Chemosphere 374 (2025) 144230

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Association of perfluoroalkyl substance (PFAS) on vitamin D biomarkers in a highly exposed population of the Veneto Region in Italy

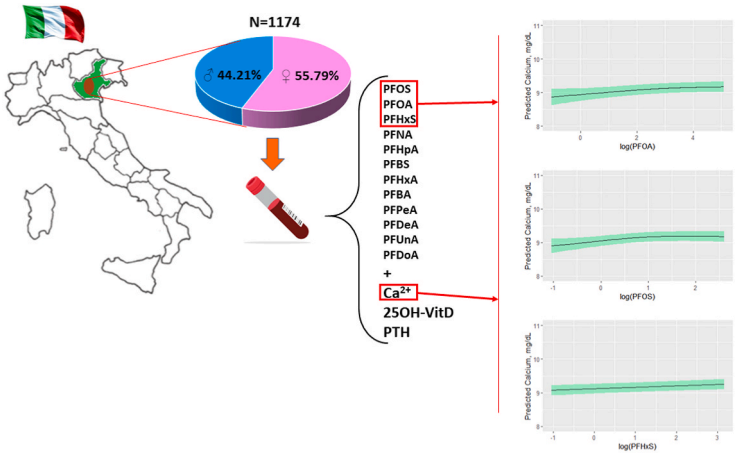
Andrea Di Nisio [a,d,1], Luca De Toni [a,1], Cristina Canova [b], Mirko Berti [b], Achille Di Falco [c], Rinaldo Zolin [c], Anna Maria Bettega [c], Iva Sabovic [a], Alberto Ferlin [a], Carlo Foresta [a,*]

[a] Department of Medicine, Operative Unit of Andrology and Medicine of Human Reproduction, University of Padova, Padova, Italy
[b] Unit of Biostatistics, Epidemiology and Public Health, Dpt. of Cardio-Thoraco-Vascular Sciences and Public Health, University of Padova, Padova, Italy
[c] Unit of Epidemiology, Hospital of Vicenza, ULSS 8 Berica, Vicenza, Italy
[d] Department of Psychology and Health Sciences, Pegaso Digital University, Napoli, Italy

HIGHLIGHTS

- PFASs are persistent and bio-accumulative environmental pollutants.
- PFAS exposure is thought to associate with early derangements of calcium metabolism.
- Environmental PFAS exposure does not associate with vitamin D or PTH alteration.
- Serum PFOA, PFOS and PFHxS levels are associated with serum calcium.
- Association with calcium is maintained independently from vitamin D supplementation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: A. Gies

Keywords:
Vitamin D status
Environmental exposure
Calcium homeostasis
Parathyroid hormone

ABSTRACT

Perfluoroalkyl substances (PFASs) raise concerns about their environmental accumulation. Experimental data have suggested that PFASs interfere with bone metabolism from the early stages of life. However, mechanisms underlying this association are unclear. The aim of this study was to evaluate the possible association between environmental exposure to PFAS and vitamin D (VitD), serum calcium and parathyroid hormone (PTH) levels in subjects residing in high-exposure area of the Veneto Region of Italy. In this cross-sectional observational study, 1174 subjects who previously adhered to the 2016–2018 Regional Surveillance Plan for plasma levels of PFASs were recalled in 2023 and evaluated for demographic, anthropometrics and blood analyses. Data on nutritional habits and VitD supplementation were obtained by a dedicated questionnaire. Serum concentrations of PFASs,

* Corresponding author. Department of Medicine, Unit of Andrology and Medicine of Human Reproduction, University of Padova, Via Giustiniani, 2, 35128, Padova, Italy.
E-mail address: carlo.foresta@unipd.it (C. Foresta).
[1] These two authors contributed equally to the study.

https://doi.org/10.1016/j.chemosphere.2025.144230
Received 11 November 2024; Received in revised form 13 February 2025; Accepted 14 February 2025

Skeletal metabolism
Supplementation

calcium, 25-hydroxy-vitamin D (25OH-VitD) and PTH were determined from blood sampling. Perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonic acid (PFHxS) were the only three PFASs, of 12, quantifiable in at least 90% of the samples and considered for further analyses. Generalized additive models, using linear regression and smoothing thin plate splines, detected a positive association between serum calcium and all considered PFAS (PFOA: $\beta = 0.03$; CI 95% 0.01–0.06; PFOS: $\beta = 0.06$; CI 95% 0.02–0.09, PFHxS: $\beta = 0.04$; CI 95% 0.01–0.06). Estimated degrees of freedom (EDF) analysis showed the approximately linear association between serum calcium with PFOA (EDF = 1.89) and PFHxS (EDF = 1.21), but not for PFOS (EDF = 3.69). Differently, PFAS levels showed no association with either 25-hydroxy-vitamin D or PTH, except for ln-transformed 25OH-D and PFOS ($\beta = 0.04$; CI 95% 0.00–0.08). Stratified analyses confirmed the positive association between all considered PFAS and calcium in subjects not taking a VitD supplementation. Results show that high exposure levels to PFAS may interfere with calcium metabolism, independently of lifestyle and dietary factors. Further elucidation on the mechanisms underlying calcium homeostasis disruption, including multiple binding-equilibrium with serum albumin, remains to be addressed.

1. Introduction

Perfluoroalkyl substances (PFASs) are a class of synthetic molecules used in a wide range of consumer products and industrial applications, due to their unique physical and chemical characteristics (Conder et al., 2008). Major concerns on the environmental spreading of these compounds derive from tissue accumulation in animal species. Experimental data have demonstrated the bio-accumulation of PFAS in the bone marrow and bone tissue of mice exposed during embryo development (Bogdanska et al., 2011). Similar findings have been reported also in humans since autopsy specimens, obtained from subjects living in exposed areas, showed significant levels of PFAS in the bone tissue, suggesting the ability of these compounds to bio-accumulate in the skeletal apparatus from the foetal to the adult age range, with possible consequences on bone metabolism in the corresponding stages of life (Koskela et al., 2017; Pérez et al., 2013). In support of this hypothesis, toxicological studies showed reduced mineralization in *in vitro* cell models and altered bone mineral density *in vivo* in mouse foetuses exposed to PFASs (Koskela et al., 2017; Lau et al., 2007). In addition, epidemiological studies have reported an association between the exposure to PFASs and skeletal derangements (Khalil et al., 2016; Lin et al., 2014). In particular, a significant correlation between high serum levels of PFAS and reduced bone mineral density has been documented, with a variable extent according to the specific PFAS molecules considered. In this frame, a higher prevalence of osteoporosis, a lower bone mineral density in the tibia and femur and a higher prevalence of osteoporosis among women was associated with exposure to perfluoro-octanoic acid (PFOA), perfluoro-nonanoic acid (PFNA), and perfluoro-hexanesulfonic acid (PFHxS) (Khalil et al., 2016). Furthermore, a study on young girls exposed to PFAS during foetal development showed reduced bone mass and reduced skeletal growth (Jeddy et al., 2018). More recently these results have been confirmed by other studies on teenager or young adults (Hu et al., 2019; Cluett et al., 2019), as has as the association with an increased risk of caries, suggestive of phospho-calcium disequilibrium (Wiener and Waters, 2019). More recently, consistent associations of PFAS exposure to osteoporosis were reported by, respectively, Fan et al. in a large group of healthy subjects from the general population residing in southern China and by Bukley et al. in 197 teenagers from a prospective pregnancy and birth cohort study conducted in Ohio, USA (Buckley et al., 2024; Fan et al., 2023). Although the increasing number of epidemiological studies supporting the adverse association between the exposure to PFASs and skeletal metabolism, the mechanistic aspects of this association are unclear. A key driver in skeletal development is vitamin D (VitD), a secosteroid hormone promoting gut reabsorption of calcium in favour of bone anabolism (Plum and DeLuca, 2010). Of note, VitD has been recently involved in multiple health outcomes, including cardiovascular health, inflammatory modulation and reproductive outcome and cancer prevention (Giustina et al., 2024). Several exogenous factors are known to influence circulating VitD levels, including obesity, dietary pattern and environmental pollution (Arabi et al., 2010). In addition, the homeostasis of VitD can be influenced by endocrine disruptors since the biologically active metabolite of VitD, 1,25-dihydroxyvitamin D (1,25OH-VitD) is similar in structure to classic steroid hormones and its biological effects rely on the transcriptional activity of a specific nuclear receptor as for thyroid or steroid hormone receptors (Wang et al., 2010). As an example, two epidemiological studies showed an inverse association between recognized endocrine disruptors, such as bisphenol A and phthalates, and VitD levels (Johns et al., 2016, 2017a). PFASs are also claimed as endocrine disruptors, having been associated with estrogen-like or anti-androgen-like activity, and the possible disrupting activity of these compounds on VitD-related pathways is currently under investigation (Ješeta et al., 2021). In this regard, few studies analyzed the association between PFAS and VitD status and most focused on early life association, such as in women during pregnancy or in subjects of perinatal age (Berger et al., 2024; Chang et al., 2021; Liu et al., 2023). The possible association between PFASs and VitD levels in the general population has been investigated only recently by two studies, showing an increased risk of hypovitaminosis D in exposed subjects (Carlsson et al., 2023; Etzel et al., 2019). A previous experimental study from our group showed the possible competition between PFOA and 1,25OH-VitD on the binding to VitD receptor, with major consequences on its structural flexibility at molecular dynamics analysis (Di Nisio et al., 2020a,b). Functional correlates of this binding activity on VitD receptor were then investigated *in vitro* on a human osteosarcoma cell line, whose mineralization activity upon stimulation with 1,25OH-VitD was significantly reduced during exposure to PFOA (Di Nisio et al., 2020a,b). This model of interference could find a clinical counterpart in the altered response to VitD in two different target cell populations of this hormone: osteoblasts and enterocytes (Cipriani and Cianferotti, 2023). Based on the possible mechanisms through which PFAS may interfere with VitD activity, we hypothesized a possible role for these pollutants in the pathogenesis of osteoporosis, the main pathology related to functional hypovitaminosis D.

