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Perfluoroalkyl substances and changes in bone mineral density: a prospective analysis in the POUNDS-LOST Study

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Abstract

Background—Recent studies suggested an inverse association between exposures to perfluoroalkyl substances (PFASs) and bone mineral density (BMD). Whether exposures to PFASs are also associated with changes in BMD has not been examined.

Methods—Five major PFASs (perfluorooctanesulfonic acid, PFOS; perfluorooctanoic acid, PFOA; perfluorohexanesulfonic acid, PFHxS; perfluorononanoic acid, PFNA; perfluorodecanoic acid, PFDA) and BMD (g/cm^2) at six bone sites (spine, total hip, femoral neck, hip intertrochanteric area, hip trochanter, and hip Ward's triangle area) were measured at baseline

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among 294 participants in the POUNDS-LOST study, a weight-loss trial, of whom a total of 175 participants had BMD measured at both baseline and year 2. Linear regression was used to model the differences or changes in BMD for each SD increment of PFAS concentrations. In a secondary analysis, interactions between PFASs and baseline body mass index (BMI), as well as a BMI-related genetic risk score (GRS) derived from 97 BMI-predicting SNPs were examined in relation to changes in BMD.

Results—At baseline, both PFOS and PFOA were significantly associated with lower BMD at several sites. For each SD increase of PFOS, the βs (95% CIs) for BMD were −0.020(−0.037, −0.003) for spine, −0.013(−0.026, 0.001) for total hip, −0.014(−0.028, 0.000) for femoral neck, and −0.013(−0.026, 0.000) for hip trochanter. For PFOA, the corresponding figures were −0.021(−0.038, −0.004) for spine, −0.015(−0.029, −0.001) for total hip, and −0.015(−0.029, −0.002) for femoral neck. After adjusting for baseline covariates and 2-year weight change, higher baseline plasma concentrations of PFOS, PFNA, and PFDA were associated with greater reduction in BMD in the hip; the βs (95% CIs) were −0.005(−0.009, −0.001), −0.006(−0.010, −0.001), and −0.005(−0.009, −0.001), respectively. Similar associations were found in hip intertrochanteric area for all PFASs except PFHxS, with βs ranging from −0.006 for PFOA to −0.008 for PFOS and PFNA. Participants with a higher GRS tended to have less PFAS-related BMD decline in total hip ($P_{interaction}$ = 0.005) and the hip intertrochanteric area ($P_{interaction}$ = 0.021). There were similar PFAS-related BMD changes by baseline BMI levels, although the interactions did not achieve statistical significance.

Conclusions—This study demonstrated that higher plasma PFAS concentrations were not only associated with a lower BMD at baseline, but also a faster BMD loss in a weight-loss trial setting. Genetic predisposition to larger body size may somewhat attenuate the deleterious effects of PFASs on BMD. Further exploration of the possible impact of PFAS exposures on bone density is warranted.

Introduction

Perfluoroalkyl substances (PFASs) have been widely used as surfactants in many consumer products due to their unique anti-stain properties (Lindstrom et al., 2011). Exposures to PFASs are ubiquitous in the U.S. population. Major PFASs, including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) are detectable in serum for 98% of the U.S. population (Calafat et al., 2007). Despite a decline of serum concentrations of PFOS because of discontinued production of PFOS since 2002, the exposures to certain PFASs, such as PFNA, increased over time in the U.S. population (Kato et al., 2011). The persistence of PFASs in the environment and human body makes these chemicals a long-term health concern (Lindstrom et al., 2011).

Studies of murine models have demonstrated that exposures to PFASs may result in adverse health effects through endocrine disruption (Post et al., 2017). Accumulating evidence from epidemiological studies also showed that exposures to PFASs were associated with multiple adverse health effects (ATSDR, 2018). In addition, emerging evidence from animal and *in vitro* models also suggests that PFOS in particular may interfere with osteoclast functions and lead to decreased bone mineral density (BMD) (Agas et al., 2018; Koskela et al., 2017).

Two recent cross-sectional epidemiological studies showed lower BMD at multiple bone sites among people with higher serum concentrations of PFASs in a representative sample of U.S. population (Khalil et al., 2016; Lin et al., 2014). Given the widespread PFAS exposures, more evidence is needed to evaluate prospective associations with the decline of BMD over time.

