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Exposure to perfluoroalkyl substances and longitudinal changes in bone mineral density in adolescents and young adults: A multi-cohort study

Emily Beglarian [a,*], Elizabeth Costello [a], Douglas I. Walker [b], Hongxu Wang [a], Tanya L. Alderete [c], Zhanghua Chen [a], Damaskini Valvi [d], Brittney O. Baumert [a], Sarah Rock [a], Bruna Rubbo [a], Max T. Aung [a], Frank D. Gilliland [a], Michael I. Goran [e], Dean P. Jones [f], Rob McConnell [a], Sandrah P. Eckel [a], David V. Conti [a], Jesse A. Goodrich [a], Lida Chatzi [a]

[a] Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States
[b] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, United States
[c] Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, United States
[d] Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY, United States
[e] Department of Pediatrics, Children's Hospital Los Angeles, The Saban Research Institute, Los Angeles, CA, United States
[f] Clinical Biomarkers Laboratory, Division of Pulmonary, Allergy Critical Care and Sleep Medicine, Emory University, Atlanta, GA, United States

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ABSTRACT

Background: Per- and polyfluoroalkyl substances (PFAS) may impair bone development in adolescence, which impacts life-long bone health. No previous studies have examined prospective associations of individual PFAS and their mixture with bone mineral density (BMD) changes in Hispanic young persons, a population at high risk of osteoporosis in adulthood.

Objectives: To examine associations of individual PFAS and PFAS mixtures with longitudinal changes in BMD in an adolescent Hispanic cohort and examine generalizability of findings in a mixed-ethnicity young adult cohort (58.4% Hispanic).

Methods: Overweight/obese adolescents from the Study of Latino Adolescents at Risk of Type 2 Diabetes (SOLAR; n = 304; mean follow-up = 1.4 years) and young adults from the Southern California Children's Health Study (CHS; n = 137; mean follow-up = 4.1 years) were included in this study. Plasma PFAS were measured at baseline and dual x-ray absorptiometry scans were performed at baseline and follow-up to measure BMD. We estimated longitudinal associations between BMD and five PFAS via separate covariate-adjusted linear mixed effects models, and between BMD and the PFAS mixture via quantile g-computation.

Results: In SOLAR adolescents, baseline plasma perfluorooctanesulfonic acid (PFOS) was associated with longitudinal changes in BMD. Each doubling of PFOS was associated with an average −0.003 g/cm^2 difference in change in trunk BMD per year over follow-up (95% CI: −0.005, −0.0002). Associations with PFOS persisted in CHS young adults, where each doubling of plasma PFOS was associated with an average −0.032 g/cm^2 difference in total BMD at baseline (95% CI -0.062, −0.003), though longitudinal associations were non-significant. We did not find associations of other PFAS with BMD; associations of the PFAS mixture with BMD outcomes were primarily negative though non-significant.

Discussion: PFOS exposure was associated with lower BMD in adolescence and young adulthood, important periods for bone development, which may have implications on future bone health and risk of osteoporosis in adulthood.

1. Introduction

Childhood and adolescence are crucial periods for development of bone mineral density (BMD) (Weaver et al., 2016). Bone mass accrued during this time predicts peak bone density in early adulthood and determines lifetime bone health (Gordon et al., 2017; Weaver et al., 2016). Low BMD in early adulthood is a risk factor for future development of

* Corresponding author. Department of Population and Public Health Sciences, University of Southern California Keck School of Medicine, 1845 N Soto St, Los Angeles, CA, 90032, United States.
E-mail address: ebeglari@usc.edu (E. Beglarian).

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osteoporosis, the most common bone disease, which affects more than 10 million adults in the US and is projected to become even greater in prevalence as our population ages (Office of the Surgeon General, 2004; Wright et al., 2014). Individuals with osteoporosis are at higher rates of bone fracture and other bone-related morbidities (Riggs and Melton, 1995; Ullom-Minnich, 1999). The prevalence of osteoporosis is higher in Hispanic individuals than in other ethnic groups (Barrett-Connor et al., 2005; Wright et al., 2014). This may be partially explained by factors such as increased prevalence of diabetes in this population (Aguayo--Mazzucato et al., 2019), which may escalate bone loss and increase risk of osteoporosis (Wongdee and Charoenphandhu, 2011). Further, high percentages of Hispanic adults report physical inactivity (CDC, 2023), a modifiable risk factor for osteoporosis (Carter and Hinton, 2014), possibly due to structural barriers such as lack of access to parks and outdoor spaces (Ramirez et al., 2019). However, these factors do not conclusively explain the increased rates of osteoporosis in Hispanic individuals, and studies on bone health often fail to include this ethnic group. Because bone accrual predominantly occurs during childhood and adolescence, it is crucial to identify risk factors that impact bone health during these life periods, especially in vulnerable populations such as Hispanic individuals. Identifying factors that hinder bone accrual in youth can assist in preserving bone health at all ages and preventing morbidities due to low BMD in adulthood.

Recent research indicates that environmental factors, including per- and polyfluoroalkyl substances (PFAS), can adversely affect bone health. PFAS are persistent synthetic chemicals to which most of the US population has been exposed due to their historic use in food packaging, cookware, and other industrial processes (Kannan et al., 2004). Although use and production of some legacy PFAS has declined over recent years (Agency for Toxic Substances and Disease Registry, 2022), the chemicals persist in the environment due to resistance to degradation, bioaccumulation and their long half-lives (Fromme et al., 2009). Humans are exposed through a variety of mechanisms, including exposure to contaminated dust, food, and water (Fromme et al., 2009). Analyses from the United States National Health and Nutrition Examination Survey (NHANES) found that several common PFAS are detectable in the serum of 97–100% of US individuals (Kato et al., 2011).

