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Supplemental Material

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

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Table S1. Characteristics of Project Viva participants who attended the midchildhood visit (n=1,116) and who were included (n=576) versus excluded (n=540) from this analysis.

	Attended the midchildhood visit (n=1,116)		Subset included in analysis (n=576)		Subset excluded from analysis (n=540)	
	N	Mean ± SD or n (%)	N	Mean ± SD or n (%)	N	Mean ± SD or n (%)
Maternal/neighborhood characteristics						
Maternal age at enrollment (years)	1116	32.1 ± 5.4	576	31.8 ± 5.7	540	32.4 ± 5.0
College graduate (%)	1110	754 (68)	572	364 (64)	538	390 (72)
Census tract median household income (%)	1109		570		539	
< US$ 30,000		55 (5)		35 (6)		20 (4)
US$ 30,000-59,999		467 (42)		248 (43)		219 (41)
US$ 60,000-99,999		487 (44)		244 (42)		243 (45)
≥ US$ 100,000		100 (9)		43 (8)		57 (11)
Individual household income (%)	1043		543		500	
< US$ 40,000		129 (12)		85 (16)		44 (9)
US$ 40,001-70,000		142 (14)		89 (16)		53 (11)
> US$ 70,000		772 (74)		369 (68)		403 (81)
Child characteristics						
Age (years)	876	7.9 ± 0.8	576	7.9 ± 0.8	300	7.9.0 ± 0.8
Female (%)	1116	557 (50)	576	280 (49)	540	277 (51)
Race/ethnicity (%)	1114		574		540	
White		707 (63)		328 (57)		379 (70)
Black		189 (17)		129 (22)		60 (11)
Asian		38 (3)		14 (2)		24 (4)
Hispanic		48 (4)		34 (6)		14 (3)
Other		132 (12)		69 (12)		63 (12)
Dairy intake (servings per week)	1059	2.3 ± 1.5	554	2.2 ± 1.5	505	2.4 ± 1.5
Physical activity (hours per week)	1063	1.8 ± 1.3	554	1.9 ± 1.4	509	1.7 ± 1.2
Year of blood draw (%)	760		576		184	
2007		77 (10)		64 (11)		13 (7)
2008		255 (34)		203 (35)		52 (28)
2009		252 (33)		189 (33)		63 (34)
2010		176 (24)		120 (21)		56 (30)
aBMD Z-score	876	-0.85 ± 0.78	576	-0.86 ± 0.77	300	-0.84 ± 0.79

Abbreviations: aBMD – areal bone mineral density; SD – standard deviation

Table S2: Variables that did not confound the PFAS-aBMD association, overall (n=576) and by quartiles of perfluorooctanoate (PFOA) plasma concentration in midchildhood

	Overall [b] n=576	Quartiles of PFOA [a]			
		Q1 n=145	Q2 n=147	Q3 n=140	Q4 n=144
	Mean ± SD or n (%)	Mean ± SD or n (%)			
Breastfeeding duration (months)	6.3 ± 4.5	4.6 ± 4.1	6.3 ± 4.6	6.8 ± 4.4	7.4 ± 4.5
Soda intake (servings per week)	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.3
Secondhand smoke exposure					
< 1 hour	530 (95)	126 (94)	137 (96)	130 (96)	137 (97)
≥ 1 hour	25 (5)	8 (6)	6 (4)	6 (4)	5 (3)
25(OH)D plasma concentration (ng/mL)	26.8 ± 7.7	24.2 ± 7.1	26.4 ± 7.5	27.6 ± 8.0	29.2 ± 7.4

Abbreviations: Q1 – first/lowest quartile; SD – standard deviation; 25(OH)D – 25-hydroxyvitamin D
[a] PFOA quartile minimum and maximum values: <0.1 (LOD)-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
[b] Missing data for participants overall (n = 576): 30 missing 25(OH)D plasma concentration, 49 breastfeeding duration, 25 soda intake, 21 secondhand smoke exposure

Table S3. Per- and polyfluoroalkyl substance (PFAS) plasma concentration distributions and Spearman correlation coefficients.