Moreover, there is an interesting relationship between body size and BMD. Cross-sectional studies have suggested that BMD is proportional to the body size (Asomaning et al., 2006; Felson et al., 1993), and longitudinal studies showed that loss of BMD at most sites was positively related to the rate of loss in fat mass (Chen et al., 1997; Reid et al., 1994). Intriguingly, accumulating evidence has suggested body mass index (BMI) and BMD share some common genetic determinants (Kemp, 2017; Locke, 2015), implying shared regulatory mechanisms between body weight and bone composition. Despite this intimate relationship between BMI and BMD, few studies have evaluated whether BMI modulates the association between PFASs and BMD.

To shed further light on these important associations, the current study aimed to evaluate the association between plasma concentrations of PFASs and baseline BMD as well as changes in BMD in the two-year Prevention of Obesity Using Novel Dietary Strategies (POUNDS) Lost trial. As a secondary aim, the potential interactions between PFASs and baseline BMI on BMD changes were also explored. We used a BMI-predicting genetic score in the interaction tests to help alleviate confounding by lifestyle and dietary factors that predict both BMI and BMD.

Methods

Study population

The POUNDS-LOST trial was a randomized dietary intervention trial, aiming to assess long-term weight change in response to diets that emphasized different macronutrient compositions among people who were overweight or obese. Detailed description of the trial design has been published elsewhere (Sacks et al., 2009; Williamson et al., 2010). Briefly, a total of 811 overweight/obese healthy participants aged 30-70 years were randomly assigned to four energy-restricted diets, and about 80% of participants completed the study at 2 years. The magnitude of weight loss and regain was not statistically different among the four diets. Dietary assignment was unrelated with baseline plasma PFAS concentrations (Liu et al., 2018). The current analysis includes 294 participants with complete data on plasma-PFASs and BMD at baseline. Of these participants, 175 had BMD measured at both baseline and year 2, allowing us to examine baseline plasma-PFAS concentrations and changes in BMD prospectively.

The protocol was approved by the Institutional Review Board at Harvard T.H. Chan School of Public Health, Brigham and Women’s Hospital, and the Pennington Biomedical Research Center of the Louisiana State University System, as well as by a data and safety monitoring board appointed by the National Heart, Lung, and Blood Institute. All participants provided written informed consent.

Measurement of bone mineral density

As per POUNDS-Lost study protocol, the BMD of the spine (L1-L4), total hip, femoral neck, hip intertrochanteric area, hip trochanter, and hip Ward's triangle area were measured using dual-energy x-ray absorptiometry (DXA) after an overnight fast (De Souza et al., 2012; Heianza et al., 2016; Zhang et al., 2012) (Hologic QDR-4500A bone densitometer; Hologic, Inc). The BMD measurements were expressed as g/cm^2.

Measurement of plasma PFAS concentrations

Plasma concentrations of five major PFASs, i.e., PFOS, PFOA, PFNA, PFHxS, and perfluorodecanoic acid (PFDA), were measured at baseline by a well-established method primarily based on column switching liquid chromatography coupled to a triple quadropole mass spectrometer.(Haug et al., 2009) The concentrations of all five PFASs exceeded the limit of detection (0.05 ng/ml), and the inter- and intra-assay coefficients of variation (CV%) were <6.3% and <6.1%, respectively. A pilot study comparing two blood samples collected 1–2 years apart from 58 participants in the Nurses' Health Study II demonstrated an excellent reproducibility of PFAS measurement: the intra-class correlation coefficients (ICCs) were 0.91 for PFOS, 0.90 for PFOA, 0.94 for PFHxS, 0.87 for PFNA, and 0.82 for PFDA (all $P < 0.001$).

Genotyping and genetic risk score calculation

DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA). Genotyping was performed using the OpenArray SNP Genotyping System (BioTrove, Woburn, MA, USA) with a success rate of 99% and genotyping concordance rate >99% from testing 10% replicate quality control samples. Ninety-seven BMI-predicting single nucleotide polymorphisms (SNPs) identified through a meta-analysis of GWAS(Locke, 2015) were selected in the current analysis to calculate the genetic score. The allele frequencies of all SNPs in total participants were in Hardy-Weinberg equilibrium (all $P > 0.05$).