PFAS are endocrine-disrupting chemicals (Jensen and Leffers, 2008; White et al., 2011) that alter hormonal regulation of many processes in the body, including those involved in bone modeling. Specifically, PFAS may disrupt pathways involved in formation and differentiation of osteoblasts, the cells that build new bone material (Kjeldsen and Bonefeld-Jørgensen, 2013; Koskela et al., 2017; Yamamoto et al., 2015). Exposure to PFAS has been linked with worse bone health in multiple cross-sectional observational analyses in both child and adult populations (Banjabi et al., 2020; Carwile et al., 2022; Cluett et al., 2019; Fan et al., 2023; Khalil et al., 2016, 2018; Lin et al., 2014; Xiong et al., 2022). In children, negative associations have been observed between PFAS and bone health measures evaluated via bone quantitative ultrasounds (Khalil et al., 2018) and dual-energy x-ray absorptiometry (DXA) (Carwile et al., 2022; Cluett et al., 2019) in primarily non-Hispanic White individuals. In NHANES adults and adolescents, higher serum PFAS concentrations have been associated with lower BMD (Carwile et al., 2022; Khalil et al., 2016; Lin et al., 2014; Xiong et al., 2022). Recent analyses in racially homogeneous Faroese and Danish birth cohorts have demonstrated negative impacts of early childhood PFAS exposure on BMD (e.g., 12 years old or younger) (Blomberg et al., 2022; Højsager et al., 2022). Emerging studies have also evaluated associations between PFAS and bone-related outcomes such as osteoporosis and increased fracture rate (Banjabi et al., 2020; Fan et al., 2023; Xu et al., 2023). Many existing studies have limitations including: 1) cross-sectional designs, and thus the inability to establish temporality in the PFAS—BMD association; 2) analyses that evaluated PFAS individually, without accounting for their correlation and mixture effect; 3) study populations that were primarily focused on older adults or young children, as opposed to the sensitive BMD development periods in adolescence and young adulthood; and/or 4) inclusion of homogenous populations with a lack of Hispanic participants, typically focused on individuals of European ancestry, therefore reducing generalizability of results to diverse populations.

The present study is the first to examine associations of individual PFAS and their mixture with longitudinal changes in BMD during adolescence and early adulthood, a critical period for bone development. We focused primarily on Hispanic individuals, a population that is understudied in this area of research and is at increased risk of low BMD and osteoporosis later in life. To examine generalizability of findings across different life periods, we evaluated two independent cohorts: Hispanic adolescents, and mixed-ethnicity young adults. We hypothesized that PFAS would be associated with lower BMD in adolescence, and these associations persist into young adulthood.

Abbreviations

BMD	bone mineral density
CHS	Children's Health Study
DAG	directed acyclic graph
DXA	dual-energy x-ray absorptiometry
FDR	false discovery rate
NHANES	National Health and Nutrition Examination Survey
LRT	likelihood ratio test
PFAS	per- and polyfluoroalkyl substances
PFDA	perfluorodecanoate
PFHxS	perfluorohexane sulphonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoate
PFOS	perfluorooctane sulfonate
SOLAR	Study of Latino Adolescents at Risk of Type 2 Diabetes
USC	University of Southern California

2. Materials and methods

2.1. Study populations

2.1.1. The SOLAR cohort

As described previously, the study included 328 Hispanic youth from urban Los Angeles, CA, who were recruited in two waves between 2001 and 2012 (Goodrich et al., 2021, 2023; Goran et al., 2004). Participants were primarily recruited through local metabolic clinics, as well as via health fairs, local advertisements, and word of mouth. Subjects were included if they were between 8 and 13 years old, had self-reported Hispanic or Latino ancestry of all four grandparents, had a body mass index (BMI) $\geq$ the 85th percentile for age and sex based on Centers for Disease Control and Prevention standards, had a family history of type 2 diabetes in at least one parent, and had an absence of type 1 or type 2 diabetes. Participants were excluded if they were taking medications known to affect insulin or glucose metabolism.

Participants underwent annual visits at the University of Sothern California (USC) General Clinic Research Center or Clinical Trials Unit for an average of 3.4 years. In the current study, analysis was subset to the participants' first and second visits only. Of 328 participants, 304 had available measurements of plasma PFAS and bone mineral density at baseline and were included in the analysis (Fig. S1). Of these, 226 had complete data from a second visit. This study was approved by the USC Institutional Review Board. Written informed assent and consent were obtained from participants and their parents, respectively.

2.1.2. The CHS cohort

Between 2014 and 2018, 158 participants between 17 and 22 years old who participated in the Southern California Children's Health Study (McConnell et al., 2015) participated in the Meta-AIR study, as described previously (Goodrich et al., 2021, 2023; Kim et al., 2019). Subjects were included if they had a history of overweight or obesity in early adolescence, had not been diagnosed with type 1 or type 2 diabetes, were not taking medications that were known to affect glucose metabolism, and did not have any other medical conditions. Between 2020 and 2022, participants were invited for a follow-up visit. All study visits took place at the Diabetes and Obesity Research Institute at USC.

Of 158 participants at baseline, 137 had available measurements of both plasma PFAS and bone mineral density and provided consent for future use of biospecimens and were included in the analysis (Fig. S1). Of these, 79 had complete data from a second visit. This study was approved by the USC Institutional Review Board. Written informed consent was obtained from participants.

2.2. PFAS measures

PFAS concentrations were measured from plasma samples collected at baseline. Details on method parameters for these measurements have been previously described (Goodrich et al., 2022, 2023). In brief, levels of 5 PFAS were quantified using liquid chromatography mass spectrometry. The PFAS analyzed in the current study included perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluorohexane sulphonic acid (PFHxS). The limits of detection (LOD) for PFOS, PFOA, PFNA, PFDA, and PFHxS were 0.02, 0.01, 0.10, 0.20, and 0.03 μg/L, respectively. Of these PFAS, PFDA was the only substance that had values below the LOD, and values below the LOD were imputed as $LOD/\sqrt{2}$. Measurement units were μg/L.

2.3. Bone mineral density measurements

Trained technicians performed DXA scans at all visits for both cohorts. In the SOLAR cohort, a whole-body scan was performed on the Hologic QDR 4500 W. Outcomes of interest were total body BMD and trunk BMD, which excludes limb and head BMD measures, at baseline or follow-up. The CHS cohort utilized the Hologic QDR 4500 W or Horizon W models at baseline and the Horizon W model at follow-up. Outcomes of interest were total body BMD and lumbar spine BMD at baseline and follow-up, following the recommendations of the World Health Organization for adults (Sözen et al., 2017). Lumbar BMD measures were not available for the SOLAR cohort. Measurements were expressed in g/cm^2.

