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Per- and Polyfluoroalkyl Substance Plasma Concentrations and Bone Mineral Density in Midchildhood: A Cross-Sectional Study (Project Viva, United States)

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Background: Identifying factors that impair bone accrual during childhood is a critical step toward osteoporosis prevention. Exposure to per- and polyfluoroalkyl substances (PFASs) has been associated with lower bone mineral density, but data are limited, particularly in children.

Methods: We studied 576 children in Project Viva, a Boston-area cohort of mother/child pairs recruited prenatally from 1999 to 2002. We quantified plasma concentrations of several PFASs and measured areal bone mineral density (aBMD) by dual-energy X-ray absorptiometry (DXA) in midchildhood. We used linear regression to examine associations between plasma concentrations of individual PFASs and aBMD z-score. We used weighted quantile sum (WQS) regression to examine the association of the PFAS mixture with aBMD z-score. All models were adjusted for maternal age, education, annual household income, census tract median household income, and child age, sex, race/ethnicity, dairy intake, physical activity, and year of blood draw.

Results: Children were [mean ± standard deviation (SD)] 7.9 ± 0.8 years of age. The highest PFAS plasma concentrations were of perfluorooctanesulfonic acid (PFOS) {median [interquartile range (IQR)]: 6.4 (5.6) ng/mL} and perfluorooctanoic acid (PFOA) [median (IQR): 4.4 (3.2) ng/mL]. Using linear regression, children with higher plasma concentrations of PFOA, PFOS, and perfluorodecanoate (PFDA) had lower aBMD z-scores [e.g., β: −0.16; 95% confidence interval (CI): −0.25, −0.06 per doubling of PFOA]. The PFAS mixture was negatively associated with aBMD z-score (β: −0.16; 95% CI: −0.28, −0.04 per IQR increment of the mixture index).

Conclusions: PFAS exposure may impair bone accrual in childhood and peak bone mass, an important determinant of lifelong skeletal health. https://doi.org/10.1289/EHP4918

Introduction

Osteoporosis affects over 200 million adults worldwide, and associated fractures are costly, with high morbidity and mortality and limited treatment options (Reginster and Burlet 2006). Because bone accrues during childhood and adolescence and is maximized by early adulthood (Gordon et al. 2017), identifying and remediating factors that lead to low bone accrual in childhood is a critical component of osteoporosis prevention efforts. However, our understanding of the role of environmental factors on childhood bone accrual is limited.

Per- and polyfluoroalkyl substances (PFASs), ubiquitous environmental contaminants added to food packaging, clothing, furniture, and carpets to make the products nonstick and stain repellant (Lindstrom et al. 2011), are universally detectable in the general U.S. population, including children (Calafat et al. 2007; Ye et al. 2018).

PFASs may influence areal bone mineral density (aBMD) through several mechanisms. PFASs activate the nuclear peroxisome proliferator–activated receptor gamma (PPARγ), which suppresses the osteoblast lineage of mesenchymal stem cells (Yamamoto et al. 2015). PFASs may also reduce BMD by acting as androgen receptor antagonists (Clarke and Khosla 2009; Kjeldsen and Bonefeld-Jorgensen 2013), inhibiting steroidogenic enzyme activity on the androgen secretion pathway (Zhao et al. 2010) or by directly intercalating into bone (Bogdanska et al. 2011; Koskela et al. 2017; Pérez et al. 2013). Consistent with these mechanistic data, two epidemiologic studies in U.S. adults reported higher PFAS serum concentrations to be associated with lower aBMD (Khalil et al. 2016; Lin et al. 2014), and in a pilot cross-sectional study of 48 obese children, higher PFAS plasma concentrations were associated with lower aBMD measured by calcaneal quantitative ultrasound (Khalil et al. 2018).

The extent to which different PFASs may affect aBMD in children is not well characterized. Based on limited existing data suggesting a contemporaneous inverse association between PFAS exposure and aBMD, we set out to test the hypothesis that higher plasma PFAS concentrations would be cross-sectionally associated with lower aBMD z-score at midchildhood in a large Boston-area cohort.