	PFAS plasma concentrations (ng/mL)					
	PFOA	**PFOS**	**PFDA**	**PFHxS**	**MeFOSAA**	**PFNA**
Median (IQR)	4.4 (3.2)	6.4 (5.6)	0.3 (0.3)	1.9 (2.3)	0.3 (0.5)	1.5 (1.2)
5th percentile	1.9	2.1	< LOD [a]	0.6	< LOD [a]	0.7
95th percentile	9.8	18.7	0.7	14.7	1.9	5.1
Detection frequency (%) [b]	99.5 [c]	99.5 [c]	88.4	99.5	65.6	99.5
	Spearman correlation coefficients					
PFOA	1.00					
PFOS	0.79	1.00				
PFDA	0.69	0.59	1.00			
PFHxS	0.60	0.67	0.34	1.00		
MeFOSAA	0.50	0.63	0.32	0.37	1.00	
PFNA	0.43	0.35	0.57	0.13	0.22	1.00

Abbreviations: PFAS – Per- and polyfluoroalkyl substances; PFOA – Perfluorooctanoic acid; PFOS – Perfluorooctane sulfonic acid; PFDA – Perfluorodecanoate; PFHxS – Perfluorohexane sulfonate; MeFOSAA – 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFNA – Perfluorononanoate

[a] The limit of detection (LOD) was 0.1 ng/mL for all PFASs

[b] Within this analytic dataset (n=576).

[c] Detection frequencies for linear and branched isomer components from which total PFOA and PFOS concentrations were obtained: n-perfluorooctanoate (n-PFOA): 99.5%; sum of perfluoromethylheptanoates and perfluorodimethylhexanoates (Sb-PFOA): 58.5%; n-perfluorooctane sulfonic acid (n-PFOS): 99.5%; sum of perfluoromethylheptane sulfonates (Sm-PFOS): 99.5%; sum of perfluorodimethylhexane sulfonates (Sm2-PFOS): 1.2%

Table S4. Associations of per- and polyfluoroalkyl substance (PFAS) plasma concentrations with areal bone mineral density (aBMD) Z-score in midchildhood in (1) single PFAS models, (2) with further adjustment for maternal plasma PFAS concentration, and (3) by sex. β (95% CIs) represent difference in aBMD Z-score per doubling of PFAS plasma concentration.

PFAS	Covariate-adjusted single PFAS models $n = 531$	Additionally adjusted for maternal PFAS $n = 423$	Stratified by sex		Interaction term p-value
			Girls $n = 280$	Boys $n = 296$	
	Effect estimate (95% confidence interval)				
PFOA	-0.16 (-0.25, -0.06)	-0.18 (-0.28, -0.07)	-0.24 (-0.4, -0.07)	-0.11 (-0.23, 0.00)	0.27
PFOS	-0.08 (-0.16, -0.01)	-0.10 (-0.18, -0.02)	-0.12 (-0.25, 0.00)	-0.06 (-0.16, 0.03)	0.52
PFDA	-0.09 (-0.16, -0.02)	N/A[a]	-0.14 (-0.25, -0.03)	-0.04 (-0.14, 0.06)	0.28
PFHxS	-0.02 (-0.07, 0.03)	-0.03 (-0.09, 0.04)	-0.01 (-0.1, 0.08)	-0.04 (-0.11, 0.03)	0.68
MeFOSAA	-0.04 (-0.08, 0.01)	-0.04 (-0.09, 0.01)	-0.06 (-0.12, 0.01)	-0.01 (-0.07, 0.05)	0.40
PFNA	0.02 (-0.06, 0.09)	0.01 (-0.08, 0.09)	0.01 (-0.11, 0.13)	0.02 (-0.08, 0.11)	0.83

Abbreviations: PFAS – Per- and polyfluoroalkyl substances; PFOA – Perfluorooctanoic acid; PFOS – Perfluorooctane sulfonic acid; PFDA – Perfluorodecanoate; PFHxS – Perfluorohexane sulfonate; MeFOSAA – 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFNA – Perfluorononanoate

[a] We were unable to adjust for maternal plasma concentrations of PFDA because maternal concentrations were above the LOD for < 60% of maternal samples.