2.4. Covariates

In both cohorts, participants (and/or their guardians) completed questionnaires regarding demographic information, as described previously (Goran et al., 2004; Kim et al., 2019; Weigensberg et al., 2003). Age, sex, and parental education level (less than high school, high school graduate, more than high school, or missing) were collected for participants in both cohorts. Additionally, CHS participants self-identified their race, with the categories including non-Hispanic White, Hispanic, and other.

In the SOLAR cohort, a physical examination was performed by a physician at each annual visit to measure child height and weight and determine participants' Tanner stage (Marshall and Tanner, 1969, 1970). In brief, individuals can be categorized into 1 of 5 categories, from Tanner 1 (prepuberty) to Tanner 5 (post puberty).

2.5. Statistical analysis

Descriptive statistics were calculated for both cohorts' characteristic at the baseline and follow-up visits; t-tests and chi-squared tests were utilized to test for differences in continuous and categorical values, respectively, between visits. Geometric means and standard deviations of baseline PFAS values were quantified for each cohort, as well as the 10th and 90th percentile of exposure to each PFAS. Distributions of the BMD outcome variables were calculated within each cohort.

To examine associations of each individual baseline plasma PFAS with changes in BMD, we utilized linear mixed-effect models with individual-level random intercepts within each cohort. This modeling strategy allows for inclusion of data from each participant, despite missing values or irregularly-spaced measurements (Diggle et al., 1994), including participants with only one (i.e., baseline) measurement of BMD. To begin, each PFAS was evaluated in separate models. Each BMD outcome was modeled as a function of age (centered at 10 years for SOLAR and 18 years for CHS) and PFAS exposure, with a random intercept to account for two visits per participant. Specifically, for $i = \{1, \ldots, N\}$ individuals and $j = \{1, 2\}$ visits, we used the following model:

$$Y_{ij} = \gamma_0 + \gamma_1 PFAS_i + \gamma_2 Age_{ij} + \gamma_3 PFAS_i Age_{ij} + \omega_i + \varepsilon_{ij},$$

where $\omega_i \sim N(0, \sigma_\omega)$ is the random intercept for each participant and $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon)$ are the residuals. In line with previous analyses (Blomberg et al., 2022; Cluett et al., 2019; Højsager et al., 2022), PFAS were log-2 transformed to aid in interpretation of model parameters. Specifically, the main effect, or γ_1, represents the difference in baseline BMD outcome (in g/cm^2) per doubling of baseline PFAS exposure, while the age interaction effect, or γ_3, represents the longitudinal change in BMD outcome (in g/cm^2) per year per each doubling of baseline PFAS exposure.

Covariates included in the models were selected via a directed acyclic graph (DAG; Fig. S2) and included sex, race/ethnicity (for the CHS cohort), and parental education (as a proxy of socioeconomic status). Additionally, models in the SOLAR cohort were adjusted for study wave and Tanner stage. Body mass index was not controlled for as it may be on the causal pathway between PFAS and BMD (Geiger et al., 2021; Palermo et al., 2016). Based on previous research on PFAS and BMD (Blomberg et al., 2022; Højsager et al., 2022; Khalil et al., 2016; Lin et al., 2014; Xiong et al., 2022), we hypothesized that associations may differ by sex and therefore performed secondary sex-stratified analyses for each chemical. Before modeling, we assessed linearity of associations using general additive models after log-2 transformation of PFAS.

Significance of associations between total PFAS exposure and BMD outcomes were assessed using likelihood-ratio tests (LRTs) comparing the full model (i.e., the model with PFAS exposure and covariates) to the null model (i.e., the model with only covariates). LRTs had two degrees of freedom and tested whether the cumulative effects of γ_1 and γ_3 improved the model compared to the model without these parameters. Significance threshold for p-values was <0.05. To account for multiple comparisons, false discovery rate (FDR)-adjusted p-values were calculated with the Benjamini-Hochberg method (Benjamini and Hochberg, 1995) within each cohort. P-values were adjusted within each BMD outcome as we hypothesized *a priori* that both total BMD and site-specific BMD were important predictors of bone health. We considered findings to be robust if their adjusted p-value was <0.20.

Estimated marginal means were calculated to compare hypothetical individuals at the 10th and 90th percentiles for each PFAS exposure. The marginal means and 95% confidence intervals (CIs) for each BMD site were calculated at the median age for each visit in both cohorts, while controlling for the previously mentioned covariates. Differences between groups were calculated and t-tests evaluated whether the differences between groups was significantly different from 0 with a p-value significance threshold of <0.05. Data analysis was performed in R (version 4.2.2; R Development Core Team).

2.6. Mixtures analysis

We estimated associations of the cumulative PFAS mixture with change in BMD outcomes via quantile g-computation, a parametric approach based on generalized linear models used to estimate mixture effects (Keil et al., 2020). Quantile g-computation estimates the change in potential outcome per 1-quantile increase in each component of the mixture while adjusting for covariates. The mixture included the PFAS of interest: PFOS, PFOA, PFNA, PFDA, and PFHxS. The outcome variables of interest within each cohort were change in each BMD outcome measure per year, calculated as: $BMD_{(visit2)} - BMD_{(visit1)}/follow - up\ time_{(years)}$. Only participants with information for both visits were included in the mixtures analysis (n = 226 for SOLAR and n = 79 for CHS). Covariates included were those used in the primary analysis, selected via DAG.

Quantile g-computation utilizing linear regression estimated the following parameters: the overall mixture effect, Ψ, interpreted as the difference in BMD change per year per quartile increase in all PFAS in the mixture (similar to γ_3 in the mixed modeling approach); the directional effect of each PFAS (positive or negative); and the absolute effect of each PFAS, interpreted as each PFAS's contribution to Ψ. A p-value significance threshold of <0.05 was used to identify whether each mixture effect (Ψ) was significantly different from 0. The mixtures analysis was performed using the R package "qgcomp".