Methods

Study Population and Design

Between 1999 and 2002, we recruited pregnant women to Project Viva, a longitudinal cohort study of prenatal exposures and child health, during their first prenatal visit at Atrius Harvard Vanguard

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Medical Associates, a multispecialty group practice in eastern Massachusetts (Oken et al. 2015). Of 2,128 mother–infant pairs, 1,116 (54%) children had a follow-up research visit in midchildhood (age range, 6–10 y; median, 7.7 y). At the midchildhood visit, 653 (59%) of these children had PFAS concentrations measured in plasma, and 576 children from this population had aBMD measured by dual-energy X-ray absorptiometry (DXA) and thus were included in the present analysis. Among participants who attended the midchildhood visit, participants who were included in the statistical analysis were less likely to be white compared with excluded participants (57% vs. 70%). Although included participants' mothers were less likely to have graduated from college (64% vs. 72%), they were more likely to belong to households with high income (total annual income >$70,000) compared with participants who were excluded (80% vs. 68%) (see Table S1).

The institutional review boards of participating institutions approved this analysis. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects research. All mothers provided written informed consent for their child's participation.

Exposure and Outcome Measurements

We measured several PFASs in plasma collected in midchildhood as previously described (Sagiv et al. 2015). Staff at the Division of Laboratory Sciences at the CDC quantified PFASs using online solid-phase extraction with isotope dilution high-performance liquid chromatography–mass spectrometry. The limit of detection (LOD) was 0.1 ng/mL for all PFASs; we replaced values below the LOD with the LOD/$\sqrt{2}$. We summed the concentrations of the isomers of perfluorooctanoic acid (PFOA) [i.e., n-PFOA and the sum of perfluoromethylheptanoates and perfluorodimethylhexanoates (Sb-PFOA)] to obtain total PFOA. We summed the concentrations of the isomers of perfluorooctanesulfonic acid (PFOS) [i.e., n-PFOS, sum of perfluoromethylheptane sulfonates (Sm-PFOS), and sum of perfluorodimethylhexane sulfonates (Sm2-PFOS)] to obtain total PFOS. We decided *a priori* to only consider PFASs with detectable concentrations in >60% of samples for the current analysis. The PFASs that met this threshold were n-PFOA, n-PFOS, Sm-PFOS, total PFOA, total PFOS, perfluorodecanoate (PFDA, previously abbreviated as PFDeA), perfluorohexane sulfonate (PFHxS), 2-(N-methyl-perfluorooctane sulfonamide) acetate (MeFOSAA, previously abbreviated as MePFOSA-AcOH), and perfluorononanoate (PFNA).

We measured total-body (excluding the skull) aBMD and bone mineral content (BMC) in midchildhood via a total-body DXA scan (Discovery A model; Hologic Inc.). In order to avoid variation in aBMD measures between DXA instruments (Brownbill and Ilich 2005), we used the same DXA scanner for all participants and calibrated it daily with a standard phantom from the manufacturer to assess for machine drift. We excluded the skull in the measurements because the skull comprises a large proportion of the skeleton in growing children and is not responsive to physical activity or other environmental influences (Crabtree et al. 2014). Total body less head measurements are also recommended for clinical pediatric skeletal assessments (Gordon et al. 2014). Intrarater reliability on a subset of measurements was high Intraclass correlation (ICC) > 0.91. We analyzed data with Hologic software (version 12.6) using the pediatric setting and used U.S. national reference data to derive age-, sex-, race-, and height-adjusted aBMD and BMC z-scores (Zemel et al. 2011).

Covariates

We administered questionnaires to collect data on maternal education, parity, smoking habits during pregnancy and child secondhand smoke exposure, breastfeeding duration, annual household income, and race/ethnicity. At the midchildhood visit, we used a PrimeScreen questionnaire to collect information on the child's soda and dairy (milk, yogurt, cheese, butter, and ice cream) intake in servings per day over the past month (Rifas-Shiman et al. 2001). In a subset of children ($n = 546$), we measured serum 25-hydroxyvitamin D [25(OH)D] using isotope dilution liquid chromatography–tandem mass spectrometry (Baker et al. 2011; Singh et al. 2006). Based on home address recorded at the midchildhood visit, we obtained median annual household income for the child's residential census tract from U.S. Census data (U.S. Census Bureau 2000).

