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Zuha Jeddy Date

Association between Prenatal Exposure to Polyfluoroalykl Compounds and Bone Health in British Girls

By

Zuha Jeddy
Master of Public Health

Epidemiology

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Terryl J. Hartman PhD, MPH, RD
Committee Chair

Association between Prenatal Exposure to Polyfluoroalykl Compounds and Bone Health in British Girls

By

Zuha Jeddy

Bachelor of Arts, Psychology
Southern Methodist University
2013

Thesis Committee Chair: Terryl J. Hartman PhD, MPH, RD

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Abstract

Association between Prenatal Exposure to Polyfluoroalykl Compounds and Bone Health in British Girls

By Zuha Jeddy

BACKGROUND
Endocrine disrupting chemicals (EDCs), such as polyfluoroalkyl compounds (PFCs), are exposures that disrupt signaling pathways during fetal development through alteration of hormonal functions (1). Previous research suggests a possible inverse relationship between various EDC exposures and bone health (2-5).

OBJECTIVES
We explored associations between prenatal serum concentrations of common PFCs, such as perfuorootane sulfonate (PFOS), perfluorooctane (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA), with bone health. We used total body and total body less head bone mineral density (BMD), bone mineral content (BMC), bone area (BA), and area adjusted BMC (ABMC) to measure bone health.

METHODS
We studied a sample of 357 mother-daughter dyads participating in the Avon Longitudinal Study of Parents and Children (ALSPAC). Maternal serum samples were obtained in 1991-1992 during pregnancy. Data on bone outcomes were obtained using whole body dual-energy x-ray absorptiometry (DXA) scans during clinic visits at age 9. We explored associations between prenatal PFC concentrations and bone outcomes at age 9 based on multivariate adjusted models.

RESULTS
PFOS (mean: 21.64 ng/mL), PFOA (mean: 2.05 ng/mL), PFHxS (mean: 1.34 ng/mL), and PFNA (mean: 0.68 ng/mL) were detected in 100% of samples. After controlling for confounders, a one ng/mL increase in PFOS, decreased total body BMC by1.944g (SE=0.975, p=0.05) and total body BA decreased by 1.799 cm^2 (SE=0.833, p=0.03). One ng/mL increase in PFOS was also associated with a 1.811 cm^2 (SE=0.817, p=0.03) decrease in total body less head BMC after controlling for the same confounders. PFOA was significantly inversely associated with total body less head BA (β=-8.577, SE=4.319, p=0.05).

CONCLUSIONS
We found significant associations between maternal exposure to PFCs and daughter's bone health outcomes at age 9, suggesting that intrauterine PFC exposures have a prolonged negative effect on the bone health of offspring.

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I. Introduction

Endocrine disrupting chemicals (EDCs) are exposures that disrupt signaling pathways during fetal development through alteration of hormonal functions (1). The U.S. Environmental Protection Agency defines an EDC as "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process" (6). Exposures to EDCs are common in everyday life through many household consumer products.

Polyfluoroalkyl compounds (PFCs), such as perfuorootane sulfonate (PFOS), perfluorooctane (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoic acid (PFNA), are common man-made EDCs. PFCs are made to produce fluoropolymers, which are used to create many household products such as food packaging, nonstick cookware, and textiles (7). Fluoropolymers are high-performance chemicals, making them commonly used for production of industrial products, including computer systems, airplanes, and automobiles (7).

PFCs are ubiquitous chemicals that can be transferred through the penetrable placenta during pregnancy (8). Studies suggest that the fetus is particularly vulnerable to chemicals like EDCs that alter hormonal pathways during critical periods of development (8). PFCs can be found in serum from circulating blood, milk of breastfeeding women and serum from cord blood (9, 10). Epidemiologic studies that have examined the relationship between maternal exposure to PFCs and offspring's growth and development have found higher maternal levels are associated with lower birth weights, but higher weights at 20 months, and increased risk of obesity through young adulthood (11-14).