The genetic risk score (GRS97) of BMI was calculated on the basis of 97 SNPs using a weighted approach (Qi et al., 2014). Each SNP was recorded as 0, 1, 2 (according to number of risk alleles) and was weighted by its relative effect size (β coefficients) obtained from the most recent meta-analysis.(Locke, 2015) An alternative GRS score (GRS6) was calculated using 6 BMI-predicting SNPs, for which the nearest genes (TMEM18, RARB, TCF7L2, CADM1, FTO, and SMG6) were also associated with BMD (Kemp, 2017). The equation for calculating GRSs was: $(\beta_1 \times SNP_1 + \beta_2 \times SNP_2 + \ldots + \beta_n \times SNP_n) \times (n/\text{sum of the } \beta \text{ coefficients})$, where β is the coefficient of each individual SNP on BMI, n is 97 (GRS97) or 6 (GRS6), and sum of the β coefficients is 2.65 (GRS97) or 0.226 (GRS6) in the current analysis. The GRS97 ranged from 73.5 to 108.6 and GRS6 ranged from 1.88 to 9.16, with a higher score indicating a higher genetic risk of having a higher BMI.

Assessment of covariates

Demographic and lifestyle information regarding age, sex, race, educational level, smoking status, alcohol consumption, menopausal status, and postmenopausal hormone use was collected using standardized questionnaires. Physical activity was assessed using the 16-

items Baecke physical activity questionnaire inquiring level of habitual physical activity(Baecke et al., 1982). Body weight was measured at baseline, 6, 12, 18, and 24 months, and BMI was calculated as body weight in kilograms divided by height in meters squared.

Statistical analysis

The baseline characteristics were described as mean ±SD for continuous variables or count (%) for categorical variables. The baseline PFAS concentrations are shown as median (interquartile range) because of the skewed distributions. Concentrations of PFASs were normalized using the rank-based inverse normal transformation (Blom, 1958). The transformed PFAS concentrations were in standard normal distribution so that each unit increment corresponds to each SD increment of the PFAS concentrations. In the cross-sectional analysis that examined plasma concentrations of PFASs in relation to BMD at baseline, a general linear regression was used to evaluate the association between PFAS concentrations and baseline BMD. The covariates included age (yrs), sex, race (white or non-white), alcohol consumption (g/day), a physical activity score, and dietary intervention group (categorical). Because previous studies suggested that the associations between PFASs and BMD were primarily observed in women, we stratified the analysis by sex in the cross-sectional analysis.

In a prospective analysis, the association between baseline plasma PFASs concentrations and 2-year changes in BMD was evaluated using a generalized estimating equation (GEE) model to account for the repeated measurements of BMD. In a model evaluating the main effects of PFASs on changes in BMD, weight change in the same period and baseline BMD were additionally adjusted in the analysis.

In a secondary analysis that explored potential interactions between body size and PFASs, we conducted stratified analyses according to the median values of baseline BMI (31.8 kg/m^2), GRS97 (87.9), or GRS6 (5.53). The interactions between PFAS concentrations and baseline BMI were assessed by including a product term between these two continuous variables in the multivariable-adjusted model, and we further examined the interactions between PFASs and BMI-predicting GRSs (GRS97 and GRS6) using the same strategy. The p value for interaction was obtained from a Wald test for the product term. A two-sided P <0.05 was considered statistically significant and all statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

There were 294 participants included in the current analysis (Table 1). On average, participants were 52.2 ± 8.8 years old with a BMI of 32.5 ± 3.8 kg/m^2 at baseline. The majority (86.7%) of the population were white and 46% were men. Less than 10% of participants did not attend college, and less than 5% of participants were current smokers. The median plasma concentrations of PFASs ranged from 0.4 ng/ml for PFDA to 26.4 ng/ml for PFOS, while the baseline BMD ranged from 0.7g/cm^2 in the hip Ward's triangle area to 1.2g/cm^2 in the hip intertrochanteric area. The prospective analysis included 175 participants

with both BMD measurements at baseline and year 2. The population characteristics at year 2 were similar to those of the participants at baseline.

The cross-sectional associations between plasma PFAS concentrations and baseline BMD are shown in Table 2. In the multiple-variable adjusted model, inverse associations were observed between baseline PFAS concentrations and BMD at all bone sites, although only the associations for PFOS and PFOA with BMD at certain bone sites were statistically significant. For example, each SD increase of the PFOS concentrations was significantly associated with a lower BMD in spine (β = −0.020 g/cm^2; 95% CI: −0.037, −0.003), femoral neck (β = −0.014 g/cm^2; 95% CI: −0.028, 0.000), and hip trochanter (β = −0.013 g/cm^2; 95% CI: −0.026, 0.000). For PFOA, the significant associations were found for spine (β = −0.021 g/cm^2; 95% CI: −0.038, −0.004), total hip (β = −0.015 g/cm^2; 95% CI: −0.029, −0.001), femoral neck (β = −0.016 g/cm^2; 95% CI: −0.030, −0.002), and hip trochanter (β = −0.015 g/cm^2; 95% CI: −0.029, −0.002). In the gender-specific analyses, inverse associations across PFASs appeared to be somewhat stronger in women for spine BMD and weaker for femoral neck and Ward's triangle area BMD, but we did not observe any significant interactions by gender ($P_{interaction}$ > 0.05 in all analyses).