Statistical Analyses

We first ran single-PFAS linear regression models to examine independent associations between each PFAS and aBMD z-score. Next, we ran a multi-PFAS linear regression model to examine associations between each PFAS and aBMD z-score, independent of the other PFASs. Finally, we examined the association between the PFAS mixture and aBMD z-score via weighted quantile sum (WQS) regression. The distributions of all plasma PFAS concentrations were right skewed. To evaluate nonlinearity, we fit penalized spline generalized additive models of the associations of individual PFASs with aBMD z-scores. Associations were linear when PFAS plasma concentrations were $\log_2$ transformed. We therefore used $\log_2$-transformed PFAS plasma concentrations in our linear regression analyses and in developing a composite weighted index in the WQS analysis.

In all models, we accounted for potential positive or negative confounders of the association between PFAS plasma concentration (Sagiv et al. 2015) and aBMD z-score (Rubin et al. 1993). We evaluated linearity of the relationship between each continuous covariate and the outcome using penalized spline generalized additive models, and we categorized covariates that were nonlinearly associated with aBMD z-score. The covariates that we included in our final models were maternal age at enrollment (years), maternal education (with or without college degree), individual household income (<$40,000, $40,000–70,000, >$70,000), census tract median household income (<$30,000, $30,000–60,000, $60,000–100,000, >$100,000), child age (years), sex (dichotomous), race/ethnicity (white, black, Asian, Hispanic, other), dairy intake (servings per week), physical activity (hours per week), and year of blood draw for PFAS measurement (2007–2010). We included census tract median household income in addition to individual household income data because we have previously shown census tract median household income to be more strongly associated with PFAS concentrations (Harris et al. 2017). Collinearity between the income variables did not impact the results, based on variance inflation factors (VIFs) < 10 in the final models (VIF < 1.5 for each category of census tract median household income, and VIF < 1.9 for each category of individual household income).

We considered but did not include the following variables (see Table S2 for descriptive data): breastfeeding duration, soda intake, secondhand smoke exposure, and 25(OH)D plasma concentration. Figure S1 demonstrates that these variables did not confound the PFAS–aBMD association (i.e., estimate for the primary exposure changed by <10%). We did not consider pubertal status or body mass index (BMI) z-score/adiposity, as these variables may be on the causal pathway between PFAS exposure and aBMD z-score (Gilsanz et al. 2011; Lopez-Espinosa et al. 2011; Domazet et al. 2016), and inclusion in the analysis could introduce collider bias (Cole et al. 2010). We substituted maternal race/ethnicity for the 10% of children in the cohort who were missing data on race/ethnicity. We performed complete case analyses. Among participants with available exposure/outcome

data, 92.2% had information on all covariates and thus were included in our models.

In our single- and multi-PFAS linear regression models, we examined associations between the following individual PFASs and aBMD z-score: total PFOA, total PFOS, PFDA, PFHxS, MeFOSAA, and PFNA. Next, we used WQS to estimate the association between the PFAS mixture and aBMD z-score. WQS assigns each PFAS within the mixture a weight that reflects the strength of that PFAS–outcome association. However, the weight of each PFAS is also affected by the collinearity between that PFAS and other PFASs in the mixture, such that a given PFAS will have a lower weight if it is highly correlated with another PFAS in the mixture. In this case, the sum of the two weights reflect the contribution of the correlated components to the overall mixture effect. (Carrico et al. 2015).Our WQS model takes the form $Y_i = \beta_0 + \beta_1 \sum_j w_j d_{ij} + \beta_2 X_i + \varepsilon_i$, where d_{ij} is the decile corresponding to the jth exposure for the ith person, w_j is a weight for the jth exposure estimated by 500 bootstraps, and β_1 represents the overall effect of the mixture. We constrained weights w_j to sum to 1.0 so that all mixture components could be incorporated into a single effect estimate, we constrained β to be negative based on findings in individual PFAS models, and we scaled β_1 by interquartile range (IQR) of the WQS index to improve interpretability.

We assessed the robustness of our findings through multiple sensitivity analyses of the single-PFAS linear regression models. To control for prenatal PFAS exposure, we adjusted final models for $\log_2$-transformed maternal PFAS concentration in early pregnancy, measured at a median of 10 wk gestation (except for the model of PFDA, as PFDA plasma concentrations were below the LOD for 44% of maternal samples). We also examined the associations of PFOS and PFOA isomers with >60% detectable concentrations (i.e., n-PFOA, n-PFOS, Sm-PFOS) with aBMD z-score. Additionally, we evaluated the association between plasma PFAS concentrations and BMC z-score. While BMC does not track as strongly as BMD with its z-score at skeletal maturity (Wren et al. 2014), it incorporates bone mass (g) and, compared with BMD, has the advantage of accounting for bone thickness and may be less susceptible to confounding due to bone size (Crabtree et al. 2014). Finally, we assessed for effect modification by child sex via an interaction term and stratification.