In this study we aimed to evaluate the circulating levels of VitD, calcium and parathyroid hormone (PTH) in subjects residing in the red zone of Veneto Region in the North East of Italy, where groundwater of a wide area was contaminated by PFASs from a manufacturing plant active since the late 1960s. As a result, subjects in this area were exposed to high levels of PFASs, particularly PFOA, through drinking water until 2013, when the pollution was discovered by authorities and water treatment by charcoal filtration was applied, successfully reducing PFAS levels in drinking water (Pitter et al., 2020b).

2. Methods

2.1. Subjects

This study aimed to evaluate the association between serum concentrations of PFAS and serum concentrations of cholecalciferol, or 25-hydroxy-vitamin D (25OH-VitD), calcium and parathyroid hormone (PTH), with a cross-sectional design, characterizing the possible

concentration-effect correlation. It was conceived in agreement with the Good Clinical Practice of the European Medicines Agency and the Declaration of Helsinki ("World Medical Association Declaration of Helsinki," 2013) and was approved by the Ethics Committee for Clinical Trials of the Province of Vicenza (trial code 45/22, approved during the June 21, 2022 session).

The study involved the prospective recall of subjects resident in high exposure areas of the Veneto Region, North-East Italy, who previously participated to the 2016–2018 PFAS levels-evaluation campaign according to the Regional Surveillance Plan (DGR 2133/2016 and DGR 691/2018). Subjects were invited by e-mail according to the fulfilment of the first-level assessment during the 2016–2018 PFAS levels-evaluation campaign, which involved a potentially recruitable population of approximately 37,200 subjects (allegato7684276.pdf (quotidianosanita.it)). Subjects fulfilling these criteria were then recalled during December 2022–June 2023 and evaluated for demographic data and anthropometrics. All subjects were then consecutively recruited and evaluated at the Unit of Epidemiology, Hospital of Vicenza - ULSS 8 Berica (Vicenza, Italy). Upon the acquisition of the signed informed consent for participating in the study, subjects were requested to provide a single blood sample for the quantification of 25OH-VitD, calcium and PTH levels in the period March–June 2023. Considering the narrow period of blood sampling, seasonal variation was not included in the subsequent analysis. Participants also received an outpatient evaluation during which were recorded sex, age, month of sampling and anthropometric data, such as height and weight for the calculation of body mass index (BMI). Lifestyle habits, including alcohol consumption, smoking, and use of dietary supplement, were also collected by a dedicated questionnaire. Inclusion criteria were: age between 18 and 65 years, both sexes, residence in the high-exposure area of the Veneto Region, and, in particular, afference to the local Social-Health Unit "ULSS 8 Berica"; previous participation in the Health Surveillance Plan of the Veneto Region (DGR 2133/2016 and DGR 691/2018) and completion of the lifestyle questionnaire. VitD supplementation, use of drugs recognized to interfere with phospho-calcium metabolism (e.g. antiepileptics, glucocorticoids, antiretrovirals, antifungals, cholestyramine, orlistat) and malabsorption disease (e.g. cystic fibrosis, celiac disease, Crohn's disease, bariatric surgery) were considered exclusion criteria.

2.2. PFAS quantification

Quantification of PFAS was performed on serum samples obtained during the Health Surveillance Plan of the Veneto Region (DGR 2133/2016 and DGR 691/2018) and subsequent second round of surveillance (DGR n. 1752 of 30.12.2022). High-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS; Prominence UFLC XR 20, Shimadzu, coupled to API 4000TM LC-MS/MS System, Sciex) was used to quantify serum levels of twelve PFASs: perfluorooctanesulfonate (PFOS), PFOA, PFHxS, PFNA, perfluoroheptanoic acid, perfluorobutanesulfonic acid, perfluorohexanoic acid, perfluorobutanoic acid, perfluoropentanoic acid (PFPeA), perfluorodecanoic acid, perfluoroundecanoic acid and perfluorododecanoic acid. Procedures of PFAS quantification are detailed elsewhere (Pitter et al., 2020a).

According to the UNI CEI EN ISO/IEC 17,025 regulation, the analytical method was validated and applied to the analyses of low- and high-concentration pooled quality control materials, standards, reagent blanks and study samples.

2.3. Vitamin D, calcium and parathyroid hormone measurements

VitD status was evaluated through the quantification of 25OH-VitD with ECLIA method (Roche – Cobas, Monza, Italy). The method has been standardized with internal standards traceable to PMR ID-LC/MS/MS for 25OH-VitD with reference Material 2972 of the National Institute of Standards and Technology Standard (Gaithersburg, MD, USA). The dosage was linear between 3.00 and 120 ng/mL.

Serum calcium ions were quantified photometrically with Gen.2 method (Roche – Cobas), a 5-nitro-5′-methyl-(1,2-bis(*o*-aminophenoxy) ethan-N,N,N′,N′-tetraacetic acid (NM-BAPTA)-based method. Briefly, in an alkaline environment, NM-BAPTA forms an optically active compound. In a second step, this complex reacts with EDTA. The change in absorbance is directly proportional to the calcium concentration and is measured. The limit of detection was 0.20 mmol/L (0.8 mg/dL) and the limit of quantification (LOQ) = 0.20 mmol/L (0.8 mg/dL). The limit of blank, limit of sensitivity and LOQ were determined in accordance with EP17 requirements-A2 of the Clinical and Laboratory Standards Institute (Malvern, PA, USA).

Serum PTH was quantified with ECLIA method (Roche - Cobas). The test is based on the sandwich principle: a biotinylated monoclonal antibody reacts with the N-terminal fragment (1–37) and a monoclonal antibody labelled with a ruthenium complex reacts with the C-terminal fragment (38–84). The antibodies used in the test react with epitopes in the amino acid regions 26–32 and 37–42 of PTH. The method was standardized against a commercially available assay (RIA) for PTH. The recovery of the NIBSC (WHO) standard 95/646 was analyzed by analysing dilutions in human serum covering the measurement range (40–4000 pg/mL) with average recovery of 100 ± 4%.