Table 3 shows the prospective associations between plasma PFAS concentrations and the 2-year changes in BMD at multiple bone sites. After adjusting for baseline covariates and 2-year weight change, significantly greater BMD reduction in total hip was observed for each SD increment in plasma concentration of PFOS (β = −0.005 g/cm^2; 95% CI: −0.009, −0.001), PFNA (β = −0.006 g/cm^2; 95% CI: −0.010, −0.001), or PFDA (β = −0.005 g/cm^2; 95% CI: −0.009, −0.001) at baseline. Similar associations were found in the hip intertrochanteric area for all PFASs, except for PFHxS, and the effect sizes ranged from −0.006 g/cm^2 for PFOA to −0.008 g/cm^2 for PFOS and PFNA.

The interactions between PFASs and baseline BMI and GRSs in relation to BMD are shown in Table 4. To alleviate the potential multiple-comparison issue in this secondary analysis, we focused on total hip and hip intertrochanteric area, for which significant associations were observed in the prospective analysis. The associations between PFASs and BMD at these two bone sites did not differ by baseline BMI. In contrast, the PFASs-related BMD reduction in total hip and the hip intertrochanteric area were largely abolished among participants with higher GRS97, except for PFNA in relation to BMD at the hip intertrochanteric area or PFHxS in relation to BMD at both bone sites. When the GRS6 was used in this analysis, the association between baseline PFOA concentrations and BMD in the total hip ($P_{interaction}$ = 0.005) or hip intertrochanteric area ($P_{interaction}$ = 0.021) were significantly attenuated among participants with a higher GRS6 score.

Discussion

In the present study, we demonstrated that higher plasma-PFAS concentrations were not only associated with lower BMD cross-sectionally, but also a faster BMD decline in a weight-loss trial setting, independent of weight changes and other covariates. Moreover, in a secondary analysis, PFASs-related declines in BMD were largely attenuated among participants who had a stronger genetic predisposition to obesity as measured by a genetic

BMI risk score derived from 97 SNPs, but not among participants who had a lower genetic score. The associations between PFASs and the decline in BMD were also generally weaker among individuals with a higher BMI, although no significant effect modifications were detected.

Our study is among the few attempts that assessed the impact of PFAS exposures on bone health. Our findings are consistent with evidence from other cross-sectional analyses. In premenstrual women, each unit increase in the natural log-transformed serum-PFOS concentration was associated with a 0.022 g/cm^2 reduction in BMD at the spine (Lin et al., 2014). This estimate is comparable to the effect size among women in the current analysis (0.025 g/cm^2 by the same increment of log-transformed PFOS). In a more recent cross-sectional study, Khalil et al. showed inverse correlations between serum concentrations of PFOS and femoral neck BMD and between PFOA concentrations and total hip BMD (Khalil et al., 2016). Our analysis demonstrated that PFASs might also influence BMD at other relevant sites. For example, we found that PFOS might affect BMD at spine, hip trochanter, and hip Ward's triangle area, while PFOA might lead to lower BMD in spine, femoral neck, and hip trochanter.

Our study is among the first that evaluated the prospective association between baseline PFAS concentrations and changes in BMD. Specifically, we observed that baseline concentrations of PFOS, PFNA or PFDA were associated with a faster decline in BMD at both total hip and hip intertrochanteric areas, and PFOA concentrations predicted a faster decline in BMD at hip intertrochanteric area. These associations were independent of weight changes during the trial. In general populations of adults, BMD decreases linearly with age, and the bone diminution is faster in women (Riggs et al., 1981). It is estimated that the BMD loss rate is <0.004 g/cm^2/year in the spine and hip before menopause in women, and both men and postmenopausal women lose 0.002–0.006 g/cm^2/year of BMD at all bone sites (Warming et al., 2002). Our findings suggest the PFAS exposure may accelerate the age-dependent bone diminution process, although whether this PFASs-related accelerated bone loss may also happen in individuals without diet-induced weight change warrants more studies.

