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## **Association of Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES 2009–2010**

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**Short running title:** Perfluoroalkyl substances and bone density

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## Abstract

**Background:** Perfluoroalkyl substances (PFAS), including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) are detectable in serum of 95% of the United States (US) population. Considering their role as endocrine disruptors, we examined their relationships with bone health.

**Methods:** Association between serum PFAS concentrations in 1914 participants and bone mineral density at total femur (TFBMD), femoral neck (FNBMD), lumbar spine (LSBMD) and physician-diagnosed osteoporosis was assessed using the National Health and Nutritional Examination Survey 2009-2010 data.

**Results:** Mean age of participants was 43 years. Men had higher serum PFAS concentrations than women ( $p < .001$ ), except for PFNA. In both genders, serum PFOS concentrations were inversely associated with FNBMD ( $p < 0.05$ ). In women, significant negative associations were observed for ln-transformed PFOS with TFBMD, and FNBMD and for ln-PFOA exposure with TFBMD ( $p < 0.05$ ). In postmenopausal women, serum PFOS was negatively associated with TFBMD, and FNBMD, and PFNA was negatively associated with TFBMD, FNBMD and LSBMD (all  $p < 0.05$ ). With one log unit increase in serum PFOA, PFHxS, and PFNA, osteoporosis prevalence in women increased [adjusted odds ratio (a OR)]; 1.84 (95% CI: 1.17, 2.905); 1.64 (95% CI: 1.14, 2.38), and 1.45 (95% CI: 1.02, 2.05), respectively. In women, the prevalence of osteoporosis was significantly higher in the highest versus the lowest quartile of PFOA, PFHxS, and PFNA, with adjusted odds ratios of 2.59 (95% CI: 1.01, 6.67), 13.20 (2.72, 64.15): and 3.23 (95% CI: 1.44, 7.21), respectively, based on 77 cases in study sample.

**Conclusion:** In a representative sample of the adult US population, serum PFAS concentrations were associated with lower bone mineral density, with variation according to specific PFAS and bone sites assessed. Most associations were limited to women. Osteoporosis in women also was associated with PFAS exposures, based on a small number of cases.

## **Introduction**

Age-associated osteoporosis is a significant public health concern as it is related with bone fractures and associated morbidity (Johnell et al. 2001). It is estimated that more than nine percent of Americans aged 50 years and over had osteoporosis either at the femoral neck or at the lumbar spine in 2005–2008 (2% of all United States [US] men, and 10% of all US women) (Looker et al. 2010). Recent evidence suggests that exposure to environmental toxicants such as lead, cadmium, and mercury are associated with higher risk for osteoporosis and fractures (Engstrom et al. 2011; Khalil et al. 2008; Pollack et al. 2013).

Perfluoroalkyl substances (PFASs) have been widely used in protective water and stain resistant coatings on clothing, furnishing, and non-stick housewares for over 60 years. PFASs are ubiquitous environmental contaminants and are detectable in humans worldwide (Fromme et al. 2009). Of the 12 PFASs assayed in the National Health and Nutrition Examination Survey (NHANES) 1999-2008, four PFASs were found in 95% of the US population: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) (Calafat et al. 2007; Kato et al. 2011). Toxicity of PFASs including tumors, liver damage, and adverse skeletal and reproductive outcomes were reported in animal studies (Cui et al. 2009; White et al. 2007; Yu et al. 2009). PFOA has recently been categorized as possibly carcinogenic in humans (2B classification) (IARC. 2015). Moreover, PFAS have been characterized as endocrine disrupting chemicals (ED) (White et al. 2011) based on their hormonal modulation and metabolic associations (Lin et al. 2009).

A recent analysis reported negative associations between environmental exposures to PFOS and LSBMD in pre-menopausal women using NHANES 2005-2008 data (Lin et al. 2014). Further, experimental and human autopsy evidence suggests accumulation of PFASs in

the skeleton (Bogdanska et al. 2011; Perez et al. 2013). In the current analysis, we tested the following hypotheses: 1) serum PFAS concentrations are negatively associated with BMD, and 2) this association differs by gender.

## **Methods**

### **Study Methods and Participants**

Publically available data from NHANES 2009-2010 cycle were utilized for this study. A detailed description of survey design and methods are available on the NHANES website (CDC 2014). Briefly, NHANES is an ongoing survey of the non-institutionalized US population collected using a stratified, multistage probability sampling design. After providing informed consent, participants visited a mobile examination center (MEC) for physical assessment, examination, and laboratory measures. Analysis of PFASs in serum was conducted at the National Center for Environmental Health in a random, one-third subsample of participants aged 12 years and above. The present study sample consisted of NHANES participants aged 12-80 years who had BMD measures available at total femur (TFBMD, n=1914), its sub region femoral neck (FNBMD, n=1914), and lumbar spine (LSBMD, n=1605), and serum concentrations of four PFASs (PFOA, PFOS, PFHxS, PFNA).

### **Dual X-Ray Absorptiometry (DXA) and Osteoporosis**

BMD ( $\text{g}/\text{cm}^2$ ) was measured using DXA Hologic QDR 4500A fan-beam densitometers in the MEC (Hologic, Inc., Bedford, MA, USA) (CDC 2014). Antero-posterior LSBMD was scanned; mean BMD was computed from the first through fourth lumbar vertebra. For TFBMD, and FNBMD, left hip was routinely scanned. If a left hip replacement or metal objects in the left leg were reported, the right hip was scanned. Participants were excluded from the femur scan if they had bilateral hip fracture, hip replacements, or pins. Participants weighing > 300 pounds

(DXA table limitation) or pregnant females (positive urine pregnancy test and/or self-report) were ineligible for the DXA examination. Each respondent's scan was reviewed in the Department of Radiology, University of California, San Francisco using standard radiologic techniques and NHANES protocols.