2.4. Statistical analysis

PFAS serum concentrations and the outcomes were summarized using mean and median values, with corresponding standard deviation and interquartile range, both in the overall population and stratified by supplementary VitD intake. Spearman's rank correlation (ρ) was reported to evaluate pairwise correlation between PFASs and between the outcomes. Since PFASs were markedly right-skewed, we considered them using a natural logarithmic transformation to improve the model fit. 25OH-VitD and PTH levels were also ln-transformed to achieve a normal distribution and again, improve model fit.

The correlation between PFASs and each outcome was assessed using Generalized Additive Models (GAMs), evaluating the association between each PFAS and each outcome separately. GAMs were chosen as they provide an accurate evaluation of nonlinear relations, which were accounted for using thin plate splines, allowing flexibility to be adapted on data. Exposures were included in the models using different approaches. (1) To better explore the shape of the associations, thin plate spline smooth terms were employed for the exposures and the plots of predicted values were obtained. The Estimated Degrees of Freedom (EDF) for each PFAS were examined. An EDF close to 1 suggests a nearly linear correlation, whereas higher EDF values indicate a departure from linearity. The corresponding p-value reflects the significance of the variable's impact. (2) A linear association between outcomes and ln-transformed PFAS levels was considered; coefficients (β) and their 95% confidence intervals (CI) were obtained. (3) Exposures were divided into quartiles to evaluate the approximate pattern of exposure-response, with the aim of minimizing the impact of outlier values.

Potential confounders of the investigated associations were chosen from available variables, based on literature review and the construction of a directed acyclic graph (Supplemental Fig. S1). For continuous variables (age in years and BMI in kg/m^2), thin plate splines were implemented to account for the non-linear correlation between these variables and the responses. In case of dietary supplementation, we investigated whether the examined associations were different when considering only subjects not taking supplementary VitD. To assess the robustness of the results, we conducted the same analysis using models different set of covariates: (1) age (years, continuous) and sex (male/female), (2) age, sex, ethnicity (Caucasian/not Caucasian), smoking habits (nonsmokers/current smokers/previous smokers), and consumption of milk and fish (never/once a month/once a week/2+ times a week), and (3) age, sex, ethnicity, smoking habits, consumption of milk and fish, continuous

BMI, and supplementary VitD intake (yes/no). A sensitivity analysis was also performed on subjects not taking VitD supplements (n = 773) to ensure that results were not influenced by variability in the outcome unrelated to PFAS exposure. An interaction term between sex and ln-PFAS linear coefficient was also added to the main models; however, all analyses were still stratified by participants' sex generalized cross-validation was used to select the degree of smoothing (CRAN - Package mgcv (r-project.org)). The software R (R Core Team (2023) was used to perform statistical analyses. The statistical significance level was set at 0.05.

3. Results

Twenty-six of the 1212 recruited subjects had missing values for all PFAS levels considered and were therefore excluded from the analysis. Among the remaining 1186 subjects, 12 had a missing value in at least one of the covariates considered and were removed (Fig. 1). Analyses were performed on a final sample size of 1174 subjects, whose demographics, clinical data and exposure variables are reported in Table 1. The population examined had an average age of 56.30 (±6.81) years, where 655 (55.79%) were females and 519 (44.21%) were males. Among these subjects, 401 (34.16%) reported any VitD supplementation, while the remaining 773 (65.84%) did not.

The measuring range of the method was 0.5–500 ng/mL. The repeatability of the method given as RSD of determined concentrations was below 10% for all measured PFAS at different levels and the accuracy of the method was between 80 and 120%. Limit of detection for all PFAS was as low as 0.1 ng/mL and LOQ was 0.5 ng/mL (Pitter et al., 2020a,b). Concentrations below the LOQ were imputed by dividing the LOQ by the square root of 2 prior to statistical analysis. PFASs detected in greater than 90% of participants were included in the main analyses, namely, PFOA (99.83%), PFOS (99.23%), and PFHxS (96.68%).

Of all 12 PFAS measured, most were quantifiable in less than 10% of samples, with the exception of PFNAs, which was quantifiable in 32.20%. Only three compounds were quantifiable in at least 90% of the samples and therefore considered for further analyses, respectively: PFOA, detected in 99.83% of participants, PFOS, detected in 99.23% of participants, and PFHxS, detected in 96.68% of participants. Details on PFAS detection rates are reported on Supplemental Table S12.

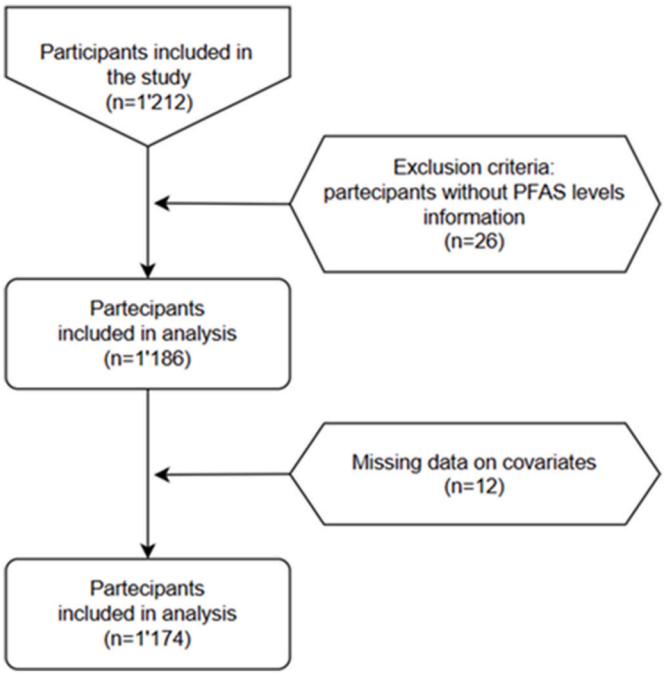


Fig. 1. Flowchart of the study population selection.

Table 1
Characteristics of the study population (n = 1174).

Exposure variables	TOTAL (n = 1174)		
	Mean (SD)	Min-max	Median (p25-p75)
PFOA (ng/mL)	52.25 (91.53)	0.35–1873.3	28.3 (12.9–59.7)
PFOS (ng/mL)	5.70 (5.33)	0.35–102	4.5 (2.7–7.1)
PFHxS (ng/mL)	7.65 (9.19)	0.35–144.5	4.9 (2.3–9.5)
Outcomes			
25OH-Vitamin D (ng/mL)	23.21 (12.10)	3–106	21.30 (14.6–29.4)
Calcium (mg/dL)	9.1 (0.41)	7.89–10.97	9.1 (8.84–9.37)
Parathyroid hormone (pg/mL)	37.42 (15.01)	2.9–112	34.9 (26.9–46.1)
Continuous covariates			
Age (years)	56.3 (6.81)	18–65	58 (55–59)
Body mass index (kg/m^2)	25.56 (4.37)	14.88–45.12	25 (22.59–27.98)
Categorical covariates	Frequency (%)		
Sex			
Female	655 (55.79)		
Male	519 (44.21)		
Ethnicity			
Caucasian	1157 (98.55)		
Not Caucasian	17 (1.45)		
Smoking habit			
Nonsmokers	744 (63.37)		
Current smokers	147 (12.52)		
Previous smokers	283 (24.11)		
Milk and derivatives consumption			
Never	47 (4.00)		
Once a month	113 (9.63)		
Once a week	564 (48.04)		
2+ times a week	450 (38.33)		
Fish consumption			
Never	106 (9.03)		
Once a month	302 (25.72)		
Once a week	675 (57.50)		
2+ times a week	91 (7.75)		
Supplementary vitamin D intake			
Yes	401 (34.16)		
No	773 (65.84)		

Among the measured PFASs, PFOA presented the highest levels (median 28.3 ng/mL), followed by PFHxS (median 4.9 ng/mL) and PFOS (median 4.5 ng/mL), as reported on Table 1. Pairwise correlations between concentrations were quite high, especially between PFOA and PFHxS ($\rho = 0.85$), whereas the correlations between PFOSs with PFHxS and PFOA were 0.73 and 0.67, respectively. When considering the outcomes, the correlations between 25OH-VitD and calcium, and 25OH-VitD and PTH were 0.17 and −0.22, respectively, while calcium and PTH exhibited a lower correlation ($\rho = -0.07$). Median values for 25OH-VitD were strongly different, as expected, between individuals taking VitD supplementation (median 30.6 ng/mL) and those who did not (median 17.3 ng/mL) (Supplemental Table S1), whilst calcium and PTH median levels were quite similar in the two groups (9.12 mg/dL, 33.6 pg/mL and 9.09 mg/dL, 35.3 pg/mL, respectively, Supplemental Table S1). After stratification for sex, males were reported having higher PFOA, PFOS and PFHxS levels compared with women, and lower 25OH-VitD (Supplemental Table S2). The distribution of outcomes, except for calcium, was not approximately normal and therefore they were ln-transformed in subsequent analyses.