Several epidemiologic studies, primarily in adults, have suggested a possible inverse relationship between various EDC exposures and bone health (2-5). With an estimated 9 million fractures due to osteoporosis annually, understanding factors that effect bone health have become increasingly important (15). Bone mineral density (BMD), bone mineral content (BMC) and bone area (BA) measured by dual-energy x-ray absorptiometry (DXA), are used to measure bone health to determine risk of osteoporosis and risk of developing fractures. Based on previous studies, reduced BMD and BMC are associated with osteoporotic fractures, while reduced bone size can contribute to increased bone fragility (16, 17).

Based on a previous epidemiologic study, there is evidence to suggest a possible association between exposures to PFCs and bone health. A population based study using data from the US National Health and Nutrition Examination Survey (NHANES) found a decrease in total lumbar spine BMD with increasing PFOS exposure in premenopausal women (2). However, the study did not find a statistically significant association between PFOS and total lumbar spine BMD in men or women in menopause or a statistically significant association between PFOA and total lumbar spine BMD in men or women. Although findings from this study indicate that exposure to EDCs may affect bone health, the findings were cross-sectional. In addition, no studies to date have examined the association between intrauterine exposure to PFCs and bone health in children. In this study, therefore, we investigate whether maternal exposure to PFCs during pregnancy is associated with bone health at age 9 in British girls using data from the Avon Longitudinal Study of Parents and Children (ALSPAC).

II. Methods

Population

Analyses for this study use data collected from the Avon Longitudinal Study of Parents and Children (ALSPAC). ALSPAC is a longitudinal birth cohort conducted in Avon, Great Britain that enrolled 14,541 pregnant women with expected delivery dates between April 1991 and December 1992. During pregnancy mothers were asked to participate in clinical assessments, provide blood samples, and complete demographic and health related questionnaires. Offspring have been followed from birth through young adulthood through questionnaires completed by parents/guardians and children completed a series of clinical assessments (18).The present study include mother-daughter dyads originally identified to participate in an ancillary study to assess the role of environmental exposures (including PFCs) in adolescent development (n=358). The study was designed as a nested-case control study including 167 girls that attained menarche before 11.5 years and 191 girls who attained menarche at ≥ 11.5 years of age.

Data collection

All of the daughters that are included in our study (n=347) completed a clinic visit at age 9 (mean age at visit of 9.8 years), which included a whole body DXA scan. GE Lunar Prodigy with pediatric scanning software was used to measure total body BMC, BMD, and BA. Scans with anomalies, such as movement or artifacts, were excluded. Weight was measured using Tanita Body Fat Analyzer; sitting and standing height was measured using Harpenden Stadiometer at the time of the scan. Variables that were derived for a previous study were used to adjust for skeletal size to calculate total body less head outcomes and adjust BMC for bone area (19).

Data for maternal and offspring covariates were collected through medical records and questionnaires. Low birth weight was characterized as less than 2,500g at delivery and preterm delivery was characterized as birth before 37 weeks gestation. Information on maternal prepregnancy body mass index (BMI kg/m^2), education, race, age at delivery, and smoking status was collected at the time maternal serum sample was acquired. Prepegnancy BMI was characterized (based on classification from the Centers for Disease Control and Prevention) as underweight (< 18.5), normal (18.5-24.9), overweight (25.0-29.9), obese (≥30.0).

Laboratory Analyses

Intrauterine exposure to PFOS, PFOA, PFHxS, and PFNA were measured from maternal serum samples collected during pregnancy (median gestational age of 15 weeks). Blood samples were transferred under controlled conditions to the National Center for Environmental Health of the Centers of Disease Control and Prevention for laboratory analyses of PFCs. A previous study has described analytical methods used to measure analytes in the serum samples (20). Limits of detection ranged between 0.1-0.2ng/ml. Quality control measures were done to ensure calibration using standards, reagent blanks, and study samples. Precision of measurements for the analytes, as relative standard deviation, ranged from 8-13%.