2.7. Sensitivity analyses

We conducted two sensitivity analyses to assess the robustness of our significant findings in the linear mixed modeling and mixtures approaches and address potential uncertainties in the structure of the DAG. First, because DXA scans are 2-dimensional and estimate measures may be affected by bone size (Alawi et al., 2021), and therefore may require additional adjustment (Crabtree et al., 2014), we further adjusted models to account for participant height to evaluate whether height-adjusted estimates remained significant. Second, to ensure that effect estimates were not being heavily influenced by individuals with measures from only one visit (i.e. baseline visit with no follow-up), we constrained the analysis to only individuals with two visits. This step was not necessary in the mixtures analysis, which only included participants with both visits.

3. Results

3.1. Characteristics of study populations

Descriptive statistics of the study populations at baseline and follow-up are provided in Table 1; outcome variables are further stratified by sex in Table S2. In SOLAR, participants were (mean ± SD) 11.3 ± 1.7 years old at baseline and 42.4% male, with the majority (47.4%) having parents with less than a high school education. There were no significant differences in demographic characteristics between baseline and the average 1.4 ± 0.9 years of follow-up, excluding age and Tanner stage, which is expected as participants matured between visits. Both total BMD and trunk BMD significantly increased between visits (both p < 0.001). The average changes in total BMD and trunk BMD per year were 0.057 ± 0.040 g/cm^2 and 0.048 ± 0.040 g/cm^2, respectively.

In CHS, participants were 19.9 ± 1.3 years old at baseline, 58.4% Hispanic, and 55.5% male, with the majority (66.4%) of participants having parents with more than a high school education. No significant differences were found in demographic characteristics between baseline and the 4.1 ± 1.1 years of follow-up, excluding age. Total BMD

Table 1
Baseline participant characteristics in Hispanic adolescents with overweight and obesity from the SOLAR cohort and young adults with a history of adolescent obesity from the CHS cohort.

	SOLAR			CHS		
Participant characteristics	Baseline (n = 304) Mean ± SD or n (%)	Follow-up (n = 226) Mean ± SD or n (%)	p-value[a]	Baseline (n = 137) Mean ± SD or n (%)	Follow-up (n = 79) Mean ± SD or n (%)	p-value[a]
Age (y)	11.3 ± 1.7	12.7 ± 2.0	<0.001	19.9 ± 1.3	24.0 ± 0.7	<0.001
Sex			0.99			0.30
Male	129 (42.4)	96 (42.5)		76 (55.5)	38 (48.1)	
Female	175 (57.6)	130 (57.5)		61 (44.5)	41 (51.9)	
Ethnicity	304 (100.0)	226 (100.0)	1.00			0.85
Hispanic				80 (58.4)	44 (55.7)	
Non-Hispanic				49 (35.8)	29 (36.7)	
Other				8 (5.8)	6 (7.6)	
Parent education			0.68			0.99
Less than high school	144 (47.4)	106 (46.9)		25 (18.2)	13 (16.5)	
High school grad	88 (28.9)	68 (30.1)		17 (12.4)	10 (12.7)	
More than high school	41 (13.5)	24 (10.6)		91 (66.4)	54 (68.4)	
Missing	31 (10.2)	28 (12.4)		4 (2.9)	2 (2.5)	
Tanner stage			<0.001	–	–	–
1 (Prepuberty)	93 (30.6)	40 (17.7)				
2 (Early puberty)	114 (37.5)	52 (23.0)				
3 (Puberty)	39 (12.8)	31 (13.7)				
4 (Late puberty)	38 (12.5)	59 (26.1)				
5 (Post puberty)	20 (6.6)	44 (19.5)				
Study wave			0.09	–	–	–
First wave	226 (74.3)	183 (81.0)				
Second wave	78 (25.7)	43 (19.0)				
Follow-up time (y)	–	1.4 ± 0.9	–		4.1 ± 1.1	
BMD (g/cm^2)						
Total body	0.927 ± 0.101	1.005 ± 0.128	<0.001	1.186 ± 0.111	1.223 ± 0.115	0.02
Trunk	0.747 ± 0.094	0.812 ± 0.118	<0.001			
Lumbar				1.143 ± 0.242	1.161 ± 0.165	0.50
BMD change/year (g/cm^2)[b]	–		–	–		–
Total body		0.057 ± 0.040			0.010 ± 0.009	
Trunk		0.048 ± 0.040				
Lumbar					0.000 ± 0.032	

[a] p-values calculated with independent t-tests for continuous variables and Pearson's chi-square tests for categorical variables, respectively.
[b] Yearly change values calculated as $BMD_{(follow-up)} - BMD_{(baseline)}$/follow-up time (years).

increased significantly between visits (p = 0.02), with an yearly change of 0.010 ± 0.009 g/cm^2. Lumbar BMD did not change significant between visits (p = 0.50), with an yearly change of 0.000 ± 0.032 g/cm^2.

Plasma PFAS concentrations at baseline are provided in Table 2. Concentrations of all PFAS were lower in the CHS cohort than the SOLAR cohort. In both cohorts, participants had the highest levels of PFOS, followed by PFOA and PFHxS. Significant positive correlations were found between multiple PFAS in both cohorts (p < 0.001; Table S1).

3.2. PFAS—BMD associations in adolescents (SOLAR cohort)

3.2.1. Individual PFAS-BMD associations in adolescents

In the SOLAR cohort, exposure to PFOS was associated with lower yearly changes in trunk BMD over the follow-up period (LRT χ^2 = 7.52, p = 0.02, FDR p = 0.12; Table 3). Each doubling of plasma PFOS was associated with an average −0.003 g/cm^2 difference in change in trunk BMD per year (95% CI: −0.005, −0.0002). At baseline, there was no significant difference in trunk BMD between individuals in the 10th and 90th percentiles of PFOS exposure, but at follow-up, individuals in the 90th percentile differed by −0.043 g/cm^2 in compared to those in the 10th percentile (p = 0.04; Fig. 1a; Table S3). Total BMD followed a similar trend, but associations were non-significant as PFOS terms did not improve the model compared to the null model (LRT χ^2 = 3.23, p = 0.20, FDR p = 0.50; Table 3). Individuals in the 90th exposure percentile at follow-up differed by −0.042 g/cm^2 total BMD compared to those in the 10th percentile, though this association did not reach statistical significance (*p* = 0.08; Table S3). No other associations between PFAS and BMD reached statistical significance (Table 3).