For penalized spline generalized additive models and WQS regression, we used R (version 3.3.2; R Development Core Team), and for all other analyses, SAS (version 9.4; SAS Institute Inc.).

Results

Population Characteristics

Children were [mean ± standard deviation (SD)] 7.9 ± 0.8 years of age at the midchildhood visit, and (mean ± SD) aBMD z-score was −0.86 ± 0.77. Forty-nine percent of children were female, 57% were white, and mothers of 64% were college graduates at the time of cohort enrollment. Children with the highest PFOA concentrations were more likely to be younger, white, live in a census tract with higher median household income, and to have

Table 1. Participant characteristics overall ($n = 576$) and by quartiles of total perfluorooctanoic acid (PFOA) plasma concentration in midchildhood.

		Quartiles of PFOA[b]			
	Overall[a]	Q1	Q2	Q3	Q4
	$n = 576$	$n = 145$	$n = 147$	$n = 140$	$n = 144$
		Mean ± SD or n (%)			
Maternal/neighborhood characteristics					
Maternal age at enrollment (y)	31.8 ± 5.7	29.8 ± 6.5	31.6 ± 5.9	32.7 ± 5.0	33.1 ± 4.5
College graduate (%)	364 (64)	59 (41)	89 (61)	101 (73)	115 (80)
Individual household income (%)					
<$40,000	85 (16)	39 (30)	20 (14)	15 (11)	11 (8)
$40,001–70,000	89 (16)	25 (19)	23 (17)	22 (16)	19 (13)
>$70,000	369 (68)	65 (51)	96 (69)	98 (73)	110 (79)
Census tract median household income (%)[c]					
<$30,000	35 (6)	15 (11)	12 (8)	4 (3)	4 (3)
$30,000–59,999	248 (44)	84 (59)	61 (42)	57 (41)	46 (32)
$60,000–99,999	244 (43)	39 (27)	64 (44)	71 (51)	70 (49)
≥$100,000	43 (7)	4 (3)	8 (6)	8 (6)	23 (16)
Child characteristics					
Age (y)	7.9 ± 0.8	8.2 ± 1.0	8.0 ± 0.8	7.8 ± 0.7	7.7 ± 0.6
Female (%)	280 (49)	73 (50)	73 (50)	101 (45)	71 (49)
Race/ethnicity (%)					
White	328 (57)	37 (26)	80 (54)	94 (68)	117 (81)
Black	129 (23)	66 (46)	32 (22)	21 (15)	10 (7)
Asian	14 (2)	6 (4)	2 (1)	2 (1)	4 (3)
Hispanic	34 (6)	13 (9)	13 (9)	6 (4)	2 (1)
Other	69 (12)	22 (15)	20 (14)	16 (12)	11 (8)
Dairy intake (servings per week)	2.2 ± 1.5	2.0 ± 1.5	2.2 ± 1.5	2.3 ± 1.6	2.4 ± 1.5
Physical activity (hours per week)	1.9 ± 1.4	1.9 ± 1.5	1.7 ± 1.3	1.9 ± 1.2	1.9 ± 1.5
Year of blood draw (%)[c]					
2007	64 (11)	6 (4)	17 (12)	16 (11)	25 (17)
2008	203 (35)	21 (14)	39 (27)	62 (44)	81 (56)
2009	189 (33)	56 (39)	58 (39)	45 (32)	30 (21)
2010	120 (21)	62 (43)	33 (22)	17 (12)	8 (6)
aBMD z-score	−0.86 ± 0.77	−0.73 ± 0.73	−0.81 ± 0.84	−0.95 ± 0.73	−0.93 ± 0.78
BMC z-score	−0.36 ± 0.74	−0.24 ± 0.70	−0.37 ± 0.84	−0.42 ± 0.69	−0.44 ± 0.70

Note: aBMD, areal bone mineral density; BMC, bone mineral content; Q1, first/lowest quartile; SD, standard deviation.
[a]Missing data for participants overall ($n = 576$): 4 missing maternal education, 6 census tract median household income, 33 individual household income, 2 race/ethnicity, 22 dairy intake, and 22 physical activity.
[b]PFOA quartile minimum and maximum values: limit of detection (LOD) < 0.1–3.0 ng/mL for Q1, 3.1–4.4 ng/mL for Q2, 4.5–6.1 ng/mL for Q3, and 6.2–14.3 ng/mL for Q4.
[c]Percentages do not add up to 100% due to rounding.

greater dairy intake and earlier blood collection. Mothers of children with the highest PFOA concentrations were more likely to be older and college graduates (Table 1).

