusted for adjusted for gender, race/ethnicity, age, body mass index category, vitamin D supplement use, poverty-to-income ratio, smoking status, and 6-month examination time period.
[c] Models were adjusted for gender, age, body mass index category, vitamin D supplement use, poverty-to-income ratio, smoking status, and 6-month examination time period.

value = 0.005), and other race/ethnicities (β: 1.3; 95% CI: −0.3, 2.8; p-value = 0.001). PFOS concentrations were more strongly associated with 25(OH)D among females (β: −1.3; 95% CI: −2.4, −0.3) compared with males (β: −0.3; 95% CI: −1.1, 0.5) but the difference was not statistically significant (p = 0.10). Gender, age, and race/ethnicity did not modify associations between PFOA, PFHxS, or PFNA with total serum 25(OH)D (all p-values ≥0.10, Table 5).

In sensitivity analyses, adjusting for educational status or continuous BMI did not appreciably change our results (results not shown). Associations of PFOS and PFHxS with total serum 25(OH)D and prevalence odds of vitamin D deficiency became stronger when we included all PFAS in the same model to estimate independent associations of each PFAS biomarker (Supplemental Table 2).

4. Discussion

We found associations of two PFAS with total serum 25(OH)D levels in a nationally representative sample of the United States population. Higher serum PFOS concentrations were monotonically associated with lower vitamin D biomarker concentrations, and the association was stronger among non-Hispanic white individuals compared to other race/ethnicities and among those older than 60 years compared to 20-60-year-old individuals. Higher serum PFOS concentrations were also associated with increased odds of being vitamin D deficient. In contrast, PFHxS was associated with higher vitamin D levels and lower odds of being vitamin D deficient.

Limited laboratory research suggests that endocrine disrupting chemicals may affect vitamin D homeostasis. The active metabolite of vitamin D, 1,25-hydroxyvitamin D [1,25(OH)D], which is important in initiating the biological response to vitamin D exposure (Bikle, 2014), is similar in molecular structure to classic steroid hormones and its nuclear receptor is similar to sex steroid and thyroid hormone receptors (Norman and Hurwitz, 1993; Norman et al., 1993). Because PFAS have been associated with altered sex steroid (Lopez-Espinosa et al., 2016) and thyroid hormone levels (Ballesteros et al., 2017; Lee and Choi, 2017; Lewis et al., 2015) we hypothesized that they may also affect vitamin D metabolism. PFOS, but not PFHxS, has been associated with lower levels of testosterone and estradiol among young children; however, these results were observed to vary by age and sex (Lopez-Espinosa et al., 2016). Both PFOS and PFHxS have been associated in the same direction with thyroid stimulating hormones and free and total triiodothyronine and thyroxine, and these associations have also been found to vary by age and sex (Ballesteros et al., 2017; Lewis et al., 2015). Other PFAS chemicals, including PFOA and PFNA have also shown some associations with sex steroid and thyroid hormone levels (Lewis et al., 2015).

While this is the first study to examine the relationships between serum PFAS and vitamin D levels, our results are consistent with previous research observing associations of other endocrine disrupting chemicals and vitamin D levels. In a cross-sectional study of NHANES participants, Johns et al. found that higher urinary levels of di(2-ethyhexyl) phthalate (DEHP) metabolites and bisphenol A (BPA) were associated with a lower serum total 25(OH)D (Johns et al., 2016). They also found a positive association between urinary MEP and total serum 25(OH)D, particularly among women (they did not assess modification by age or race and ethnicity) (Johns et al., 2016). In a subsequent prospective, repeated measures analysis among pregnant women, Johns et al. replicated their previous findings of associations between urinary DEHP metabolites and BPA concentrations with lower total 25(OH)D (Johns et al., 2017). In contrast to the cross-sectional study, MEP was not associated with total 25(OH)D in the prospective analysis (Johns et al., 2017). Similar to our findings, associations of DEHP and BPA biomarkers with vitamin D levels differed by race and ethnicity, with stronger associations among white compared to black or other race/ethnicity individuals.