Statistical Analysis

The sample used for our analysis was weighted to account for under-representation of the true number of girls without early menarche (weight for cases was 1 and for controls 15.1) due to the nested-case control study design. The markers of bone health studied were total body and total body less head BMD, BMC, BA, and BMC

adjusted for area. To investigate the association between each marker of bone health with maternal exposure to the PFCs, we developed stratum-weighted linear regression models with the bone measures as continuous outcomes. We examined data for potential outliers for all variables of interest and examined PFC concentrations across levels of covariates. We first examined crude associations between PFC analytes and bone outcomes using univariate regression analysis. We then conducted backwards elimination with potential covariates to identify relevant confounders, with $p<0.10$ as the cutoff for retention. The following covariates were considered for the model: maternal prepregnancy BMI (continuous), maternal education (categorical; <O level, O level, and >O level), maternal race (categorical; white and nonwhite), maternal age at delivery (continuous), maternal smoking status (categorical; smoked before pregnancy and did not smoke), birth weight (continuous), preterm birth (categorical; preterm and term), ever breastfed status (categorical; ever breastfed and never breastfed), age at DXA scan (continuous), daughter's BMI (continuous). Model 1 adjusts for variables that were found to be significant confounders, including: maternal education, sample gestation, and age at DXA scan. We then assessed possible causal effects in addition to the confounders. Model 2 adjusted for the confounders with the addition of significant variables that may be on the causal pathway, including preterm birth, birth weight, and daughter's BMI. We tested whether associations between PFC analytes and bone outcomes were different from the null using a $p<0.05$. Maternal education was considered as a potential effect modifier, however, we found to be not statistically significant.

SAS version 9.2 (SAS Instutite Inc.,Cary,NC) was used to conduct all analyses. ALSPAC Law and Ethics Committee, the local research ethics committees, and the CDC

Institutional Review board assessed and approved human subject protection. Mothers provided informed consent at the time of enrollment; however daughters did not provide consent because they were children.

III. Results

Exposure analytes were detected in all of the maternal samples with PFOS present at the highest concentration, followed by PFOA, PFHxS and PFNA (Table 1). Mean (standard deviation) concentration in maternal samples of PFOS was 21.64 (10.55) ng/mL; PFOA was 4.05 (1.83) ng/mL; PFHxS was 1.34 (1.84) ng/mL; and PFNA was 0.68 (0.33) ng/mL (Table 1). Spearman correlation coefficients showed a high level of correlation between PFOS and PFOA (r =0.69). Moderate correlations were found between PFOS and PFHxS (r =0.36), PFOS and PFNA (r =0.39), PFOA and PFHxS (r =0.32), and PFOA and PFNA (r =0.37). PFHxS and PFNA were only modestly correlated (r =0.15). Table 2 shows mean and standard deviation for the bone outcomes measured in girls.

Crude and adjusted associations between the PFC analytes and the total body DXA outcomes are shown in Table 3. Crude analysis showed a significant relationship between PFOS and BMC (β=-2.045, SE=0.889, p=0.02); PFOS and BA (β=-1.952, SE=0.753, p=0.01); and PFOA and BA (β=-8.785, SE=4.057, p=0.03). After controlling for significant potential confounders (maternal education, sample gestation, and age at DXA scan), the relationship between PFOS with BMC and BA remained statistically significant. For every one ng/mL increase in PFOS, BMC decreased by 1.944g (SE=0.975, p=0.05) and BA decreased by 1.799 cm^2 (SE=0.833, p=0.03). The observed relationships were attenuated and no longer remained significant after additional

adjustments for variables potentially on the causal path (preterm birth status, birth weight, and BMI at age 9).