Most PFASs considered in our study (except PFDA and MeFOSAA) were detectable in the plasma of >99% of children. Plasma PFAS concentrations in our study were similar to those reported in U.S. children in the National Health and Nutrition Examination Survey (NHANES) during the same period, from 2007 to 2008 (CDC 2018). The highest PFAS plasma concentrations in Project Viva were of PFOA [median (IQR) 4.4 (3.2) ng/mL] and PFOS [median (IQR) 6.4 (5.6) ng/mL]. PFAS plasma concentrations were moderately correlated [Spearman's rank order correlation coefficient (r_s) = 0.13–0.79], with the strongest correlations between PFOS and PFOA (r_s = 0.79) (see Table S3).

Single-Per- and Polyfluoroalkyl Substance Models

Children with higher plasma PFAS concentrations had lower aBMD *z*-scores in covariate-adjusted models (Figure 1), with strongest associations for PFOA (β: −0.16; 95% CI: −0.25, −0.06 per doubling of PFOA), PFOS (β −0.08; 95% CI −0.16, −0.01 per doubling of PFOS), and PFDA (β −0.09; 95% CI −0.16, −0.02 per doubling of PFDA). Each doubling of MeFOSAA was associated with 0.04 unit lower aBMD *z*-score [95% confidence interval (CI): −0.08, 0.01], but CIs included the null. PFHxS and PFNA were not associated with aBMD *z*-score.

Multi-Per- and Polyfluoroalkyl Substance Model

In the covariate-adjusted multi-PFAS model, each doubling of PFOA was associated a 0.24-unit-lower aBMD *z*-score (95% CI: −0.42, −0.06), whereas each doubling of PFNA was associated with a 0.12-unit-higher aBMD *z*-score (95% CI: 0.03, 0.21) (Figure 2). Other PFASs were not associated with aBMD *z*-score.

Weighted Quantile Sum Regression Model

The WQS index was negatively associated with aBMD *z*-score. Each IQR increment in the WQS index was associated with a 0.16 lower aBMD *z*-score (95% CI: −0.28, −0.04). Within the mixture, PFDA had the highest weight (37%), followed by MeFOSAA (29%). As a result of their strong correlation with each other (r_s = 0.79), PFOA and PFOS had lower individual weights (23% and 8%, respectively). However, in combination, PFOS and PFOA contributed 31% of the total strength of the association between the PFAS mixture and aBMD *z*-score. Weights of PFHxS (3%) and PFNA (0%) were lower.

Sensitivity Analyses

When we adjusted single-PFAS models for maternal plasma concentrations of each PFAS in early pregnancy, associations between midchildhood plasma PFAS concentrations and aBMD *z*-score were slightly stronger. For example, among participants with data on maternal PFOA plasma concentration (n = 423), per doubling of PFOA, aBMD *z*-score was 0.18 units lower (95% CI: −0.28, −0.07) adjusting for maternal PFOA plasma concentration, vs. 0.16 units lower (95% CI: −0.25, −0.06) without adjustment (see Table S4).

The isomers of PFOA and PFOS we considered in this study had modestly stronger covariate-adjusted associations with aBMD *z*-score than their parent compounds. Areal BMD *z*-score was 0.23 units lower (95% CI: −0.37, −0.09) per doubling of n-PFOA vs. 0.16 units lower (95% CI: −0.25, −0.06) per doubling of total PFOA. Similarly, aBMD *z*-score was 0.12 units lower (95% CI: −0.22, −0.01) per doubling of n-PFOS and 0.12 units lower (95% CI: −0.23, −0.01) per doubling of Sm-PFOS vs. 0.08 units lower (95% CI: −0.16, −0.01) per doubling of total PFOS.

The directionality of associations between PFAS plasma concentrations and BMC *z*-score were similar to aBMD *z*-score in single-PFAS models, although CIs crossed the null for all PFAS–aBMC *z*-score associations (see Figure S2). For example, BMC *z*-score was 0.08 units lower (95% CI: −0.17, 0.01), whereas aBMD *z*-score was 0.16 units lower (95% CI: −0.25, −0.16) per doubling of PFOA.