3.2.2. Sex-specific individual PFAS-BMD associations in adolescents

In males in SOLAR, PFOS exposure was associated with lower changes in trunk BMD after adjusting for multiple comparisons (LRT χ^2 = 8.88, p = 0.01, FDR p = 0.06; Table S4). Each doubling of PFOS was associated with an average of −0.004 g/cm^2 (95% CI: −0.006, −0.001) yearly change in trunk BMD. Similarly, PFOA demonstrated associations with lower changes in trunk BMD after adjustment (LRT χ^2 = 7.15, p = 0.03, FDR p = 0.07; Table S4) and each doubling of PFOA was associated with an average −0.005 g/cm^2 (95% CI: −0.009, −0.001) change in trunk BMD per year. No other associations between PFAS and BMD reached statistical significance in either females or males (Table S4).

3.2.3. Mixture models

In SOLAR, the PFAS mixture was negatively associated with lower change in BMD per year, though most associations did not reach statistical significance (Table 4 and S5). In SOLAR males, the PFAS mixture was significantly associated with lower total BMD change per year: per each quartile increase in all PFAS, average total yearly BMD change differed by −0.008 g/cm^2 (95% CI -0.016, −0.001; Table 4 and S5).

Table 2
Baseline plasma levels of PFAS (ng/mL) in n = 304 Hispanic adolescents with overweight and obesity from the SOLAR cohort and n = 137 young adults with a history of adolescent obesity from the CHS cohort.

	SOLAR		CHS	
PFAS	Geometric mean (SD)	[10th, 90th percentile]	Geometric mean (SD)	[10th, 90th percentile]
PFOS	11.6 (2.2)	[3.1, 27.1]	3.3 (1.6)	[1.9, 6.1]
PFOA	3.3 (1.8)	[1.5, 6.7]	1.3 (1.4)	[0.9, 2.1]
PFNA	0.6 (1.4)	[0.4, 0.9]	0.5 (1.3)	[0.3, 0.7]
PFDA	0.2 (1.6)	[0.2, 0.4]	0.2 (1.8)	[0.1, 0.3]
PFHxS	1.4 (2.0)	[0.6, 3.3]	1.1 (2.1)	[0.5, 3.1]

3.3. PFAS—BMD associations in young adults (CHS cohort)

3.3.1. Individual PFAS-BMD associations in young adults

PFOS exposure demonstrated an association with lower total BMD at baseline in the CHS cohort. Each doubling of plasma PFOS was associated with an average −0.032 g/cm^2 difference in total BMD (95% CI -0.062, −0.003), though rates of change over follow-up were non-significant and the overall model failed to reach significance (LRT χ^2 = 5.00, p = 0.08, FDR p = 0.41; Table 5). At baseline, those in the 90th percentile of PFOS exposure differed by −0.051 g/cm^2 total BMD than those in the 10th percentile (p = 0.043; Fig. 1b; Table S6). At follow-up, differences between the groups were similar but did not reach the threshold for statistical significance; those with higher exposure had a −0.041 g/cm^2 difference in total BMD than those with lower exposure (p = 0.10). Associations between PFOS and lumbar BMD were non-significant and BMD associations with other PFAS did not reach statistical significance (Table 5).

3.3.2. Sex-specific individual PFAS associations in young adults

In CHS females, PFDA exposure was associated with lower changes in total BMD: females had an average −0.004 g/cm^2 change in total BMD per year with each doubling of plasma PFDA (95% CI: −0.007, −0.0001), though the overall model failed to reach significance (LRT χ^2 = 5.37, p = 0.068, FDR p = 0.33; Table S7). In CHS males, however, exposure to PFOS was associated with higher changes in total BMD after adjustment for multiple comparisons (LRT χ^2 = 6.58, p = 0.037, FDR p = 0.19; Table S7). Each doubling of PFOS was associated with an average increase of 0.005 g/cm^2 in total BMD per year (95% CI: 0.0003, 0.011). Associations between other PFAS and BMD in either females or males were non-significant (Table S7).

3.3.3. PFAS mixture models

The PFAS mixture was not significantly associated with change in BMD in CHS, though associations were primarily negative (Table 4 and S8). In CHS females, each quartile increase in all PFAS was associated with a −0.003 g/cm^2 difference in total yearly BMD change, though this association did not reach statistical significance (95% CI -0.006, 00001; Table 4 and S8).

3.4. Sensitivity analyses

In the sensitivity analysis to examine associations after adjustment for participant height, results remained largely unchanged in both cohorts in regards to individual PFAS associations (Tables S9 and S10) and mixtures associations (Tables S11 and S12). Similarly, in the sensitivity analysis to examine associations exclusively in participants with two visits, results of individual PFAS associations remained qualitatively unchanged in both cohorts (Tables S9 and S10).

4. Discussion

To our knowledge, this is the first study to evaluate the associations of exposure to individual PFAS and their mixture with longitudinal changes BMD in adolescence and young adulthood, two important periods of bone development and accrual that predict life-long bone health. Additionally, this is the first study to include primarily Hispanic participants. We found that adolescents with higher PFOS exposure exhibited lower changes in trunk BMD between visits. In these adolescents, the mixture of all five PFAS was associated with negative, non-significant changes in BMD per year; in males, the mixture was significantly associated with lower change in total BMD per year. Associations with PFOS were further supported in the young adult cohort, where PFOS was associated with lower total BMD at baseline, though not associated with change in total BMD per year. Negative associations with longitudinal BMD outcomes were also demonstrated by PFOA and PFDA in sex-specific analyses, and the associations of the PFAS mixture

Table 3
Adjusted associations between PFAS with changes in BMD outcomes in Hispanic adolescents with overweight and obesity from the SOLAR cohort (n = 304).