Crude and adjusted associations between the PFC analytes and the total body less head DXA outcomes are shown in Table 4. Crude analysis showed a significant relationship between PFOS and BMC (β=-1.844, SE=0.825, p=0.03); PFOS and BA (β=-1.981, SE=0.745, p=0.01); and PFOA and BA (β=-9.206, SE=4.013, p=0.02). After controlling for significant potential confounders (maternal education, sample gestation, and age at DXA scan), the relationship between PFOS with BMC and BA and the relationship between PFOA and BA remained significant. In contract, the null relationship between PFNA and ABMC observed in the crude models became significant in the multivariable adjusted models. For every ng/mL increase in PFOS, BMC decreased 1.781g (SE=0.894, p=0.05) and BA decreased by 1.811 cm^2 (SE=0.817, p=0.03) after controlling for maternal education, sample gestation, and age at DXA scan. For every ng/mL increase in PFOA, BA decreased by 8.577 cm^2 (SE=4.319, p=0.05) after controlling for the same confounders. Finally, we found a marginal positive association between PFNA exposure and area adjusted BMC in Model 1 (β=10.945, SE=5.510, p=0.05). After additional adjustments for variables potentially on the causal pathway (preterm birth status, birth weight, and BMI at age 9), the positive association between PFNA and area adjusted BMC became statistically significant (β=11.245, SE=5.471, p=0.04).

IV. Discussion

Using data from the ALSPAC study, we found significant associations between maternal exposure to PFCs during pregnancy and daughter's bone health outcomes at age

9. We observed an inverse association between PFOS exposure with total body and total body less head BMC and BA. We also found an inverse association between PFOA and total body BA and an inverse association between PFOA and total body less head BA. Our findings suggest that intrauterine PFC exposure has a negative effect on the bone health of daughters. However, the potential biological significance of this effect appears to be modest. Although our findings may be subclinical at the population level, they may be important on an individual level. Additional research is needed to examine whether negative effects on bone health from intrauterine PFC exposure seen in our study exacerbate, or diminish, over time.

PFC exposure levels in our study population were similar to those found using NHANES data from 2003-2004 (n=2,094). PFOA, PFOS, and PFNA were slightly higher in our study population than compared to the females of childbearing ages in the NHANES population. Table 1 lists mean concentrations of maternal PFC exposure that were found in the serum of our study participants. Mean concentration in the females from the NHANES study was 18.5 μg/L (95% CI: 17.1-20.0 μg/L) for PFOS and 0.9 μg/L (95% CI: 0.7-1.0 μg/L) for PFNA. Mean concentration in the female's aged 26-41 was 3.5 μg/L (95% CI: 3.3-3.8 μg/L) for PFOA and 1.7 μg/L (95%CI: 1.5-1.9 μg/L) for PFHxS (21). Bone health outcomes, including total body and total body less head BMD, BMC, and BA, were also similar to NHANES data from 1999-2006 (n=22,667). Mean values of bone outcomes were measured in 1,465 in females aged 8-11. Total body and total body less head BMD and BMC were slightly higher in our study population; however, total body and total body less head BA were slightly lower when compared to the NHANES data (22). Based on data from the NHANES study, mean total body BMD

was 0.83 g/cm^2 (SD: 0.09 g/cm^2), BMC was 1,179.36 g (SD: 270.66 g), and BA was 1,399.15 cm^2 (SD: 188.77 cm^2). Mean total body less head BMD was 0.73 g/cm^2 (SD: 0.09 g/cm^2), BMC was 885.53 g (SD: 245.33 g), and BA was 1.189.20 cm^2 (SD: 184.41 cm^2) (22).

Biologic evidence suggests that PFC exposure during critical developmental windows in utero can affect growth and development of a fetus and subsequent risk of osteoporosis later in life. A critical period for bone development occurs during the second trimester of gestation, which is characterized by a period of rapid cell division (23). Biologic plausibility involves EDC exposure disrupting metabolic processes involved in the activation of estrogen receptors, which play a role in development of bone (24). Altered development of bone may explain the inverse association found in our study between maternal serum concentrations PFOS and PFOA with bone outcomes in childhood.