Supplemental Figures S2, S3 and S4 show the adjusted concentration-response curves in the whole study group between PFASs and the outcomes, respectively 25OH-VitD, calcium and PTH. These analyses highlighted the positive associations between serum calcium and all considered PFASs (all p-values <0.05) (Fig. S3). In particular, the correlation was approximately linear with PFOA (EDF = 1.89) and PFHxS (EDF = 1.21), but not for PFOS (EDF = 3.69, Supplemental Table S3). For 25OH-VitD and PTH, associations with PFASs were not significant, except for the one between ln-transformed 25OH-D and PFOS ($\beta = 0.04$, 95%CI 0.00–0.08) (Table 2). The trends were persisted when PFAS levels were considered as ln-transformed or categorical

Table 2
Association between PFASs and considered outcomes from GAM models (n = 1174), adjusted[a] β coefficients and 95% confidence intervals (CI).

PFASs	Natural log of 25-OH vitamin D (ln-ng/mL)		Calcium (mg/dL)		Natural log of parathyroid hormone (ln-pg/mL)	
	β[a]	CI (95%)	β[a]	CI (95%)	β[a]	CI (95%)
PFOA per ln-ng/mL	0.02	(-0.01; 0.04)	**0.03**	**(0.01; 0.06)**	0.00	(-0.02; 0.02)
1Q (0.35–12.9)	*2.75*[b]		*9.13*[b]		*3.67*[b]	
2Q (12.9–28.3)	−0.04	(-0.12; 0.03)	0.04	(-0.02; 0.11)	−0.02	(-0.09; 0.05)
3Q (28.3–59.7)	0.03	(-0.05; 0.11)	**0.09**	**(0.02; 0.16)**	0.00	(-0.07; 0.07)
4Q (59.7–1874)	−0.01	(-0.09; 0.08)	**0.08**	**(0.01; 0.16)**	0.01	(-0.06; 0.08)
PFOS per ln-ng/mL	**0.04**	**(0.00; 0.08)**	**0.06**	**(0.02; 0.09)**	−0.02	(-0.05; 0.01)
1Q (0.35–2.7)	*2.73*[b]		*9.15*[b]		*3.67*[b]	
2Q (2.7–4.5)	0.06	(-0.02; 0.13)	0.06	(-0.01; 0.12)	0.02	(-0.05; 0.08)
3Q (4.5–7.1)	−0.01	(-0.08; 0.07)	0.06	(-0.00; 0.13)	0.01	(-0.05; 0.08)
4Q (7.1–102)	0.05	(-0.03; 0.13)	**0.08**	**(0.01; 0.15)**	−0.02	(-0.09; 0.05)
PFHxS per ln-ng/mL	0.02	(-0.01; 0.04)	**0.04**	**(0.01; 0.06)**	0.01	(-0.02; 0.03)
1Q (0.35–2.3)	*2.74*[b]		*9.15*[b]		*3.66*[b]	
2Q (2.3–4.9)	0.01	(-0.06; 0.09)	0.02	(-0.04; 0.09)	0.01	(-0.06; 0.08)
3Q (4.9–9.5)	0.04	(-0.04; 0.12)	0.07	(-0.00; 0.14)	0.03	(-0.04; 0.10)
4Q (9.5–144)	0.02	(-0.07; 0.10)	**0.08**	**(0.00; 0.15)**	0.02	(-0.05; 0.10)

Figures in bold are statistically significant results (p-value <0.05).
[a] Adjusted by sex, age, ethnicity, smoking habits, milk and fish consumption, BMI and supplementary vitamin D intake.
[b] Predicted values of each outcome for the 1st quartile of the PFAS distribution.

predictors (Table 2). The ln-transformed 25OH-VitD appeared to be positively associated with PFOS levels: a unitary ln-increase in PFOS was associated to an increase of 0.04 ln-ng/mL (95% CI 0.00–0.08 ln-ng/mL) in the ln-transformed 25OH-VitD. However, there is no clear dose-response effect, as evidenced by the estimated coefficients for the PFOS quartiles (Table 2). On the other hand, all three considered PFASs were positively associated with serum calcium levels. A unitary ln-increase increase in PFOA, PFOS and PFHxS was associated, respectively, with 0.03 mg/dL (95% CI 0.01–0.06), 0.06 mg/dL (95% CI 0.02–0.09), and 0.04 mg/dL (95% CI 0.01–0.06) increased calcium levels and exposure quartiles' coefficients were greater for upper quartiles groups (Table 2). No significant associations were found between different PFASs and ln-transformed PTH. Explorative analyses indicated no issues of multicollinearity among the covariates included in the models. The robustness of the main analysis was tested through several sensitivity analyses, involving different sets of confounding variables, which showed similar results, confirming the above-mentioned findings (Supplemental Tables S4, S5, and S6).

The inclusion of the interaction term between sex and exposure in the models did not yield p-values lower than 0.05. Nevertheless, results stratified by sex for the overall population are presented in Supplemental Tables S7, S8, and S9, whilst Supplemental Tables S10, S11, and S12 show sex-stratified results in subjects not taking VitD supplements. In the analysis focused only on those subjects who did not take VitD supplementation (n = 773), the positive association between all considered PFAS and calcium was confirmed, whereas 25OH-VitD and PTH showed no significant associations with any of the PFASs (Table 3). On the other hand, in stratified analysis on VitD-supplemented subjects, no significant association was reported (Table 4).

4. Discussion

This study aimed to systematically analyse the data collected within the framework of the Regional Health Surveillance plan on a large population of subjects highly exposed to PFAS in Italy, addressing the possible interference of these compounds on phospho-calcium metabolism. We found significant associations between PFOA, PFOS and PFHxS concentrations with serum calcium in the whole sample and in subgroup stratified analyses. Only PFOS showed a positive association with 25OH-VitD in the whole group, which was not maintained after exclusion of subjects taking VitD oral supplementation.

The peculiar environmental context in which the study was conducted, together with the large size of the study population and the wide range of PFAS serum values, offered a unique opportunity to re-evaluate associations already shown in different contexts and to better investigate the possible dose-response correlation between PFAS exposure levels and health outcomes. The study involved residents of the red area of the Veneto Region (North-Eastern Italy), featured by a severe environmental

Table 3
Association between PFASs and considered outcomes from GAM models (n = 773), adjusted[a] β coefficients and 95% confidence intervals (CI) considering only subjects not taking supplemental vitamin D.