	Trunk BMD					Total BMD				
PFAS	Main effect[a] (95% CI)	Age Interaction[b] (95% CI)	χ^2	p[c]	FDR p[d]	Main effect[a] (95% CI)	Age Interaction[b] (95% CI)	χ^2	p[c]	FDR p[d]
PFOS	−0.007 (−0.021, 0.007)	−0.003 (−0.005, −0.0002)	7.52	0.02	0.12	−0.012 (−0.027, 0.003)	−0.000 (−0.003, 0.002)	3.23	0.20	0.50
PFOA	−0.002 (−0.015, 0.011)	−0.002 (−0.005, 0.001)	2.50	0.29	0.49	−0.007 (−0.021, 0.008)	0.001 (−0.002, 0.005)	0.93	0.63	0.83
PFNA	0.002 (−0.017, 0.022)	0.001 (−0.005, 0.007)	0.47	0.79	0.82	0.009 (−0.012, 0.030)	−0.002 (−0.009, 0.004)	0.82	0.66	0.83
PFDA	−0.003 (−0.017, 0.010)	0.003 (−0.001, 0.008)	2.44	0.29	0.49	0.001 (−0.013, 0.016)	0.001 (−0.004, 0.005)	0.21	0.90	0.90
PFHxS	0.003 (−0.006, 0.012)	−0.000 (−0.003, 0.002)	0.41	0.82	0.82	0.003 (−0.008, 0.013)	0.003 (−0.000, 0.005)	5.01	0.08	0.41

[a] Estimates represent the change in BMD (in g/cm^2) per each doubling of the PFAS substance at baseline.
[b] Interaction term between age (centered on 10) and each PFAS substance; estimates represent the change in BMD (in g/cm^2) per year per each doubling of the PFAS substance.
[c] p-value for likelihood-ratio χ^2 testing nested models. For detailed description of statistical models, see main text.
[d] False Discovery Rate (FDR) corrected p-value for likelihood-ratio χ^2. A value < 0.20 was considered statistically significant.

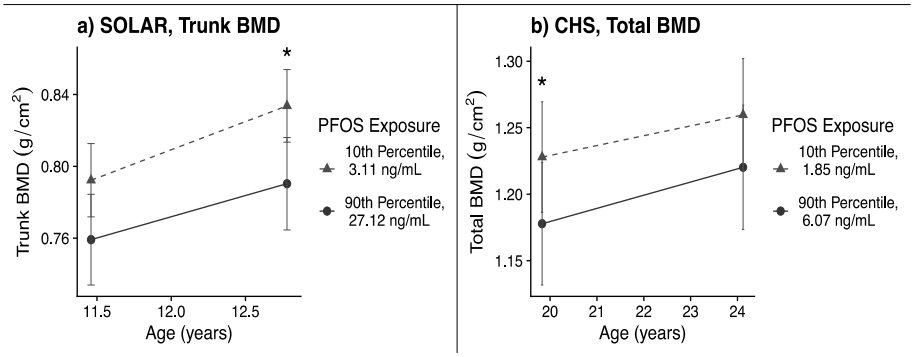

Fig. 1. Association between a) plasma PFOS and trunk BMD at the median age for baseline and follow-up visits for n = 226 Hispanic adolescents with overweight and obesity recruited from Sothern California between 2001 and 2012 (SOLAR cohort); and b) plasma PFOS and total BMD at the median age for baseline and follow-up visits for n = 79 young adults recruited from the Children's Health Study between 2014 and 2018 (CHS cohort). Figure represents point estimates and 95% confidence intervals for BMD at each age calculated using linear mixed effect models corrected for a) sex, parental education, Tanner stage, and study wave; and b) sex, ethnicity, and parental education. * denotes *p*-values for differences <0.05, calculated using t-statistics to evaluate whether the difference between individuals with low (10th percentile) vs. high (90th percentile) of PFOS was significantly different from 0.

Table 4
Adjusted associations[a] between the PFAS mixture[b] with yearly change in BMD outcomes (g/cm^2/year) in Hispanic adolescents with overweight and obesity from the SOLAR cohort estimated via quantile g-computation, overall and by sex, and young adults with a history of adolescent obesity from the CHS cohort, overall and by sex.

	SOLAR			CHS		
Subset	Trunk BMD (95% CI)	Total BMD (95% CI)	n	Lumbar BMD (95% CI)	Total BMD 95% CI)	n
Cohort	−0.002 (−0.010, 0.005)	−0.004 (−0.011, 0.003)	226	−0.003 (−0.013, 0.006)	−0.001 (−0.004, 0.002)	79
Females	−0.008 (−0.022, 0.006)	−0.007 (−0.021, 0.007)	96	−0.004 (−0.017, 0.008)	−0.003 (−0.006, 0.00001)	41
Males	−0.004 (−0.012, 0.004)	−0.008 (−0.016, −0.001)	130	0.001 (−0.016, 0.019)	0.000 (−0.004, 0.004)	38

[a] Estimates (95% CI) represent the change in BMD (in g/cm^2) per 1-quantile increase in each component of the mixture while adjusting for covariates. Intervals that do not contain 0 are considered statistically significant with p < 0.05.
[b] PFAS mixture included PFOS, PFOA, PFNA, PFDA, and PFHxS.

with change in BMD per year were primarily negative (though non-significant). These results support the hypothesis that PFAS impair bone development and bone mineral density during adolescence, and these associations persist into adulthood.