To our knowledge, there have not been any studies in humans examining the association between intrauterine exposure to PFCs and bone health. Current research exists using cross-sectional NHANES data on the association between serum levels of PFC exposure, including PFOA and PFOS, and lumbar spine and total hip BMD in premenopausal women (n=2,339) (2). This study found an association between serum PFOS levels and total lumbar spine BMD (β=-0.022 g/cm^2 ; 95% confidence interval (CI): -0.038, -0.007; p=0.006). This study did not find any other significant associations between serum PFC levels and bone outcomes. Although our study investigates maternal exposure and offspring outcomes, findings from the NHANES study are consistent with our findings suggesting a link between PFC exposure, specifically PFOS, and bone

health. Although no research exists on intrauterine PFC exposure and bone health, findings from previous animal studies suggest that in vivo exposure to other various EDCs affects fetal bone tissue development, including organic tin compounds and alkylphenols (25, 26). In utero exposure to organic tin compounds in rats were found to delay ossification of fetal skeleton (26). Alkylphenols exposure in pregnant mice were associated with inhibited bone formation and caused a decrease in width of bone growth (25).

There are several strengths to the study we conducted. The ALSPAC dataset used involves a comprehensive list of covariate data for both the mothers and the offspring who participated in the study. Data was collected on a wide range of characteristics including education, physical activity, and dietary intake. Data was available for all possible covariates being adjusted for in the models. Additionally, maternal blood samples were collected at around 15 weeks of gestation, thus we were able to measure exposure during the critical window for bone development.

There are also some potential considerations regarding our research. We conducted analysis for this study using a previously selected sample of mother-daughter pairs. Although mean values of participant characteristics from our sample were similar to the entire cohort, this sampling technique may introduce bias to our analysis. Due to the potential for sampling bias, we have adjusted the models by weighting the cases and controls. Additionally, an inadvertent limitation to using data from a longitudinal cohort study is the inability to draw causal conclusions. Results from our study suggest an association between maternal PFC exposure during pregnancy and daughter's bone

health; however there may be residual confounding by unknown or unmeasured covariates.

This sample population was limited to the daughters; an important question to be addressed in future research includes the impact of maternal PFC exposure on bone health in the boys that participated in ALSPAC. Additionally, future research should consider investigating additional time points in order to assess risk of osteoporosis in adulthood. Because our study involved bone outcomes only at age 9, results from our study are unable to conclude effects of maternal PFC exposure during pregnancy on bone health later in life and into adulthood.

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VI. Tables

Table 1. Characterisics of study population(n=357)