PFASs	Natural log of vitamin D (ln-ng/mL)		Calcium (mg/dL)		Natural log of PTH (ln-pg/mL)	
	β[a]	CI (95%)	β[a]	CI (95%)	β[a]	CI (95%)
PFOA per ln-ng/mL	0.01	(-0.02; 0.05)	**0.04**	**(0.01; 0.06)**	0.01	(-0.02; 0.03)
1Q (0.35–12.5)	*2.77*[b]		*9.11*[b]		*3.62*[b]	
2Q (12.5–28.7)	**−0.11**	**(-0.21; -0.01)**	0.02	(-0.07; 0.10)	−0.00	(-0.09; 0.08)
3Q (28.7–61.6)	0.06	(-0.04; 0.17)	0.08	(-0.01; 0.17)	0.04	(-0.05; 0.13)
4Q (61.6–1874)	−0.03	(-0.14; 0.08)	0.09	(-0.00; 0.18)	0.01	(-0.08; 0.10)
PFOS per ln-ng/mL	0.05	(-0.00; 0.10)	**0.06**	**(0.02; 0.11)**	−0.01	(-0.05; 0.03)
1Q (0.35–2.7)	*2.77*[b]		*9.12*[b]		*3.61*[b]	
2Q (2.7–4.5)	0.03	(-0.07; 0.12)	0.06	(-0.02; 0.15)	0.07	(-0.01; 0.15)
3Q (4.5–7.1)	−0.05	(-0.15; 0.05)	0.06	(-0.02; 0.15)	0.07	(-0.02; 0.16)
4Q (7.1–102)	0.06	(-0.05; 0.16)	**0.10**	**(0.01; 0.18)**	−0.02	(-0.11; 0.07)
PFHxS per ln-ng/mL	0.02	(-0.02; 0.05)	**0.04**	**(0.01; 0.07)**	0.01	(-0.02; 0.04)
1Q (0.35–2.3)	*2.76*[b]		*9.12*[b]		*3.61*[b]	
2Q (2.3–5.1)	0.05	(-0.06; 0.15)	0.03	(-0.05; 0.11)	0.01	(-0.07; 0.10)
3Q (5.1–9.9)	0.05	(-0.05; 0.16)	**0.09**	**(0.00; 0.18)**	0.07	(-0.01; 0.16)
4Q (9.9–144)	0.00	(-0.11; 0.11)	0.09	(-0.01; 0.18)	0.04	(-0.06; 0.13)

Figures in bold are statistically significant results (p-value <0.05).
[a] Adjusted by sex, age, ethnicity, smoking habits, milk and fish consumption and BMI.
[b] Predicted values of each outcome for the 1st quartile of the PFAS distribution.

Table 4
Association between PFASs and considered outcomes from GAM models (n = 401), adjusted[a] β coefficients and 95% confidence intervals (CI) considering only subjects taking supplemental vitamin D.

PFASs	Natural log of vitamin D (ln-ng/mL)		Calcium (mg/dL)		Natural log of PTH (ln-pg/mL)	
	β[a]	CI (95%)	β[a]	CI (95%)	β[a]	CI (95%)
PFOA per ln-ng/mL	0.02	(-0.01; 0.06)	0.02	(-0.02; 0.06)	−0.00	(-0.04; 0.03)
1Q (0.35–13.6)	*3.23*[b]		*9.17*[b]		*3.71*[b]	
2Q (13.6–27.3)	0.03	(-0.08; 0.14)	0.08	(-0.04; 0.20)	−0.00	(-0.11; 0.11)
3Q (27.3–55.8)	0.00	(-0.11; 0.11)	0.11	(-0.01; 0.23)	−0.05	(-0.16; 0.06)
4Q (55.8–802)	0.05	(-0.07; 0.17)	0.05	(-0.07; 0.18)	0.02	(-0.10; 0.14)
PFOS per ln-ng/mL	0.03	(-0.02; 0.09)	0.04	(-0.02; 0.10)	−0.03	(-0.09; 0.02)
1Q (0.35–2.7)	*3.22*[b]		*9.21*[b]		*3.72*[b]	
2Q (2.7–4.5)	0.10	(-0.01; 0.20)	0.06	(-0.06; 0.17)	−0.07	(-0.18; 0.03)
3Q (4.5–7)	0.07	(-0.04; 0.18)	0.07	(-0.04; 0.19)	−0.07	(-0.18; 0.04)
4Q (7–60.4)	0.03	(-0.08; 0.14)	0.04	(-0.08; 0.16)	−0.01	(-0.13; 0.10)
PFHxS per ln-ng/mL	0.02	(-0.02; 0.06)	0.03	(-0.01; 0.08)	0.01	(-0.04; 0.05)
1Q (0.35–2.4)	*3.24*[b]		*9.17*[b]		*3.71*[b]	
2Q (2.4–4.7)	−0.00	(-0.11; 0.10)	0.04	(-0.08; 0.15)	−0.00	(-0.11; 0.11)
3Q (4.7–8.9)	0.02	(-0.10; 0.13)	0.11	(-0.01; 0.23)	−0.04	(-0.15; 0.07)
4Q (8.9–51.4)	0.02	(-0.10; 0.14)	0.05	(-0.08; 0.18)	0.03	(-0.09; 0.15)

[a] Adjusted by sex, age, ethnicity, smoking habits, milk and fish consumption and BMI.
[b] Predicted values of each outcome for the 1st quartile of the PFAS distribution.

exposure to PFASs, particularly PFOA, due to water contamination since the late 1960s (Pitter et al., 2020b).

Evidence on environmental exposure to PFASs show wide variation of serum levels according to the geographical and political setting. Data from Fromme et al., in 2017, evaluating serum PFAS levels in 396 blood donors living in three different areas of South Germany, showed that median PFOS level ranged between 1.5 and 6.0 ng/mL, whilst PFHxS and PFOA were 0.2–0.7 ng/mL and 1.1–20.2 ng/mL (Fromme et al., 2017). Differently, Li et al., in a study cohort of 1945 Swedish adults exposed to PFASs by contaminated drinking water, reported median level of serum PFOS, PFHxS and PFOA of respectively 157 ng/mL, 136 ng/mL and 8.6 ng/mL (Li et al., 2020). On the other hand, recent North American data from Hall et al. on exposed subject resident in Central North Carolina document serum levels of PFOS, PFHxS and PFOA of respectively 9.8 ng/g, 2.6 ng/g and 5.3 ng/g (Hall et al., 2023). Similar data are those from Daly et al., obtained in exposed subjects to contaminated drinking water in New Hampshire, showing median serum levels of the three contaminants of respectively 8.9 ng/mL, 4.2 ng/mL and 3.2 ng/mL (Daly et al., 2018). Here we report median levels of 4.5 ng/mL, 4.9 ng/mL and 28.3 ng/mL, showing some consistency with Midde-Europe data.

Consistent experimental evidence suggests that PFAS may affect VitD signalling *in vitro* (Azhagiya Singam et al., 2023; Di Nisio et al., 2020a, b), but epidemiological evidence is scarce. The largest study to date investigating the association between serum levels of a panel of PFAS, composed by PFOA, PFOS, PFHxS, and PFNA, and vitamin D has found the associations of two PFASs with total serum 25OH-VitD levels in a nationally representative sample of 7040 subjects from the United States. In particular, higher serum PFOS concentrations were monotonically associated with lower 25OH-VitD levels, whereas PFHxS was associated with higher 25OH-VitD levels (Etzel et al., 2019). Interestingly, stronger associations were observed in particular demographic subpopulations, such as in elder individuals (>60 years of age) and in non-Hispanic Caucasian subjects. However, different from our findings, serum PFOA or PFNA was not associated with 25(OH)-VitD concentrations. In a second study on a smaller cohort of 442 healthy pregnant African American women, evaluating serum levels of a larger panel of PFASs including PFHxS, PFOS, PFOA, PFNA, PFPeA, PFDA, PFUnDA, and NMeFOSAAb, positive associations of 25OH-VitD levels with PFHxS and PFOS were reported (Chang et al., 2021). Interestingly, in the latter, sensitivity analyses involving the adjustment for fish and VitD supplementation, did significantly change the associations. Consistent with these studies, we found a marginally positive association between PFOS and 25OH-VitD in the whole sample, which was not confirmed at subgroup analyses after exclusion of subjects receiving oral VitD supplementation. The EDF in the VitD model for PFOA and PFOS, respectively, equal to 5.65 and 3.02, indicate a non-linear relationship. Positive PFAS association with 25OH-VitD concentrations may be unexpected, given the majority of environmental pollutants showing inverse associations with VitD metabolism, such as BPA, phthalates (Johns et al., 2016), polychlorinated biphenyl (Morales et al., 2013) and organochlorine pesticides (Yang et al., 2012). A possible explanation of these associations could rely on compensatory mechanisms due to competitive binding of VitD to its receptor and in turn the reduced receptor signalling may reduce the activation of VDR-responsive genes causing a functional hypovitaminosis D, despite normal or even increased VitD levels (Di Nisio et al., 2020a,b). Indeed, a compensatory increase of PTH was observed in a small population of young men from the same polluted area of Italy of our study (Di Nisio et al., 2020a,b).