In single-PFAS models, no associations between plasma PFAS concentrations and aBMD *z*-score were modified by child

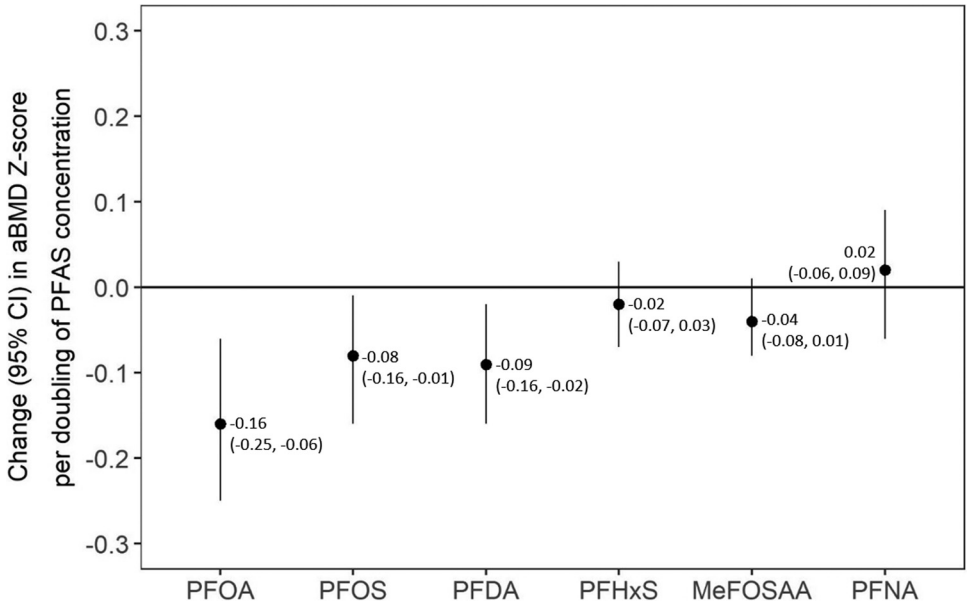

Figure 1. Single-per- and polyfluoroalkyl substance (PFAS) models showing adjusted associations of individual PFAS plasma concentrations with areal bone mineral density (aBMD) *z*-score. Note: Adjusted for maternal age, education, census tract median household income, individual household income, and child age, sex, race/ethnicity, year of blood draw, dairy intake, and physical activity. n = 531 for all single-PFAS models. CI, confidence interval; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFDA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamide) acetate; PFNA, perfluorononanoate.