	Frequency [n(%)]	PFOS (ng/mL) [Mean (SD)]	PFOA (ng/mL) [Mean (SD)]	PFHxS (ng/mL) [Mean (SD)]	PFNA (ng/mL) [Mean (SD)]
Overall	357 (100)	21.64 (10.55)	4.05 (1.83)	1.34 (1.84)	0.68 (0.33)
Maternal prepregnancy BMI					
Underweight (<18.5)	16 (4.97)	19.44 (8.33)	3.97 (1.74)	1.28 (0.46)	0.59 (0.24)
Normal (18.5-24.9)	227 (70.50)	21.88 (10.39)	4.09 (1.89)	1.35 (0.48)	0.69 (0.35)
Overweight (25.0-29.9)	54 (16.77)	22.39 (6.76)	4.01 (1.12)	1.35 (0.49)	0.72 (0.32)
Obese (≥30.0)	25 (7.76)	19.73 (12.51)	3.95 (1.87)	1.34 (0.39)	0.66 (0.28)
Missing	35 (7.00)	21.25 (15.15)	3.98 (2.45)	1.27 (0.44)	0.63 (0.25)
Maternal education					
Less than O level	52 (14.57)	20.73 (9.61)	3.88 (1.76)	1.39 (0.48)	0.69 (0.28)
O level	115 (32.21)	22.17 (11.78)	3.95 (1.38)	1.33 (0.47)	0.71 (0.31)
Greater than O level	168 (47.06)	21.71 (8.74)	4.16 (1.97)	1.34 (0.48)	0.67 (0.36)
Missing	22 (6.16)	20.45 (17.24)	4.20 (2.93)	1.23 (0.39)	0.58 (0.21)
Maternal Race					
White	339 (94.96)	21.87 (10.73)	4.10 (1.87)	1.34 (0.47)	0.69 (0.33)
Nonwhite	6 (1.68)	17.20 (4.86)	2.63 (0.55)	1.22 (0.40)	0.63 (0.25)
Missing	12 (3.36)	17.28 (4.74)	3.50 (0.99)	1.33 (0.50)	0.59 (0.24)
Maternal age at delivery					
<25 years	67 (18.77)	18.19 (5.76)	3.95 (1.23)	1.30 (0.42)	0.62 (0.22)
25-29 years	136 (38.10)	22.71 (12.85)	4.15 (1.97)	1.31 (0.46)	0.70 (0.31)
≥29 years	153 (42.86)	21.87 9.76)	4.01 (1.95)	1.39 (0.49)	0.69 (0.37)
Missing	1 (0.28)	22.70 (-)	4.40 (-)	1.0 (-)	0.80 (-)
Maternal Smoking Status before pregnancy					
Yes	72 (20.17)	18.27 (6.51)	3.71 (1.24)	1.29 (0.47)	0.58 (0.22)
No	272 (76.19)	22.39 (9.95)	4.15 (1.97)	1.35 (0.47)	0.71 (0.35)
Missing	13 (3.64)	24.55 (26.92)	3.92 (1.70)	1.34 (0.47)	0.70 (0.27)
Low Birth Weight (<2,500g at delivery)					
Yes	17 (4.76)	31.41 (25.66)	4.79 (2.19)	1.51 (0.49)	0.75 (0.22)
No	340 (95.24)	21.15 (8.99)	4.01 (1.82)	1.33 (0.47)	0.68 (0.33)
Preterm Delivery (<37 weeks gestation)					
Yes	13 (3.64)	30.21 (26.33)	4.56 (1.69)	1.25 (0.44)	0.70 (0.27)
No	344 (96.36)	21.31 (9.40)	4.03 (1.84)	1.34 (0.47)	0.68 (0.33)
Ever Breastfed					
Yes	276 (77.31)	21.87 (11.38)	4.04 (1.94)	1.33 (0.47)	0.68 (0.34)
No	61 (17.06)	21.28 (6.96)	4.12 (1.21)	1.36 (0.46)	0.67 (0.25)
Missing	20 (5.60)	19.43 (7.18)	4.08 (2.02)	1.40 (0.51)	0.70 (0.30)
Daughter's BMI at age 9					
Underweight (<18.5)	196 (54.90)	22.12 (11.23)	4.07 (1.93)	1.35 (0.48)	0.66 (0.26)
Normal (18.5-24.9)	145 (40.62)	21.00 (10.11)	4.06 (1.78)	1.36 (0.46)	0.70 (0.40)
Overweight (25.0-29.9)	15 (4.20)	21.31 (6.27)	3.74 (1.17)	1.07 (0.27)	0.77 (0.27)
Missing	1 (0.28)	22.70 (-)	4.40 (-)	1.00 (-)	0.80 (-)

Table 2. Mean and standard deviation of bone outcomes in the girls at age 9

	n	Mean (SD)
Total BMD (g/cm^2)	357	0.90 (0.05)
Total BMC (g)	357	1259.22 (221.05)
Total area (cm^2)	357	1387.50 (186.58)
Total BMC adjusted for area (g)	357	1212.91 (58.01)
Total body less Head BMD (g/cm^2)	357	0.79 (0.06)
Total body less Head BMC (g)	357	944.60 (207.56)
Total body less Head area (cm^2)	357	1185.73 (184.57)
Total body less Head BMC adjusted for area (g)	357	897.80 (42.31)

Table 3. Associations between PFC exposures and total body bone outcomes.