The homeostasis of 25OH-VitD is tightly regulated through feedbacks involving PTH and calcium, suggesting the possible role of PFASs in modifying 25OH-VitD levels through the interaction with these metabolites (Christakos et al., 2010; Di Nisio et al., 2022; Johns et al., 2017b). To this end, we aimed to evaluate calcium metabolism by assessing plasma PTH and serum calcium content. We observed a consistent positive association of PFOA, PFOS and PFHxS, the three major PFASs detected in >90% of subjects, with calcium levels, with no involvement of PTH, which was confirmed also in sensitivity analyses. This result is consistent with that from a large cross-sectional study, which reported a small but significant increase of calcium levels with increased PFOA, PFOS, PFDA, PFHxS, and PFNA levels (Cakmak et al., 2022). Interestingly, authors suggested the role of PFOA endocrine disruption of thyroid function, which in turn affects serum calcium. This observation may be important given that even subclinical hyperthyroidism is associated with osteoporosis and an increased risk of bone fractures (Blum et al., 2015). However, this hypothesis requires further investigation and other mechanisms could explain the reported association of PFOA with calcium. For example, a possible interference of PFASs on PTH receptor has been reported (Galloway et al., 2015), which could have an indirect effect on intracellular calcium levels by interaction with calmodulin 1, mediating intracellular interactions between calcium and downstream targets, without modifying circulating PTH levels (Chin and Means, 2000). Alternatively, increased levels of serum calcium might be the result of a direct effect of PFASs on bone osteoclasts. Indeed, a general tropism of these compounds for hydroxyapatite has been documented by several authors, resulting in variable, though specific, PFAS accumulation in bone tissue [Koskela et al., 2016; Yang et al., 2025]. Among the functional consequences associated with this evidence, an increased osteoclast activity and bone resorption has been described by *in vitro* approaches upon PFOA exposure [Koskela et al., 2016, 2017]. Despite this model requiring further confirm, the increased osteoclast activity could justify the higher prevalence of both bone derangements and increased calcium levels associated with PFAS exposure.

Limitations of this study are related to the cross-sectional study

design and lack of complete biochemical data to better elucidate phospho-calcium metabolism, such as urinary calcium and phosphorus levels, and the possible involvement of other branches of its tight regulation (Rana et al., 2022). In addition, given the large correlation between VitD status and nutritional/lifestyle habits, we decided to exclude socio-economic status descriptors and ethnic characterization of study participants as possible confounders. Finally, serum albumin is recognized as a major binding protein for both calcium and PFASs (Erstad, 2021; Maso et al., 2021; Moro et al., 2022). On this base, the analysis of sex-stratified adjustments of calcium would have benefit from the evaluation of serum albumin to disclose possible multiple equilibrium influencing the correlation between serum PFAS concentrations and serum calcium. Unfortunately, serum albumin dosing was beyond the aims of the present study and we acknowledge that as a major drawback. Nonetheless, this study shows that in a large population of subjects exposed to high doses of PFASs through drinking water contamination lasting more than 30 years, PFASs may interfere with the phospho-calcium balance, independent of lifestyle and dietary factors. Although typically exposure to PFASs in the general population is mainly due to daily contact with consumer products, diet and/or occupational settings, it's not surprising that in our study these factors do not interfere with PFAS associations in our results. The study population herein considered represents a very specific case with long-lasting PFAS exposure form a single-source and highly-polluted underground water basin, leading to much higher serum PFAS levels in the residents, and specifically PFOA, PFOA and PFHxS, compared to the general population, and therefore probably reducing the magnitude effect of daily sources of PFAS exposure. Future studies are necessary to better elucidate the mechanisms of PFAS interference on calcium homeostasis in order to address the clinical and public health relevance of these findings.

CRediT authorship contribution statement

Andrea Di Nisio: Writing – review & editing, Investigation, Data curation, Conceptualization. **Luca De Toni:** Writing – review & editing, Project administration, Investigation. **Cristina Canova:** Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation. **Mirko Berti:** Investigation, Formal analysis, Data curation. **Achille Di Falco:** Validation, Supervision. **Rinaldo Zolin:** Investigation, Formal analysis, Data curation. **Anna Maria Bettega:** Writing – original draft, Supervision, Investigation, Formal analysis, Data curation. **Iva Sabovic:** Project administration, Investigation. **Alberto Ferlin:** Validation, Supervision, Conceptualization. **Carlo Foresta:** Validation, Supervision, Funding acquisition, Conceptualization.

Funding

REGIONE VENETO (IT) through "Consorzio per la Ricerca Sanitaria – CORIS" (DGRV n. 1894, 29/12/2020) supported this research with a grant to CF. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carlo Foresta reports financial support was provided by Consortium for Health Research - CORIS. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge the contributions of Matteo Chinellato (ARPAV), Elena Zocca (CERBA health care), Donatella Sorio (University of Padova).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2025.144230.

Data availability

Data will be made available on request.

References

Arabi, A., El Rassi, R., El-Hajj Fuleihan, G., 2010. Hypovitaminosis D in developing countries—prevalence, risk factors and outcomes. Nat. Rev. Endocrinol. 6, 550–561. https://doi.org/10.1038/nrendo.2010.146.

Azhagiya Singam, E.R., Durkin, K.A., La Merrill, M.A., Furlow, J.D., Wang, J.-C., Smith, M.T., 2023. The vitamin D receptor as a potential target for the toxic effects of per- and polyfluoroalkyl substances (PFASs): an in-silico study. Environ. Res. 217, 114832. https://doi.org/10.1016/j.envres.2022.114832.

Berger, K., Bradshaw, P.T., Poon, V., Kharrazi, M., Eyles, D., Ashwood, P., Lyall, K., Volk, H.E., Ames, J., Croen, L.A., Windham, G.C., Pearl, M., 2024. Mixture of air pollution, brominated flame retardants, polychlorinated biphenyls, per- and polyfluoroalkyl substances, and organochlorine pesticides in relation to vitamin D concentrations in pregnancy. Environ. Pollut. 340, 122808. https://doi.org/10.1016/j.envpol.2023.122808.

Blum, M.R., Bauer, D.C., Collet, T.-H., Fink, H.A., Cappola, A.R., da Costa, B.R., Wirth, C. D., Peeters, R.P., Åsvold, B.O., den Elzen, W.P.J., Luben, R.N., Imaizumi, M., Bremner, A.P., Gogakos, A., Eastell, R., Kearney, P.M., Strotmeyer, E.S., Wallace, E. R., Hoff, M., Ceresini, G., Rivadeneira, F., Uitterlinden, A.G., Stott, D.J., Westendorp, R.G.J., Khaw, K.-T., Langhammer, A., Ferrucci, L., Gussekloo, J., Williams, G.R., Walsh, J.P., Jüni, P., Aujesky, D., Rodondi, N., 2015. Subclinical thyroid dysfunction and fracture risk. JAMA 313, 2055. https://doi.org/10.1001/jama.2015.5161.