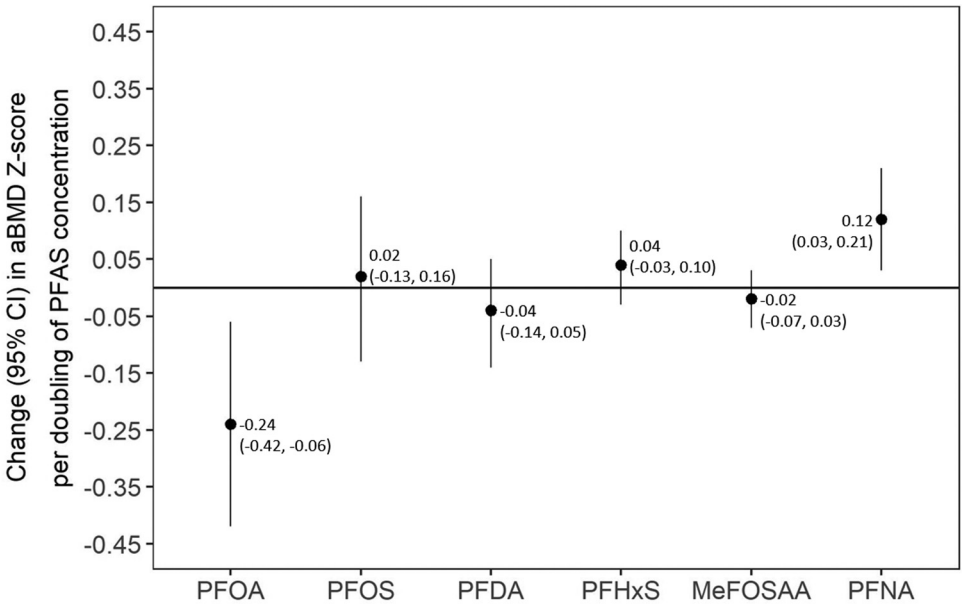

Figure 2. Multi-per- and polyfluoroalkyl substance (PFAS) model showing adjusted associations of PFAS plasma concentrations with areal bone mineral density (aBMD) *z*-score. Note: Adjusted for maternal age, education, census tract median household income, individual household income, and child age, sex, race/ethnicity, year of blood draw, dairy intake, and physical activity. $n = 531$. CI, confidence interval; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFDA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; MeFOSAA - 2-(N-methyl-perfluorooctane sulfonamide) acetate; PFNA, perfluorononanoate.

sex (*p*-interaction terms were all >0.05). However, we observed stronger associations of PFOA and PFDA plasma concentrations with aBMD *z*-score in girls as compared with boys in stratified analyses. In girls, aBMD *z*-score was 0.18 units lower (95% CI: −0.32, −0.04) per doubling of PFOA [vs. 0.09 units lower (95% CI: −0.21, 0.02) in boys, *p*-interaction = 0.27] and 0.13 units lower (95% CI: −0.23, −0.02) per doubling of PFDA [vs. 0.04 lower (95% CI: −0.13, 0.05) in boys, *p*-interaction = 0.28] (see Table S4).

Discussion

Our analysis of data from a large Boston-area cohort is among the first to investigate the role of an environmental exposure on bone mineral density in childhood. We found that children with greater plasma concentrations of select PFASs had lower aBMD *z*-scores during midchildhood.

Our findings align with our *a priori* hypothesis that higher PFAS exposure would be associated with lower BMD in childhood and are consistent with established biological pathways of action. For example, PFASs have been shown to activate PPARγ (Vanden Heuvel et al. 2006), which triggers mesenchymal stem cells to differentiate into adipocytes at the expense of osteoblasts and may thereby reduce BMD (Marciano et al. 2015; Yamamoto et al. 2015). PFASs also act as androgen receptor antagonists (Kjeldsen and Bonefeld-Jorgensen 2013), which may further lower BMD (Clarke and Khosla 2009). In addition, animal models suggest PFOS at environmentally relevant doses rapidly deposits into bone tissue (Bogdanska et al. 2011). In a study of human bone bank and cadaver samples, PFOA and PFOS were sequestered in all femoral head samples and detected in bone marrow, with an association between higher bone PFOS concentration and lower bone volume, although this result was not statistically significant (Koskela et al. 2017). Our findings substantiate existing evidence of biological pathways through which PFAS exposure may lower aBMD *z*-score.

Our research confirms and extends one relatively small study in children and two studies in older adults that found a cross-sectional association between greater PFAS plasma concentrations and lower BMD. In a pilot study of 48 obese children aged 8–12 y, greater plasma concentrations of PFOA, PFOS, PFNA, and PFHxS were associated with poorer bone health (Khalil et al. 2018). However, this study was limited by a small sample size, limited statistical precision, and the use of calcaneal ultrasound bone measurements, which is not a standard bone assessment tool for use in children (Gordon et al. 2014). Similarly, in 2,339 NHANES adults, greater PFOS but not PFOA was associated with lower lumbar spine BMD in premenopausal women but not postmenopausal women or men (Lin et al. 2014). In a different study of 1,914 NHANES adolescents and adults, higher serum PFOS concentrations were associated with lower femoral neck BMD in men, and higher serum PFOS and PFOA concentrations were associated with lower femur BMD in women (Khalil et al. 2016). Thus, our finding of an association between greater PFAS plasma concentrations and lower BMD are in line with the limited existing epidemiologic literature.

To put our findings into perspective, the magnitude of the association between PFAS exposure and aBMD *z*-score in the present study [0.16-unit-lower aBMD *z*-score (95% CI: −0.28, −0.04) per IQR increment in the WQS index] was greater than that of other well-known determinants of aBMD evaluated in the Bone Mineral Density in Childhood Study (Kalkwarf et al. 2007). For example, spine aBMD *z*-score was 0.04 higher (95% CI: 0.02, 0.06) per additional hour per day of high-impact physical activity and −0.04 lower (95% CI: −0.05, −0.02) per 1% increase in a BMD genetic risk score (Mitchell et al. 2016). Future studies of PFAS exposure and fracture incidence will help to elucidate the clinical relevance of these findings.