All models adjusted for maternal age, education, individual household income, census tract median household income, and child age, sex, race/ethnicity, physical activity, dairy intake, and year of blood draw (sex-specific models do not include child sex as a covariate)

Figure S1: Comparison of effect estimates from single per- and polyfluoroalkyl substance (PFAS) models examining associations between PFAS plasma concentrations and aBMD Z-score in midchildhood, with and without additional adjustment for variables that were considered but not included in the primary analysis [a]
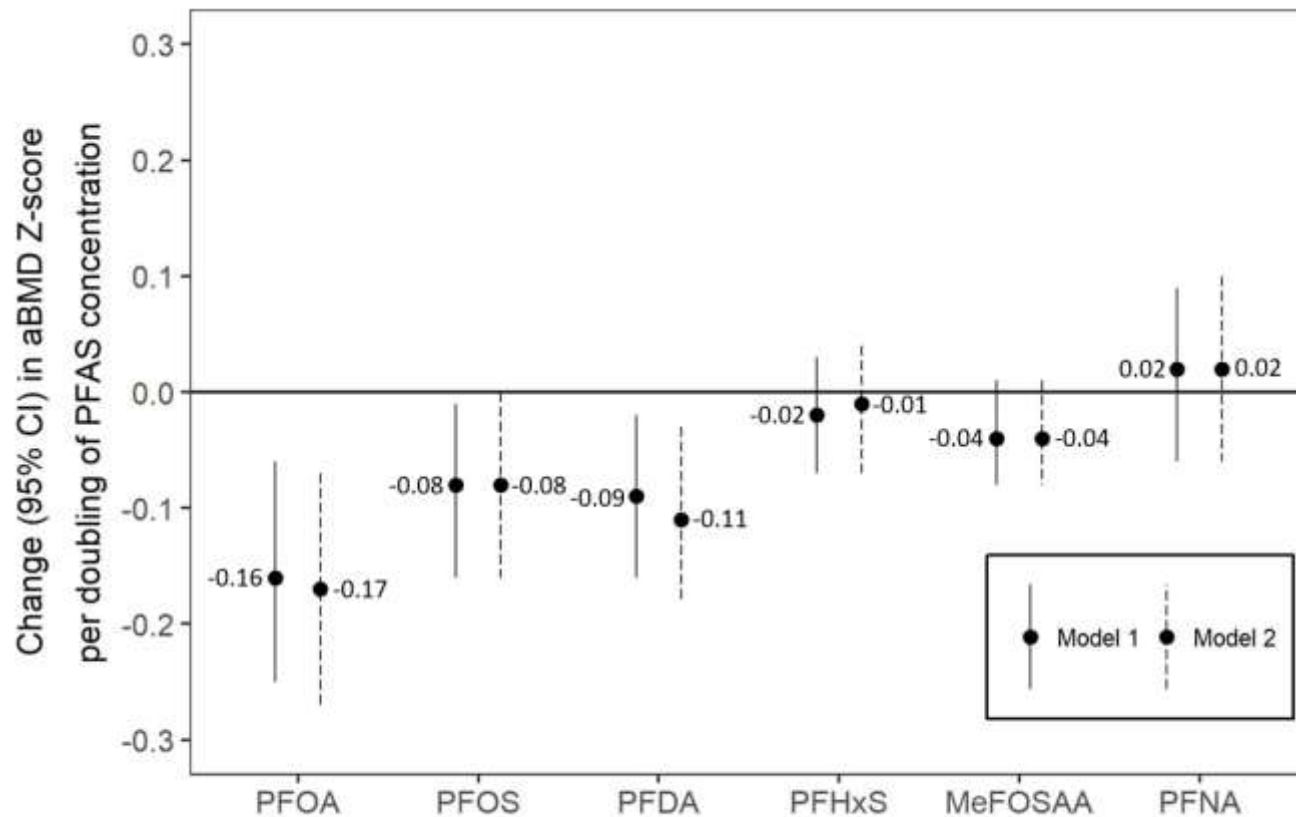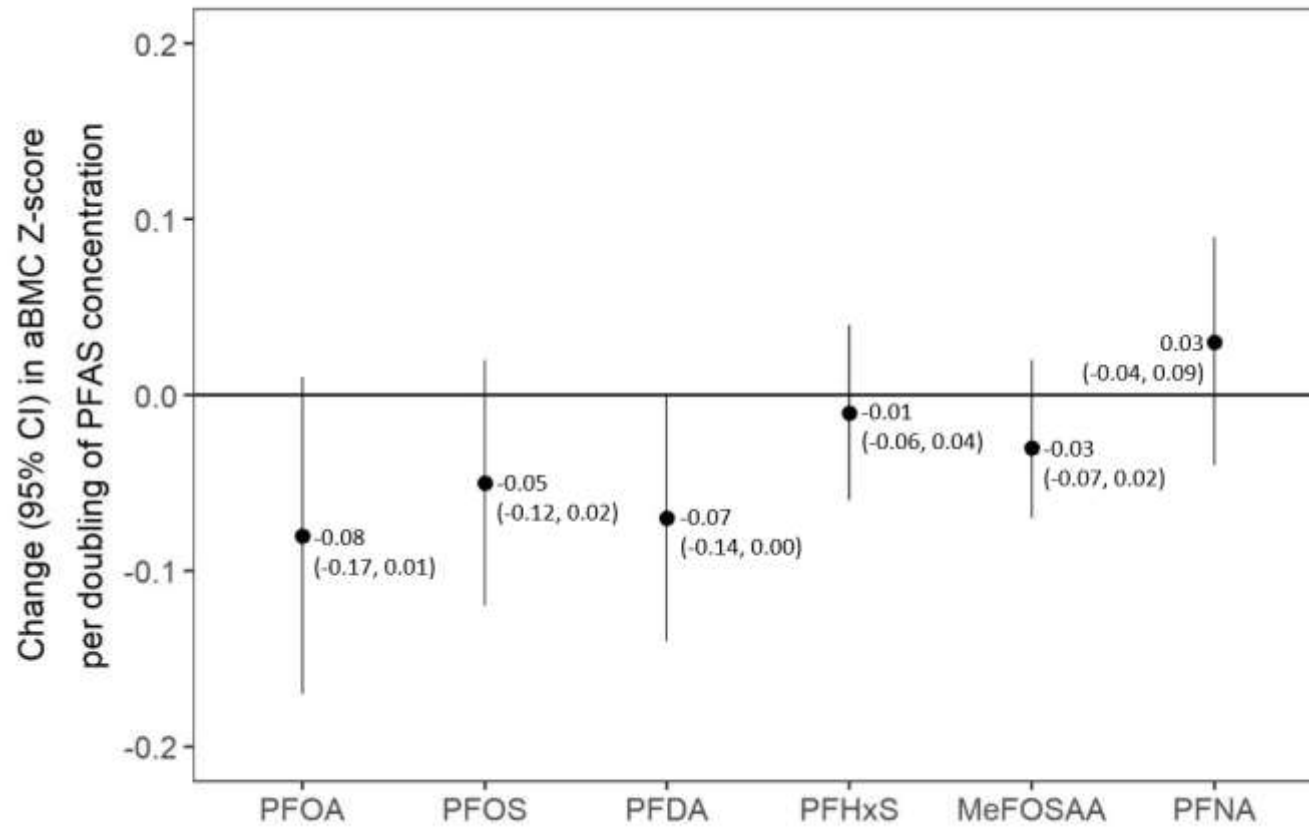

Abbreviations: PFOA – Perfluorooctanoic acid; PFOS – Perfluorooctane sulfonic acid; PFDA – Perfluorodecanoate; PFHxS – Perfluorohexane sulfonate; MeFOSAA – 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFNA – Perfluorononanoate
[a] Model 1: Adjusted for maternal age, education, individual household income, census tract median household income, and child age, sex, race/ethnicity, physical activity, dairy intake, and year of blood draw (n = 531)
 Model 2: Additionally adjusted for breastfeeding duration, soda intake, secondhand smoke exposure, and 25-hydroxyvitamin D plasma concentration (n = 463)

Figure S2. Single PFAS models showing adjusted associations of per- and polyfluoroalkyl substance (PFAS) plasma concentrations with bone mineral content (BMC) Z-score in midchildhood (n=531).[a]

Abbreviations: PFOA – Perfluorooctanoic acid; PFOS – Perfluorooctane sulfonic acid; PFDA – Perfluorodecanoate; PFHxS – Perfluorohexane sulfonate; MeFOSAA – 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFNA – Perfluorononanoate
[a]Adjusted for maternal age, education, individual household income, census tract median household income, and child age, sex, race/ethnicity, dairy intake, physical activity, and year of blood draw.