Bogdanska, J., Borg, D., Sundström, M., Bergström, U., Halldin, K., Abedi-Valugerdi, M., Bergman, Å., Nelson, B., DePierre, J., Nobel, S., 2011. Tissue distribution of 35S-labelled perfluorooctane sulfonate in adult mice after oral exposure to a low environmentally relevant dose or a high experimental dose. Toxicology 284, 54–62. https://doi.org/10.1016/j.tox.2011.03.014.

Buckley, J.P., Zhou, J., Marquess, K.M., Lanphear, B.P., Cecil, K.M., Chen, A., Sears, C.G., Xu, Y., Yolton, K., Kalkwarf, H.J., Braun, J.M., Kuiper, J.R., 2024. Per- and polyfluoroalkyl substances and bone mineral content in early adolescence: modification by diet and physical activity. Environ. Res. 252, 118872. https://doi.org/10.1016/j.envres.2024.118872.

Cakmak, S., Lukina, A., Karthikeyan, S., Atlas, E., Dales, R., 2022. The association between blood PFAS concentrations and clinical biochemical measures of organ function and metabolism in participants of the Canadian Health Measures Survey (CHMS). Sci. Total Environ. 827, 153900. https://doi.org/10.1016/j.scitotenv.2022.153900.

Carlsson, H., Sreenivasan, A.P., Erngren, I., Larsson, A., Kultima, K., 2023. Combining the targeted and untargeted screening of environmental contaminants reveals associations between PFAS exposure and vitamin D metabolism in human plasma. Environ. Sci. Process Impacts 25, 1116–1130. https://doi.org/10.1039/D3EM00060E.

Chang, C.-J., Barr, D.B., Zhang, Q., Dunlop, A.L., Smarr, M.M., Kannan, K., Panuwet, P., Tangpricha, V., Shi, L., Liang, D., Corwin, E.J., Ryan, P.B., 2021. Associations of single and multiple per- and polyfluoroalkyl substance (PFAS) exposure with vitamin D biomarkers in African American women during pregnancy. Environ. Res. 202, 111713. https://doi.org/10.1016/j.envres.2021.111713.

Chin, D., Means, A.R., 2000. Calmodulin: a prototypical calcium sensor. Trends Cell Biol. 10, 322–328. https://doi.org/10.1016/S0962-8924(00)01800-6.

Christakos, S., Ajibade, D.V., Dhawan, P., Fechner, A.J., Mady, L.J., 2010. Vitamin D: metabolism. Endocrinol Metab. Clin. N. Am. 39, 243–253. https://doi.org/10.1016/j.ecl.2010.02.002.

Cipriani, C., Cianferotti, L., 2023. Vitamin D in hypoparathyroidism: insight into pathophysiology and perspectives in clinical practice. Endocrine 81, 216–222. https://doi.org/10.1007/s12020-023-03354-2.

Cluett, R, Seshasayee, SM, Rokoff, LB, Rifas-Shiman, SL, Ye, X, Calafat, AM, Gold, DR, Coull, B, Gordon, CM, Rosen, CJ, Oken, E, Sagiv, SK, Fleisch, AF, 2019 Aug. Per- and Polyfluoroalkyl Substance Plasma Concentrations and Bone Mineral Density in Midchildhood: a Cross-Sectional Study (Project Viva, United States). Environ. Health Perspect. 127 (8), 87006. https://doi.org/10.1289/EHP4918.

Conder, J.M., Hoke, R.A., Wolf, W. de, Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environ. Sci. Technol. 42, 995–1003. https://doi.org/10.1021/es070895g.

Daly, E.R., Chan, B.P., Talbot, E.A., Nassif, J., Bean, C., Cavallo, S.J., Metcalf, E., Simone, K., Woolf, A.D., 2018. Per- and polyfluoroalkyl substance (PFAS) exposure assessment in a community exposed to contaminated drinking water, New Hampshire, 2015. Int. J. Hyg Environ. Health 221, 569–577. https://doi.org/10.1016/j.ijheh.2018.02.007.

Di Nisio, A., De Rocco Ponce, M., Giadone, A., Rocca, M.S., Guidolin, D., Foresta, C., 2020a. Perfluoroalkyl substances and bone health in young men: a pilot study. Endocrine 67, 678–684. https://doi.org/10.1007/s12020-019-02096-4.

Di Nisio, A., Lopez-Espinosa, M.J., Foresta, C., 2022. Editorial: emerging chemical risks for human health: endocrine disruption by per- and poly-fluorinated alkyl substances (PFAS). Front. Endocrinol. https://doi.org/10.3389/fendo.2021.813785.

Di Nisio, Andrea, Rocca, M.S., De Toni, L., Sabovic, I., Guidolin, D., Dall'Acqua, S., Acquasaliente, L., De Filippis, V., Plebani, M., Foresta, C., 2020b. Endocrine disruption of vitamin D activity by perfluoro-octanoic acid (PFOA). Sci. Rep. 10, 16789. https://doi.org/10.1038/s41598-020-74026-8.

Erstad, B.L., 2021. Serum albumin levels: who needs them? Ann. Pharmacother. 55, 798–804. https://doi.org/10.1177/1060028020959348.

Etzel, T.M., Braun, J.M., Buckley, J.P., 2019. Associations of serum perfluoroalkyl substance and vitamin D biomarker concentrations in NHANES, 2003–2010. Int. J. Hyg Environ. Health 222, 262–269. https://doi.org/10.1016/j.ijheh.2018.11.003.

Fan, S., Wu, Y., Bloom, M.S., Lv, J., Chen, L., Wang, W., Li, Z., Jiang, Q., Bu, L., Shi, J., Shi, T., Zeng, X., Zhang, L., Zhang, Z., Yang, B., Dong, G., Feng, W., 2023. Associations of per- and polyfluoroalkyl substances and their alternatives with bone mineral density levels and osteoporosis prevalence: a community-based population study in Guangzhou, Southern China. Sci. Total Environ. 862, 160617. https://doi.org/10.1016/j.scitotenv.2022.160617.

Fromme, H., Wöckner, M., Roscher, E., Völkel, W., 2017. ADONA and perfluoroalkylated substances in plasma samples of German blood donors living in South Germany. Int. J. Hyg Environ. Health 220, 455–460. https://doi.org/10.1016/j.ijheh.2016.12.014.

Galloway, T.S., Fletcher, T., Thomas, O.J., Lee, B.P., Pilling, L.C., Harries, L.W., 2015. PFOA and PFOS are associated with reduced expression of the parathyroid hormone 2 receptor (PTH2R) gene in women. Chemosphere 120, 555–562. https://doi.org/10.1016/j.chemosphere.2014.09.066.

Giustina, A., Lazaretti-Castro, M., Martineau, A.R., Mason, R.S., Rosen, C.J., Schoenmakers, I., 2024. A view on vitamin D: a pleiotropic factor? Nat. Rev. Endocrinol. 20, 202–208. https://doi.org/10.1038/s41574-023-00942-0.

Hall, S.M., Zhang, S., Tait, G.H., Hoffman, K., Collier, D.N., Hoppin, J.A., Stapleton, H. M., 2023. PFAS levels in paired drinking water and serum samples collected from an exposed community in Central North Carolina. Sci. Total Environ. 895, 165091. https://doi.org/10.1016/j.scitotenv.2023.165091.

Hu, Y, Liu, G, Rood, J, Liang, L, Bray, GA, de Jonge, L, Coull, B, Furtado, JD, Qi, L, Grandjean, P, Sun, Q, 2019. Perfluoroalkyl substances and changes in bone mineral density: A prospective analysis in the POUNDS-LOST study. Environ Res 179 (Pt A), 108775. https://doi.org/10.1016/j.envres.2019.108775.

Jeddy, Z., Tobias, J.H., Taylor, E.V., Northstone, K., Flanders, W.D., Hartman, T.J., 2018. Prenatal concentrations of perfluoroalkyl substances and bone health in British girls at age 17. Arch. Osteoporosis 13, 84. https://doi.org/10.1007/s11657-018-0498-5.