Our results demonstrate that PFAS have similar impacts on BMD as substances with established osteotoxicity that have been associated with increased likelihood of bone fracture or osteoporosis in later life (Briot and Roux, 2015; Mortimer et al., 2005; Wang et al., 2019). For example, one study on corticosteroids, drugs that have been demonstrated to negatively affect bone health, found that each doubling of inhaled corticosteroid dose was associated with an average 0.032 lower g/cm^2 lumbar BMD in adults with asthma (Wong et al., 2000). We found similar results for exposure to PFOS in our analysis, where each doubling of PFOS was associated with an average 0.032 g/cm^2 baseline lower total BMD in young adults. Additionally, environmental toxicants such as lead have demonstrated reductions in BMD in studied populations. In NHANES children from 2005 to 2010, each 1 mg/dl increase of blood lead levels was associated with an average 0.011 g/cm^2 decrease in total spine BMD (Cui et al., 2022). Our findings imply that PFAS may have similar harmful impacts on BMD as other established substances, including exposure to other environmental toxicants. Individuals with higher PFAS exposure may have lower BMD through childhood and adolescence, which may lead to higher rates of bone fractures or other injuries during this time period (Clark et al., 2006). Low BMD in adolescence may additionally lead to higher rates of osteoporosis and bone fractures in later adulthood, an important concern for our aging population (Rowe et al., 2022). PFAS should be considered in addition to other established osteotoxic substances when developing policies and interventions to preserve bone health at all ages.

4.1. Previous evidence

Our study contribute longitudinal findings to previous evidence demonstrating negative relationships between PFAS and bone health outcomes in both adults and children. In adults, a cross-sectional study of NHANES individuals from 2005 to 2008 demonstrated an average

Table 5
Adjusted associations between PFAS with changes in BMD outcomes in young adults with a history of adolescent obesity from the CHS cohort (n = 137).

	Lumbar BMD					Total BMD				
PFAS	Main effect[a] (95% CI)	Age Interaction[b] (95% CI)	χ^2	p[c]	FDR p[d]	Main effect[a] (95% CI)	Age Interaction[b] (95% CI)	χ^2	p[c]	FDR p[d]
PFOS	0.000 (−0.068, 0.069)	0.001 (−0.009, 0.011)	0.04	0.98	0.98	−0.032 (−0.062, −0.003)	0.001 (−0.001, 0.004)	5.00	0.08	0.41
PFOA	0.011 (−0.073, 0.094)	−0.001 (−0.014, 0.012)	0.07	0.97	0.98	−0.017 (−0.053, 0.020)	−0.001 (−0.005, 0.003)	1.54	0.46	0.67
PFNA	−0.077 (−0.189, 0.034)	0.003 (−0.016, 0.023)	2.17	0.34	0.98	−0.042 (−0.090, 0.006)	0.002 (−0.004, 0.008)	3.10	0.21	0.53
PFDA	−0.033 (−0.089, 0.023)	0.005 (−0.006, 0.016)	1.43	0.49	0.98	−0.008 (−0.031, 0.015)	0.000 (−0.003, 0.003)	0.50	0.78	0.78
PFHxS	0.003 (−0.037, 0.044)	−0.000 (−0.006, 0.006)	0.03	0.98	0.98	−0.009 (−0.027, 0.008)	−0.000 (−0.002, 0.002)	1.26	0.53	0.67

[a] Estimates represent the change in BMD (in g/cm^2) per each doubling of the PFAS substance at baseline.
[b] Interaction term between age (centered on 10) and each PFAS substance; estimates represent the change in BMD (in g/cm^2) per year per each doubling of the PFAS substance.
[c] p-value for likelihood-ratio χ^2 testing nested models. For detailed description of statistical models, see main text.
[d] FDR corrected p-value for likelihood-ratio χ^2. A value < 0.20 was considered statistically significant.

0.022 g/cm^2 decrease in lumbar BMD per each unit increase of natural log-transformed PFOS in premenopausal women (Lin et al., 2014). A later NHANES analysis of adolescents and adults from 2009 to 2010 demonstrated negative associations between serum PFOS and femoral neck BMD in men, and between serum PFOS and PFOA and femur BMD in women (Khalil et al., 2016). A longitudinal analysis of almost 300 overweight/obese adults found PFOS, PFNA, and PFDA to be associated with greater reductions of BMD in the hip (Hu et al., 2019). In children, observational findings have also demonstrated negative relationships between PFAS and BMD. The cross-sectional analysis of participants aged 6–10 in the Project Viva cohort found that higher concentrations of serum PFOA, PFOS, and PFDA were associated with lower BMD z-scores (Cluett et al., 2019). A study of obese 8–12 year old children exhibited a negative relationship between serum PFNA and bone density assessed by bone qualitative ultrasounds (Khalil et al., 2018). Few studies have longitudinally evaluated the PFAS-BMD association in child populations, and cohorts typically contain racially-homogenous children. A study following 366 children in the Faroese Cohort over 9 years identified a negative relationship between PFNA and BMD z-scores at age 5, though associations were no longer significant at age 9 (Blomberg et al., 2022). More recently, early postnatal exposure to PFDA was associated with lower BMD z-scores at age 7 in the Odense Child Cohort (Højsager et al., 2022). Evidence from our study supports the relationships established in previous studies and contributes longitudinal findings from primarily Hispanic adolescents and young adults to the literature.

Experimental evidence supports the hypothesis that PFAS have a negative effect on BMD. PFAS are endocrine-disrupting chemicals (White et al., 2011) that alter hormonal regulation of many processes in the body, including bone modeling. PFAS have been shown to activate peroxisome proliferator-activated receptor gamma (PPARγ), stimulating mesenchymal stem cell differentiation into adipocytes instead of osteoblasts (Lecka-Czernik et al., 2002; Yamamoto et al., 2015). Additionally, PFAS act as antagonists of androgen receptors that regulate androgen-mediated osteoblastogenesis (the production of osteoblasts), which may inhibit osteoblast formation (Kjeldsen and Bonefeld-Jørgensen, 2013). Lower levels of osteoblasts may disrupt the homeostasis of bone remodeling and lead to increased bone resorption by osteoclasts, resulting in lower BMD. PFAS may also impact BMD by persisting in bone tissues; human autopsy studies have demonstrated concentrations of PFAS in bone, implying that the substances accumulate in bone tissue and may lead to osteotoxicity (Koskela et al., 2017; Pérez et al., 2013).