Baseline BMI did not significantly modulate the associations between PFASs and BMD change, although the faster decline of BMD by higher PFASs concentrations appeared to be significantly attenuated among participants who had a higher BMI-related GRS. It is well-established that higher body weight is associated with higher BMD (Edelstein and Barrett-connor, 1993; Felson et al., 1993). A number of mechanisms have been proposed to explain this relationship, including the effect of soft tissue mass on skeletal loading, the association of fat mass with the secretion of bone-active hormones from the pancreas (including insulin, amylin, and preptin), and the secretion of bone-active hormones (e.g., estrogens and leptin) from adipocytes (Reid, 2002). Interestingly, an early study among twins estimated that genetic factors that affected lean mass or fat mass also had positive correlations with BMD (Nguyen et al., 1998; Seeman et al., 1996), implying some shared genetic regulation for body mass and BMD. Indeed, the most recent meta-analysis of GWAS data demonstrated shared genetic predictors between BMI and BMD (Kemp, 2017; Locke, 2015). In our GRS6, the six genes are either directly implicated in bone cell remodeling (RAAB) (Henning et al.,

2015) and bone formation (CADM1) (Mentink et al., 2013) or indirectly involved in bone health through affecting body growth and energy balance (FTO and TMEM18)(Gao et al., 2010) and in crosstalk with *Wnt* signaling pathway, a key mechanisms in bone formation (TCF7L2) (Ip et al., 2012). Our additional analysis of the GRS97 showed that it was positively correlated with baseline BMD ($\beta>0$ for all bone sites, although this correlation was statistically significant only for spine, $p=0.034$). Taken together, our analysis suggested that individuals who are predisposed to have a higher BMI might be more resistant to PFASs-related BMD loss.

Few mechanistic studies have been conducted to specifically elucidate the role of PFASs on bone health, although current evidence from animal models suggests that PFASs may influence BMD through their adverse effects via modulating hormone functions (Post et al., 2017), e.g., sex hormones and thyroid hormones, both of which play a critical role in maintaining bone health (Greendale et al., 1997; Schneider et al., 1994). Animal studies have shown that exposures to PFOA increased serum estradiol concentrations while inhibiting testosterone release (Biegel et al., 1995). Moreover, higher dose of PFOA reduced estradiol production and downregulated the expression of some key genes responsible for estrogen synthesis (Shi et al., 2009). The estrogen-like properties of PFOA (Benninghoff et al., 2011) enable this chemical to directly modulate ovary function and lead to decreased steroid hormonal synthetic enzyme levels and reduced expression of estrogen- or progesterone-induced mammary growth factors (Zhao et al., 2012). PFASs can also disrupt the functions of thyroid hormones by competing with certain hormone binding proteins including albumin and transthyretin, which indirectly interferes with the hypothalamus-pituitary-thyroid axis (Jones et al., 2003; Weiss et al., 2009). Some population-based studies suggested that PFASs could alter the levels of triiodothyronine (T3) and thyroxine (T4) (Jain, 2013; Shrestha et al., 2015; Webster et al., 2016), which are essential for the normal development of endochondral and intramembranous bone and play an important role in the linear growth and maintenance of bone mass (Bassett and Williams, 2003). Despite these potential mechanisms, human studies did not demonstrate consistent evidence associating PFAS with abnormalities in estrogen and thyroid hormone metabolism (ATSDR, 2018). Clearly, more investigations are needed to further shed light on mechanistic pathways that underlie the link between PFASs and bone health.

The strengths of our study include repeated measurements of BMD, inclusion of a comprehensive assessment of major PFASs and BMD at different bone sites, adjustment for multiple potential confounding factors, including genetic risk score. The major limitation is the modest sample size that might render insufficient power, particularly for gene-environment interaction analyses. Moreover, we could not rule out the possibility of chance findings due to multiple comparisons. The conservative Bonferroni correction was not applied because the PFASs and the BMD assessments at different sites were inter-correlated. Nonetheless, our findings were generally consistent with previous reports. Furthermore, the lack of diagnostic data on osteoporosis prohibited us from evaluating whether the plasma PFAS concentrations were directly associated with clinically recognized osteoporosis. In the secondary analysis that examined GRSs and PFASs interactions on BMD decline, we did not genotype the SNPs that were associated with BMD in previous GWAS studies and thus could not examine whether the BMD-specific GRS would also modify the associations of

PFASs with BMD decline. Finally, some degree of residual confounding is still possible despite a relatively comprehensive adjustment for demographic and lifestyle factors.

In conclusion, our study within the POUNDS-LOST trial showed that higher plasma PFAS concentrations were associated with lower BMD cross-sectionally and a faster BMD decline in two years prospectively. Although these associations were largely independent of sex and of body weight changes during the weight-loss trial, a higher BMI-related GRS significantly attenuated the associations between baseline PFAS concentrations and faster BMD decline. Further studies with a larger sample size and more comprehensive assessments of genetic predictors of BMD are needed to replicate this observation.