	BMD (g/cm^2)			BMC (g)			Area (cm^2)			ABMC (g)		
	β	SE	*P* value	β	SE	*P* value	β	SE	*P* value	β	SE	*P* value
PFOS												
Crude	-0.0002	0.0002	0.44	-2.045	0.889	0.02	-1.952	0.753	0.01	0.244	0.256	0.34
Model 1[a]	-0.0002	0.0003	0.42	-1.944	0.975	0.05	-1.799	0.833	0.03	0.166	0.289	0.57
Model 2[b]	-0.0002	0.0002	0.52	-1.364	0.848	0.11	-1.221	0.714	0.09	0.068	0.292	0.82
PFOA												
Crude	-0.0004	0.0012	0.75	-8.655	4.790	0.07	-8.785	4.057	0.03	1.647	1.377	0.23
Model 1[a]	-0.0005	0.0013	0.69	-8.247	5.158	0.11	-8.136	4.403	0.07	1.293	1.523	0.40
Model 2[b]	-0.0002	0.0013	0.87	-4.577	4.566	0.32	-4.498	3.846	0.24	0.698	1.567	0.66
PFHxS												
Crude	-0.0026	0.0053	0.62	-7.361	20.661	0.72	-3.095	17.538	0.86	-3.731	5.924	0.53
Model 1[a]	-0.0014	0.0053	0.79	0.290	20.375	0.99	3.606	17.414	0.84	-3.938	5.994	0.51
Model 2[b]	-0.0007	0.0051	0.89	8.764	17.810	0.62	12.126	15.000	0.42	-5.456	6.100	0.37
PFNA												
Crude	0.0053	0.007	0.45	-1.288	27.472	0.96	-7.0879	23.314	0.76	7.0240	7.8711	0.37
Model 1[a]	0.00544	0.0069	0.43	-3.106	26.665	0.91	-9.153	22.786	0.69	7.6276	7.8385	0.33
Model 2[b]	0.00542	0.0066	0.41	-6.855	22.809	0.76	-13.198	19.211	0.49	8.6217	7.8057	0.27

[a] Adjusted for maternal education, sample gestation, and age at DXA scan

[b] Adjusted for maternal education, sample gestation, age at DXA scan, preterm birth, birthweight, and BMI

Table 4. Associations between PFC exposures and total body less head bone outcomes.

	BMD (g/cm^2)			BMC (g)			Area (cm^2)			ABMC (g)		
	β	SE	*P* value	β	SE	*P* value	β	SE	*P* value	β	SE	*P* value
PFOS												
Crude	-0.0002	0.0003	0.48	-1.844	0.825	0.03	-1.981	0.745	0.01	0.327	0.183	0.07
Model 1[a]	-0.0002	0.0003	0.36	-1.781	0.894	0.05	-1.811	0.817	0.03	0.203	0.204	0.32
Model 2[b]	-0.0002	0.0002	0.46	-1.277	0.761	0.09	-1.266	0.698	0.07	0.111	0.205	0.59
PFOA												
Crude	-0.0003	0.0014	0.80	-7.960	4.448	0.07	-9.206	4.013	0.02	2.124	0.981	0.03
Model 1[a]	-0.0008	0.0014	0.59	-7.894	4.729	0.10	-8.577	4.319	0.05	1.501	1.073	0.16
Model 2[b]	-0.0004	0.0013	0.76	-4.878	4.097	0.23	-5.271	3.755	0.16	0.896	1.103	0.42
PFHxS												
Crude	-0.0013	0.0058	0.82	-5.739	19.183	0.76	-3.708	17.362	0.83	-1.677	4.239	0.69
Model 1[a]	0.0001	0.0056	0.98	1.520	18.684	0.94	3.550	17.094	0.84	-2.368	4.233	0.58
Model 2[b]	0.0001	0.0051	0.98	7.418	15.992	0.64	11.207	14.660	0.45	-4.858	4.292	0.26
PFNA												
Crude	0.0090	0.0077	0.24	0.953	25.506	0.97	-8.438	23.078	0.71	10.195	5.611	0.07
Model 1[a]	0.0093	0.0074	0.21	-0.797	24.453	0.97	-10.720	22.365	0.63	10.945	5.510	0.05
Model 2[b]	0.0085	0.0065	0.19	-4.977	20.482	0.81	-14.810	18.769	0.43	11.245	5.471	0.04

[a] Adjusted for maternal education, sample gestation, and age at DXA scan

[b] Adjusted for maternal education, sample gestation, age at DXA scan, preterm birth, birthweight, and BMI