Ješeta, M., Navrátilová, J., Franzová, K., Fialková, S., Kempisty, B., Ventruba, P., Žáková, J., Crha, I., 2021. Overview of the mechanisms of action of selected bisphenols and perfluoroalkyl chemicals on the male reproductive axes. Front. Genet. 12. https://doi.org/10.3389/fgene.2021.692897.

Johns, L.E., Ferguson, K.K., Cantonwine, D.E., McElrath, T.F., Mukherjee, B., Meeker, J. D., 2017a. Urinary BPA and phthalate metabolite concentrations and plasma vitamin D levels in pregnant women: a repeated measures analysis. Environ. Health Perspect. 125. https://doi.org/10.1289/EHP1178.

Johns, L.E., Ferguson, K.K., Cantonwine, D.E., McElrath, T.F., Mukherjee, B., Meeker, J. D., 2017b. Urinary BPA and phthalate metabolite concentrations and plasma vitamin D levels in pregnant women: a repeated measures analysis. Environ. Health Perspect. 125. https://doi.org/10.1289/EHP1178.

Johns, L.E., Ferguson, K.K., Meeker, J.D., 2016. Relationships between urinary phthalate metabolite and bisphenol A concentrations and vitamin D levels in U.S. Adults: national health and nutrition examination survey (NHANES), 2005–2010. J. Clin. Endocrinol. Metab. 101, 4062–4069. https://doi.org/10.1210/jc.2016-2134.

Khalil, N., Chen, A., Lee, M., Czerwinski, S.A., Ebert, J.R., DeWitt, J.C., Kannan, K., 2016. Association of perfluoroalkyl substances, bone mineral density, and osteoporosis in the U.S. Population in NHANES 2009–2010. Environ. Health Perspect. 124, 81–87. https://doi.org/10.1289/ehp.1307909.

Koskela, A., Finnilä, M.A., Korkalainen, M., Spulber, S., Koponen, J., Håkansson, H., Tuukkanen, J., Viluksela, M., 2016. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. Toxicol. Appl. Pharmacol. 301, 14–21. https://doi.org/10.1016/j.taap.2016.04.002.

Koskela, A., Koponen, J., Lehenkari, P., Viluksela, M., Korkalainen, M., Tuukkanen, J., 2017. Perfluoroalkyl substances in human bone: concentrations in bones and effects on bone cell differentiation. Sci. Rep. 7, 6841. https://doi.org/10.1038/s41598-017-07359-6.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–394. https://doi.org/10.1093/toxsci/kfm128.

Lin, L.-Y., Wen, L.-L., Su, T.-C., Chen, P.-C., Lin, C.-Y., 2014. Negative association between serum perfluorooctane sulfate concentration and bone mineral density in US premenopausal women: NHANES, 2005–2008. J. Clin. Endocrinol. Metab. 99, 2173–2180. https://doi.org/10.1210/jc.2013-3409.

Liu, H., Huang, Y., Pan, Y., Cheng, R., Li, X., Li, Y., Lu, S., Zhou, A., Dai, J., Xu, S., 2023. Associations between per and polyfluoroalkyl ether sulfonic acids and vitamin D biomarker levels in Chinese newborns. Sci. Total Environ. 866, 161410. https://doi.org/10.1016/j.scitotenv.2023.161410.

Li, Y., Barregard, L., Xu, Y., Scott, K., Pineda, D., Lindh, C.H., Jakobsson, K., Fletcher, T., 2020. Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environ. Health 19, 33. https://doi.org/10.1186/s12940-020-00588-9.

Maso, L., Trande, M., Liberi, S., Moro, G., Daems, E., Linciano, S., Sobott, F., Covaceuszach, S., Cassetta, A., Fasolato, S., Moretto, L.M., De Wael, K., Cendron, L., Angelini, A., 2021. Unveiling the binding mode of perfluorooctanoic acid to human serum albumin. Protein Sci. 30, 830–841. https://doi.org/10.1002/pro.4036.

Morales, E., Gascon, M., Martinez, D., Casas, M., Ballester, F., Rodríguez-Bernal, C.L., Ibarluzea, J., Marina, L.S., Espada, M., Goñi, F., Vizcaino, E., Grimalt, J.O., Sunyer, J., 2013. Associations between blood persistent organic pollutants and 25-hydroxyvitamin D3 in pregnancy. Environ. Int. 57–58, 34–41. https://doi.org/10.1016/j.envint.2013.03.011.

Moro, G., Liberi, S., Vascon, F., Linciano, S., De Felice, S., Fasolato, S., Foresta, C., De Toni, L., Di Nisio, A., Cendron, L., Angelini, A., 2022. Investigation of the interaction between human serum albumin and branched short-chain perfluoroalkyl compounds. Chem. Res. Toxicol. 35, 2049–2058. https://doi.org/10.1021/acs.chemrestox.2c00211.

Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J.L., Barceló, D., Farré, M., 2013. Accumulation of perfluoroalkyl substances in human tissues. Environ. Int. 59, 354–362. https://doi.org/10.1016/j.envint.2013.06.004.

Pitter, G., Da Re, F., Canova, C., Barbieri, G., Zare Jeddi, M., Daprà, F., Manea, F., Zolin, R., Bettega, A.M., Stopazzolo, G., Vittorii, S., Zambelli, L., Martuzzi, M., Mantoan, D., Russo, F., 2020a. Serum levels of perfluoroalkyl substances (PFAS) in adolescents and young adults exposed to contaminated drinking water in the Veneto Region, Italy: a cross-sectional study based on a health surveillance program. Environ. Health Perspect. 128. https://doi.org/10.1289/EHP5337.

Pitter, G., Da Re, F., Canova, C., Barbieri, G., Zare Jeddi, M., Daprà, F., Manea, F., Zolin, R., Bettega, A.M., Stopazzolo, G., Vittorii, S., Zambelli, L., Martuzzi, M., Mantoan, D., Russo, F., 2020b. Serum levels of perfluoroalkyl substances (PFAS) in adolescents and young adults exposed to contaminated drinking water in the Veneto Region, Italy: a cross-sectional study based on a health surveillance program. Environ. Health Perspect. 128. https://doi.org/10.1289/EHP5337.

Plum, L.A., DeLuca, H.F., 2010. Vitamin D, disease and therapeutic opportunities. Nat. Rev. Drug Discov. 9, 941–955. https://doi.org/10.1038/nrd3318.

Rana, Z.H., Bourassa, M.W., Gomes, F., Khadilkar, A., Mandlik, R., Owino, V., Pettifor, J. M., Roth, D.E., Shlisky, J., Thankachan, P., Weaver, C.M., 2022. Calcium status assessment at the population level: candidate approaches and challenges. Ann. N. Y. Acad. Sci. 1517, 93–106. https://doi.org/10.1111/nyas.14886.

Wang, X.X., Jiang, T., Levi, M., 2010. Nuclear hormone receptors in diabetic nephropathy. Nat. Rev. Nephrol. 6, 342–351. https://doi.org/10.1038/nrneph.2010.56.

Wiener, R.C., Waters, C., 2019. Perfluoroalkyls/polyfluoroalkyl substances and dental caries experience in children, ages 3–11 years, National Health and Nutrition Examination Survey, 2013–2014. J. Publ. Health Dent. 79, 307–319. https://doi.org/10.1111/jphd.12329.

World Medical Association Declaration of Helsinki, 2013. JAMA 310, 2191. https://doi.org/10.1001/jama.2013.281053.

Yang, J.-H., Lee, Y.-M., Bae, S.-G., Jacobs, D.R., Lee, D.-H., 2012. Associations between organochlorine pesticides and vitamin D deficiency in the U.S. Population. PLoS One 7, e30093. https://doi.org/10.1371/journal.pone.0030093.

Yang, S., Chen, M., Yang, D., Guo, X., 2025. Perfluorooctanoic acid and its alternatives disrupt the osteogenesis and osteoclastogenesis balance: evidence from the effects on cell differentiation process. Sci. Total Environ. 959, 178331. https://doi.org/10.1016/j.scitotenv.2024.178331.