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- Higher plasma concentrations of perfluoroalkyl substances were not only associated with lower bone mineral density cross-sectionally but also predicted faster reduction of bone mineral density following weight changes in a weight-loss trial setting.
- Participants with a higher genetic risk score of body mass index tended to have less perfluoroalkyl substances-related bone mineral density decline over time.

Table 1.

Baseline characteristics of participants in the POUNDS LOST study

	Cross-sectional analysis	Prospective analysis
N	294	175
Age (years)	52.2±8.8	54.5±8.1
Sex, men, %	45.6	45.1
Race, White, %	86.7	91.4
BMI (kg/m^2)	32.5±3.8	32.1±3.9
Waist circumference (cm)	104.2±13.1	103.8±13.4
Education level, high school or less, %	9.5	11.4
Current smoker, yes, %	4.1	4.6
Alcohol consumption (drinks/week)	2.3±3.1	2.2±3.1
Physical activity *	1.6±0.1	1.6±0.1
GRS97	88.1±6.2	88.3±6.3
GRS6	5.6±1.5	5.6±1.6
Plasma PFAS concentrations (ng/ml)		
- PFOS (ng/ml)	32.2 (16.8, 43.1)	33.9 (19.2, 44.3)
- PFOA (ng/ml)	5.2 (3.5, 6.5)	5.4 (3.7, 6.6)
- PFHxS (ng/ml)	3.1 (1.7, 3.9)	3.2 (1.7, 3.9)
- PFNA (ng/ml)	2.0 (1.1, 2.5)	2.1 (1.1, 2.6)
- PFDA (ng/ml)	0.4 (0.3, 0.5)	0.5 (0.3, 0.5)
Baseline BMD (g/cm^2)		
- Spine	1.1±0.1	1.0±0.2
- Total hip	1.0±0.1	1.0±0.1
- Femoral neck	0.8±0.1	0.8±0.1
- Hip intertrochanteric area	1.2±0.2	1.2±0.2
- Hip trochanter	0.8±0.1	0.8±0.1
- Hip Ward's triangle area	0.7±0.2	0.7±0.2

Data are mean ± SD, median (interquartile range), or percentage (%).

* Physical activity was estimated by the Baecke Questionnaire.

Abbreviations: BMI, body mass index; TG, Triglycerides; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate; PFHxS, perfluorohexanesulfonate; PFNA, perfluorononanoic acid.

Table 2.

Cross-sectional association between plasma PFAS concentrations and bone mineral density at multiple bone sites at baseline.[1]

BMD by sites [β for one SD increase of PFASs (95% CI)]	Plasma PFAS concentrations, ng/ml				
	PFOS	**PFOA**	**PFHxS**	**PFNA**	**PFDA**
Spine					
Total	−0.020(−0.037, −0.003)	−0.021(−0.038, −0.004)	−0.013(−0.032, 0.006)	−0.011(−0.028, 0.006)	−0.009(−0.026, 0.008)
Women	−0.015(−0.036, 0.005)	−0.017(−0.039, 0.004)	−0.010(−0.034, 0.015)	−0.006(−0.026, 0.014)	−0.008(−0.027, 0.012)
Men	−0.01l(−0.041, 0.018)	−0.008(−0.040, 0.023)	−0.001(−0.032, 0.029)	−0.007(−0.037, 0.023)	0.001(−0.029, 0.031)
***P* for interaction**	0.68	0.71	0.63	0.95	0.76
Total hip					
Total	−0.013(−0.026, 0.001)	−0.015(−0.029, −0.001)	−0.006(−0.022, 0.009)	−0.006(−0.020, 0.007)	−0.005(−0.018, 0.009)
Women	−0.010(−0.028, 0.008)	−0.013(−0.031, 0.006)	−0.011(−0.032, 0.010)	−0.005(−0.023, 0.012)	−0.006(−0.023, 0.012)
Men	−0.011(−0.033, 0.011)	−0.013(−0.036, 0.011)	0.006(−0.016, 0.029)	−0.007(−0.029, 0.016)	0.000(−0.023, 0.022)
***P* for interaction**	0.98	0.67	0.51	0.99	0.64
Hip intertrochanteric area					
Total	−0.012(−0.028, 0.004)	−0.016(−0.032, 0.000)	−0.007(−0.025, 0.010)	−0.006(−0.021, 0.010)	−0.003(−0.019, 0.012)
Women	−0.013(−0.033, 0.008)	−0.017(−0.038, 0.005)	−0.015(−0.040, 0.009)	−0.007(−0.027, 0.013)	−0.007(−0.027, 0.013)
Men	−0.008(−0.033, 0.018)	−0.010(−0.037, 0.017)	0.008(−0.019, 0.034)	−0.003(−0.030, 0.023)	0.005(−0.021, 0.031)
***P* for interaction**	0.76	0.97	0.42	0.74	0.42
Femoral neck					
Total	−0.014(−0.028, 0.000)[2]	−0.016(−0.030, −0.002)	−0.007(−0.022, 0.009)	−0.008(−0.022, 0.006)	−0.005(−0.019, 0.009)
Women	−0.004(−0.022, 0.014)	−0.008(−0.027, 0.010)	−0.005(−0.025, 0.016)	0.000(−0.018, 0.017)	−0.002(−0.019, 0.015)
Men	−0.021(−0.044, 0.001)	−0.019(−0.043, 0.005)	−0.001(−0.024, 0.023)	−0.016(−0.039, 0.007)	−0.004(−0.027, 0.019)
***P* for interaction**	0.24	0.26	>0.99	0.31	0.98
Hip trochanter					
Total	−0.013(−0.026, 0.000)[2]	−0.015(−0.029, −0.002)	−0.003(−0.018, 0.011)	−0.008(−0.020, 0.005)	−0.007(−0.020, 0.006)
Women	−0.011(−0.028, 0.006)	−0.010(−0.028, 0.007)	−0.005(−0.025, 0.015)	−0.006(−0.022, 0.010)	−0.006(−0.022, 0.010)
Men	−0.008(−0.028, 0.013)	−0.013(−0.035, 0.009)	0.008(−0.013, 0.030)	−0.007(−0.028, 0.014)	−0.003(−0.024, 0.018)