Stronger associations of PFOS with vitamin D levels among non-Hispanic whites compared to other race/ethnicities could be due to differences in vitamin D levels or susceptibility to altered vitamin D metabolism. Vitamin D insufficiency is more prevalent among African Americans and most young healthy African Americans do not have recommended levels of 25(OH)D concentrations any time of year (Harris, 2006). In addition to physical differences, such as skin pigmentation, which can alter vitamin D production in the skin (Harris, 2006), there are vitamin D receptor (VDR) polymorphisms that differ by race and ethnicity (Cooper and Umbach, 1996; Nelson et al., 2000). Because non-Hispanic white individuals have higher levels of vitamin D compared to all other race and ethnicities, they have the potential for a greater decrease in 25(OH)D levels whereas those with low 25(OH)D may be less susceptible to further reductions. Finally, it is also possible that there is unmeasured confounding within categories of race and ethnicity.

We also found differences in the relationship between PFOS and 25(OH)D by age, with stronger associations among individuals 12-< 20 years of age and individuals older than > 60 years of age. While the reason for this difference is unclear, bone turnover is greater in children due to bone mass accrual and linear growth and among older adults due to age-related bone loss. Given the role of vitamin D in bone remodeling, early life and old age may be periods of heightened susceptibility to chemical impacts on vitamin D metabolism. If PFOS does interfere with vitamin D metabolism during these age periods, it could lead to impaired skeletal development and osteoporosis. Indeed, prior studies have observed associations of PFAS concentrations with shorter stature in children (Lee et al., 2018; Shoaff et al., 2018; Wang et al., 2016) and with reduced bone mineral density and osteoporosis in older adults (Khalil et al. 2016, 2018; Lin et al., 2014).

We observed a positive association between serum concentrations of PFHxS and serum total 25(OH)D levels, which was in the opposite direction of the PFOS results and remained after co-adjusting for PFAS biomarkers. One explanation for this directional difference is residual confounding given that PFHxS concentrations were lower compared to the other PFAS and that associations did not increase monotonically with greater exposure quintile. It is also possible that compared to PFOS, PFHxS may have different actions on the vitamin D endocrine system.

There are several limitations in our analysis. First, our study is cross-sectional study and temporality is a concern as serum concentrations of both PFAS biomarkers and total 25(OH)D were measured at the same time. However, the half-lives of these longer chain PFAS range from 3.8 to 7.3 years (Olsen et al., 2007) whereas the half-life of 25(OH)D is about three weeks (Zerwekh, 2008). Therefore, the likelihood of reverse causality due to the cross-sectional study design is low. Additionally, factors related to sunlight exposure are major predictors of vitamin D levels but are not well-captured in the NHANES survey data. Because NHANES does not release information on month of year or season, we used the six-month examination time period as a crude proxy for seasonal differences in exposure to sunlight. However, given the long half-lives of PFAS, biomarker concentrations are not likely to vary strongly by season and thus, we expect any confounding to be small. We were also unable to account for geographical location or other factors such as time spent outdoors, which could confound associations if they are also related to PFAS concentrations. While previous research suggests that other endocrine disrupting chemicals such as phthalates and BPA are associated with altered vitamin D levels, we were unable to account for potential confounding by these chemicals due to nonoverlap of the PFAS subsamples with the phthalate and BPA subsamples in NHANES. However, correlations of PFAS with these non-persistent compounds have been reported to be low (Woods et al., 2017). Women of childbearing age may excrete more PFAS when pregnant or menstruating (Wong et al., 2014) and may be more likely to be vitamin D deficient (De-Regil et al., 2016). We were unable to assess the influence of menstruation or pregnancy among women due to the small number of pregnant women in our study and missing or unmeasured data on

women's reproductive health. However, age-stratified results among females suggested that associations were stronger among women < 20 and 60+ years of age (Supplemental Table 3). Finally, while we excluded individuals who reported having a history of kidney or liver disease, we were limited in our ability to account for liver or gall bladder functioning, which is important for xenobiotic and vitamin D metabolism. Therefore, there may be potential confounding by pharmacokinetics.

Our analysis also has several strengths. Our study utilized a nationally-representative sample, adjusted for many potential confounders, had a large sample size, and assessed effect measure modification by gender, age, and race/ethnicity.

5. Conclusion

To our knowledge, this is the first study to observe associations of PFAS and vitamin D biomarker concentrations, we observed associations of two PFAS biomarkers with altered vitamin D levels and found differences in associations of PFOS with vitamin D by age and race/ethnicity. Laboratory and prospective epidemiological studies are needed to confirm our findings and determine their implications for vitamin D-associated health outcomes in children and adults.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2018.11.003.

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