4.2. Sex-specific associations

Sex-specific analyses also demonstrated that both PFOS and PFOA were negatively associated with yearly change in trunk BMD in males in the adolescent cohort, and PFDA was negatively associated with yearly change in total BMD in females in the young adult cohort. Previous studies have reported sex-specific differences in PFAS-BMD associations (Blomberg et al., 2022; Højsager et al., 2022; Khalil et al., 2016; Lin et al., 2014; Xiong et al., 2022). Unexpectedly, in the adolescent CHS population, PFOS demonstrated a significant positive association with total yearly BMD change in males. These results contradict the general findings in both the CHS and SOLAR cohorts that PFOS is negatively associated with BMD at baseline and over time. Due to the small sample size after stratification (n = 38), it is possible that associations may have been spurred by participants with high BMD values. Repeated analyses with a larger sample size are warranted.

4.3. Strengths and limitations

Several limitations are worth noting. First, the small sample sizes in each cohort likely reduced statistical power to detect associations, especially after stratifying for sex-specific models. Second, PFAS measures can change over time, and exposure was only measured at one timepoint in the current analysis. New research has demonstrated that prenatal (Buckley et al., 2021; Højsager et al., 2022) and early childhood (Blomberg et al., 2022; Højsager et al., 2022) PFAS exposures have been associated with lower BMD, but we were only able to collect plasma PFAS values in adolescence/young adulthood. However, PFAS have long biological half-lives (Fenton et al., 2021), meaning that measurement at one time is likely sufficient to provide a good estimate of long-time exposure, and exposure misclassification risk is likely low. A third limitation is related to the different measures of BMD sites between cohorts, which creates the inability to compare effect estimates across the two groups. The SOLAR cohort is a historical cohort, and information on lumbar BMD measurements was not available, though the more general measure of trunk BMD was. Although both are relevant measures of BMD that exclude limb measurements, lumbar BMD is preferred for clinical diagnostics (Sözen et al., 2017). Finally, despite adjustment for known confounders such as sex, parental education, and race (when applicable), it is possible that unmeasured factors may have caused residual confounding and impacted the true relationship between exposure and outcome.

Despite these limitations, strengths of the study should be recognized. Our study is the first to our knowledge to evaluate the relationship between PFAS and longitudinal changes in BMD in childhood and young adulthood, two important periods of bone accrual that impact bone health throughout the lifespan. A primary strength in this analysis is the repeated measurements of BMD, and the ability to establish temporality due to measurement of PFAS at baseline and BMD at both baseline and follow-up. Most of the previous studies on this topic have been cross-sectional, collecting values of PFAS and BMD at the same time point, meaning that temporality of the relationship cannot be established, and reverse causation is impossible to rule out. Additionally, the repeated BMD measures were collected using dual x-ray absorptiometry, the gold-standard method for measuring BMD to diagnose osteoporosis (Roux and Briot, 2017). These measures are preferred over other methods such as bone qualitative ultrasounds, which have been used in other studies

on children (Di Nisio et al., 2020; Khalil et al., 2018). Finally, a major strength of this project was the inclusion of mixed-ethnicity participants. While most other studies on this topic contain populations that were non-Hispanic White, the SOLAR and CHS cohorts were 100% and 58.4% Hispanic, respectively. Due to structural and environmental racism, individuals of Hispanic/Latino ethnicity may bear the burden of increased environmental health risks such as poorer air or water quality (Quintero-Somaini et al., 2004). For example, the US Government Accountability Office found that "disadvantaged communities" (defined by percentages of the populations that identified as low income and that identified as non-White or Hispanic/Latino) were more likely to have PFAS in their drinking water in some states (U. S. Government Accountability Office, 2022). Therefore it is of utmost important to include minority populations in research as opposed to generalize findings found in non-Hispanic White populations.

5. Conclusions

In two independent cohorts of adolescents and young adults, PFOS was associated with lower BMD. In a cohort of overweight/obese Hispanic adolescents, PFOS was associated with lower yearly change in trunk BMD during puberty. In a cohort of mixed-ethnicity young adults, PFOS was associated with lower total BMD at baseline, though longitudinal associations were not significant. These results suggest that minimizing PFAS exposure in adolescence may improve adolescent bone density, thereby preserving bone health at all ages and leading to long-term reductions in osteoporosis and fractures later in life. Developing interventions to prevent disturbance of BMD development or treat those with lower BMD before morbidities develop has the potential to significantly improve individual quality of life, and is of utmost important in at-risk ethnic groups such as Hispanic individuals.

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CRediT authorship contribution statement

Emily Beglarian: Conceptualization, Methodology, Software, Formal analysis, Writing - original draft, Visualization. **Elizabeth Costello:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Douglas I. Walker:** Methodology, Investigation, Resources, Writing - review & editing, Funding acquisition. **Hongxu Wang:** Software, Data curation, Writing - review & editing. **Tanya L. Alderete:** Methodology, Investigation, Writing - review & editing, Funding acquisition. **Zhanghua Chen:** Methodology, Investigation, Writing - review & editing, Funding acquisition. **Damaskini Valvi:** Methodology, Writing - review & editing. **Brittney O. Baumert:** Methodology, Writing - review & editing. **Sarah Rock:** Methodology, Investigation, Project administration. **Bruna Rubbo:** Methodology, Writing - review & editing. **Max T. Aung:** Methodology, Writing - review & editing. **Frank D. Gilliland:** Methodology, Investigation, Writing - review & editing, Funding acquisition. **Michael I. Goran:** Methodology, Investigation, Writing - review & editing, Funding acquisition. **Dean P. Jones:** Resources, Writing - review & editing, Funding acquisition. **Rob McConnell:** Methodology, Investigation, Writing - review & editing. **Sandrah P. Eckel:** Methodology, Formal analysis, Writing - review & editing. **David V. Conti:** Methodology, Formal analysis, Writing - review & editing, Funding acquisition. **Jesse A. Goodrich:** Conceptualization, Methodology, Software, Formal analysis, Writing - original draft, Visualization, Supervision, Funding acquisition. **Lida Chatzi:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at 10.1016/j.envres.2023.117611.

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