BMD by sites [β for one SD increase of PFASs (95% CI)]	Plasma PFAS concentrations, ng/ml				
	PFOS	PFOA	PFHxS	PFNA	PFDA
***P* for interaction**	0.84	0.45	0.60	0.91	0.83
Ward's triangle area					
Total	−0.017(−0.034, 0.001)	−0.015(−0.033, 0.003)	−0.005(−0.024, 0.015)	−0.008(−0.025, 0.009)	−0.008(−0.025, 0.009)
Women	−0.003(−0.027, 0.020)	−0.003(−0.028, 0.02l)	−0.00l(−0.029, 0.027)	0.003(−0.019, 0.026)	0.000(−0.022, 0.022)
Men	−0.028(−0.055, 0.000)	−0.02l(−0.050, 0.008)	0.002(−0.027, 0.030)	−0.022(−0.050, 0.006)	−0.015(−0.043, 0.013)
***P* for interaction**	0.22	0.24	0.95	0.24	0.48

[1]Model were adjusted for age (years), sex (men, women), race (White, non-White), alcohol consumption (g/day), physical activity factor (continuous), body mass index (kg/m^2), and dietary intervention groups (categorical).

[2]P=0.05

Table 3.

Prospective association between plasma PFAS concentrations and 2-year changes in bone mineral density at multiple bone sites.[1]

2-year change of BMD, g/cm^2 [β for one SD increase of PFASs (95% CI)]	Plasma PFAS concentrations, ng/ml				
	PFOS	**PFOA**	**PFHxS**	**PFNA**	**PFDA**
Spine	0.002(−0.003, 0.008)	−0.002(−0.007, 0.004)	0.003(−0.003, 0.009)	0.003(−0.002, 0.009)	0.004(−0.002, 0.009)
Total hip	**−0.005(−0.009, −0.001)**	−0.004(−0.008, 0.000)	0.000(−0.005, 0.004)	**−0.006(−0.010, −0.001)**	**−0.005(−0.009, −0.001)**
Hip intertrochanteric area	**−0.008(−0.013, −0.003)**	**−0.006(−0.011, −0.001)**	−0.002(−0.008, 0.004)	**−0.008(−0.013, −0.003)**	**−0.007(−0.013, −0.002)**
Femoral neck	−0.001(−0.007, 0.004)	−0.001(−0.007, 0.004)	0.003(−0.002, 0.008)	−0.003(−0.008, 0.002)	−0.003(−0.008, 0.002)
Hip trochanter	−0.002(−0.006, 0.002)	−0.003(−0.007, 0.001)	0.001(−0.003, 0.005)	−0.003(−0.007, 0.001)	−0.003(−0.007, 0.001)
Ward's triangle area	−0.003(−0.011, 0.004)	−0.004(−0.012, 0.005)	0.004(−0.005, 0.012)	−0.005(−0.013, 0.003)	−0.005(−0.013, 0.003)

[1]Model were adjusted for age (years), sex (men, women), race (White, non-White), alcohol consumption (continuous), physical activity (continuous), body mass index (continuous), dietary intervention groups (categorical), baseline respective BMD, and 2-year weight change.