An advantage of our analysis was our ability to examine associations of several individual PFASs and the entire mixture with aBMD *z*-score. In single-PFAS models, we observed plasma concentrations of PFOA, PFOS, and PFDA to be most strongly associated with lower aBMD *z*-score. When we examined associations of individual PFASs independent of the other PFASs in a multi-PFAS model, only PFOA remained significantly associated with lower aBMD *z*-score. In our mixture model, we found the association

between the PFAS mixture and lower aBMD z-score to be driven by PFDA. The different results may be due to high collinearity between PFOS and PFOA, as the mixture model accounts for collinearity between compounds, while the multi-PFAS model does not. It is important to note that PFDA plasma concentrations had relatively small variability in our cohort, with a range of 0.07–1.9 ng/mL. Few epidemiologic studies have evaluated PFDA, and additional research would increase our understanding of the potential role of PFDA on aBMD and other health outcomes. Overall, our findings are in line with prior studies in our cohort that have shown PFOA, PFOS, and PFDA to have the strongest associations with other health outcomes, including insulin resistance (Fleisch et al. 2017), lipid profile (Mora et al. 2018), and visual–motor abilities (Harris et al. 2018).

Interestingly, in our multi-PFAS model, we found PFNA to be associated with higher (rather than lower) aBMD z-score. A similar inference may be made from the results of our WQS model, in which PFNA received a weight of zero in the composite weighted index, indicative of no (or negligible) negative association (Carrico et al. 2015). This finding is consistent with the fact that PFNA activates PPARα more strongly than the other PFASs (Vanden Heuvel et al. 2006; Wolf et al. 2010), and PPARα activation stimulates bone development and increases aBMD (Stunes et al. 2011). Simultaneous activation of PPARα and PPARγ may reduce the negative bone effects of PPARγ alone (Smith et al. 2012). The opposite direction of effect observed for PFNA in relation to aBMD z-score in our study, when compared with other PFAS, supports existing evidence of variation in mechanisms of action between PFASs and their consequent association with health outcomes.

We observed a stronger association of PFASs with aBMD in females than males, although these differences were not statistically significant. The direction and magnitude of our results are consistent with findings from the two prior adult NHANES analyses in which PFAS serum concentrations were associated with lower aBMD (Khalil et al. 2016; Lin et al. 2014) and higher osteoporosis risk (Khalil et al. 2016) in females relative to males. Rodent studies have also shown differences in the pharmacokinetic characteristics and tissue distribution of PFASs (specifically PFOA, PFOS, and PFHxS), with more rapid absorption in female as compared with male rats (Kim et al. 2016). Our study results align with these observations and strengthen the case for investigating sex-based differences in biological PFAS activity in humans.

As far as we are aware, our study is the largest data set to date to examine the association between childhood PFAS exposures (estimated from PFAS plasma concentrations) and aBMD, and the only pediatric study to evaluate total body (excluding skull) aBMD z-score using DXA, which is widely considered the gold standard for pediatric bone density measurements (Gordon et al. 2014). A particular strength of this study was our evaluation of several PFASs and our ability to consider multiple potential confounding factors, including prenatal PFAS exposure. We were also able to account for determinants of bone mass, such as dairy intake and physical activity, although physical activity is challenging to quantify in children (Rowlands and Eston 2007) and limited by questionnaire measures in this cohort. In addition, we used WQS regression, a novel statistical approach to address highly correlated mixtures of exposures. A limitation of our cross-sectional analysis is that we were not temporally positioned to assess for mediation by pubertal status or BMI, which have both been associated with PFAS plasma concentration (Lopez-Espinosa et al. 2011; Domazet et al. 2016) and aBMD (Rokoff et al. 2019; Cousminer et al. 2018). In addition, our sample size was not considered robust to splitting into training and validation data sets in the WQS analysis, and therefore, weights were estimated in the same data used to test for significance. Our results may have been influenced by multiple testing for associations between several PFAS and BMD, although consistent patterns in our results reduce this concern.

In summary, we are among the first to evaluate environmental exposures in relation to bone health in childhood. We observed higher exposure to PFASs to be associated with lower aBMD z-scores in children. While replication of our findings is necessary, lower exposures to environmental toxicants such as PFASs during childhood may improve childhood bone accrual and thereby optimize peak bone mass and lifelong skeletal health.

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