Table 4.

Interactions between plasma PFAS concentrations and baseline BMI and GRS in relation to 2-year changes in bone mineral density at multiple bone sites. [1]

2-year change of BMD, g/cm^2 [β for one SD increase of PFASs (95% CI)]	Plasma PFAS concentrations, ng/ml				
	PFOS	**PFOA**	**PFHxS**	**PFNA**	**PFDA**
Total hip					
BMI> 31.8 kg/m^2	−0.006(−0.011, 0.000)	−0.005(−0.011, 0.001)	0.000(−0.006, 0.006)	−0.003(−0.008, 0.002)	−0.002(−0.007, 0.004)
BMI≤ 31.8 kg/m^2	−0.007(−0.012, −0.002)	−0.006(−0.010, −0.001)	−0.002(−0.007, 0.004)	−0.007(−0.012, −0.002)	−0.009(−0.014, −0.004)
***P* for interaction**[2]	0.88	0.41	0.60	0.15	0.07
GRS97>87.9	−0.001(−0.006, 0.004)	−0.001(−0.006, 0.004)	−0.001(−0.006, 0.003)	−0.001(−0.006, 0.003)	0.000(−0.005, 0.004)
GRS97≤ 87.9	−0.008(−0.015, −0.002)	−0.007(−0.013, 0.000)	0.000(−0.008, 0.007)	−0.010(−0.017, −0.003)	−0.011(−0.017, −0.004)
***P* for interaction**[2]	**0.03**	**<0.001**	0.22	**0.02**	**<0.01**
GRS6 > 5.53	−0.005(−0.011, 0.001)	−0.001(−0.007, 0.004)	−0.001(−0.007, 0.004)	−0.005(−0.012, 0.001)	−0.004(−0.011, 0.003)
GRS6 ≤ 5.53	−0.005(−0.010, 0.000)	−0.006(−0.012, −0.001)	0.001(−0.005, 0.008)	−0.006(−0.011, −0.001)	−0.007(−0.012, −0.001)
***P* for interaction**[2]	0.37	**<0.01**	0.59	0.35	0.27
Hip intertrochanteric area					
BMI> 31.8 kg/m^2	−0.010(−0.017, −0.003)	−0.006(−0.013, 0.002)	−0.003(−0.011, 0.006)	−0.006(−0.013, 0.001)	−0.004(−0.010, 0.003)
BMI≤ 31.8 kg/m^2	−0.010(−0.016, −0.004)	−0.009(−0.015, −0.003)	−0.003(−0.010, 0.004)	−0.010(−0.016, −0.004)	−0.011(−0.017, −0.005)
***P* for interaction**[2]	0.66	0.82	0.98	0.51	0.34
GRS97>87.9	−0.004(−0.010, 0.001)	−0.003(−0.010, 0.003)	−0.005(−0.012, 0.002)	−0.004(−0.010, 0.001)	−0.003(−0.008, 0.002)
GRS97≤ 87.9	−0.012(−0.020, −0.005)	−0.009(−0.016, −0.001)	−0.002(−0.011, 0.008)	−0.013(−0.022, −0.004)	−0.014(−0.022, −0.006)
***P* for interaction**[2]	**0.05**	**0.01**	0.49	0.06	**0.01**
GRS6 > 5.53	−0.008(−0.015, 0.000)	−0.003(−0.011, 0.004)	−0.002(−0.010, 0.006)	−0.007(−0.016, 0.001)	−0.004(−0.013, 0.005)
GRS6 ≤ 5.53	−0.008(−0.014, −0.002)	−0.008(−0.014, −0.002)	0.000(−0.009, 0.009)	−0.008(−0.015, −0.002)	−0.010(−0.017, −0.003)
***P* for interaction**[2]	0.18	**0.02**	0.53	0.27	0.10

[1] Model were adjusted for age (years), sex (men, women), race (White, non-White), alcohol consumption (continuous), physical activity (continuous), body mass index (continuous), dietary intervention groups (categorical), baseline respective BMD, and 2-year weight change.

[2] P value for interaction was obtained from a Wald test for the product term between continuous BMI/GRS and PFASs.