Participants answering 'yes' to the question "Has a doctor or other health professional ever told you that you have osteoporosis?" were coded as having 'self-reported physician-diagnosed' osteoporosis. Validity of self-reported osteoporosis is moderate to good in mid-age and older adults, respectively (Peeters et al. 2013). In a study of Australian women, the agreement between self-reported osteoporosis and medication claims was moderate in women 56-71 years of age (kappa statistic 0.51)

### **PFAS Assay**

Briefly, serum PFAS (non-fasting) were measured using automated solid-phase extraction coupled to isotope-dilution high-performance liquid chromatography-tandem mass spectrometry, as published elsewhere (Calafat et al. 2007). Serum measures of four PFASs (PFOA, PFOS, PFHxS, and PFNA), which were detectable in > 98% of the 2009-2010 survey participants, were included in this analysis.

### **Covariates**

Covariates selected *a priori* (Cummings et al. 1985; Hannan et al. 2000; Khalil et al. 2008) included age, race/ethnicity, gender, body mass index (BMI), smoking (serum cotinine), daily milk intake, physical activity (PA), menopause, and blood lead concentration. Socio-demographic information such as age, gender, race/ethnicity, and reproductive history were recorded using interviewer-administered questionnaires. Age was used concurrently both as a continuous variable and categorized in three levels as 12-20 years, 21-50 years, and 50-years or

greater (referent). Race/ethnicity was self-reported as non-Hispanic White, non-Hispanic Black, Mexican American, Other Hispanic, and other multi-racial (referent).

Body weight was measured to the nearest 0.01 kg using an electronic load cell scale and standing height was measured with a fixed stadiometer. BMI was calculated as body weight (kilograms) divided by height (meters squared). Serum cotinine levels <1.0 ng/mL were categorized as non-smoker (referent), 1.0-9.9 ng/mL as environmental tobacco smoke (ETS) exposure, and  $\geq 10.0$  ng/mL as current smoker (CDC 2015a) (Hukkanen et al. 2005).

Menopause status was ascertained as self-reported cessation of regular menstruation over the past 12 months. If women stated 'no' to a question "have you had regular periods in the past twelve months?", and stated that the reason for not having regular periods due to "menopause/hysterectomy", then they were categorized as post-menopausal.

Separate questions assessed the history of hysterectomy and bilateral oophorectomy as answering yes to a question about having had a hysterectomy (have you had a hysterectomy, including a partial hysterectomy that is, surgery to remove your uterus or womb)? Or answering yes to a question about having had both ovaries removed (have had both of your ovaries removed either when you had your uterus removed or at another time)? Women who answered reason for not having regular periods as "menopause / hysterectomy" also included women who had a hysterectomy and/or bilateral oophorectomy.

Self-reported vigorous or moderate recreational physical activity (PA), was categorized as *inactive* (<10 minutes consecutive minutes per week: referent), *low activity* (10 to 149 active minutes per week), *sufficient / medium activity* (150 to 299 active minutes per week), and *high activity* ( $\geq 300$  active minutes per week) according to 2008 Physical Activity Guidelines for Americans (USDHHS 2008).. This activity categorization was derived from six PA variables

using an algorithm (Tucker et al. 2011). These six variables assessed vigorous and moderate recreational PA using NHANES questionnaire data. For example *vigorous* recreational PA evaluated answers to the following questions: 1) “Do you do any vigorous-intensity sports, fitness, or recreational activities that cause large increases in breathing or heart rate like running or basketball for at least 10 minutes continuously?”; 2) “in a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational activities?”; 3) “how much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?”. The same three variables were incorporated in the algorithm for *moderate* intensity PA.

Milk intake was ascertained as answering “yes” (“no”: referent) to a question “have you been a regular (5 times per week) milk drinker for most or all of your life, including childhood?” Blood lead concentration (ug/dL), and BMI were used as continuous variables (except Supplemental Material Table S1, presented as quartiles [lead], or by weight category [BMI]).

### **Statistical Analysis**

Population characteristics, outcomes, and exposures were summarized as means  $\pm$  standard errors (SE) or numbers of observations (%), and differences according to gender were tested using Student’s 2-tailed t- test or Rao Scott Chi-square test as recommended by the National Center for Health Statistics (NCHS) (NCHS 2015). As decided *a priori*, analyses were conducted to examine relationships between PFASs, BMD, and osteoporosis stratified by gender. Due to significant non-normal distribution, natural log-transformation (Ln-PFAS) was performed on PFAS concentrations. Analyses were repeated by categorizing PFASs in quartiles using gender-specific quartile (Q1: referent, lowest; Q4 highest) cut points for men and women. We constructed full multivariable linear regression models with TFBMD, FNBMD, and LSBMD as dependent variables and individual PFAS concentrations as natural log-transformed continuous

predictors or in quartiles while adjusting for covariates related to BMD described previously in literature. Each PFAS was modeled separately. Results are reported as regression coefficients and 95% confidence intervals (CI).

In multiple logistic regression models, adjusted odds ratios (aOR) and 95% CI of osteoporosis were calculated for each PFAS separately as natural log-transformed predictors or as gender-specific quartiles (using lowest quartile as referent). To explore if PFAS, BMD, and osteoporosis associations differed by menopause status, stratified multiple linear regression analysis by menopause status was completed (as described above). Supplemental Material Table S1 shows mean untransformed PFAS concentration by covariate categories for men and women. Table S2 shows unadjusted mean serum PFAS concentration (log transformed) by osteoporosis diagnosis in men and women.

To account for the complex NHANES survey design, sampling weights, strata and primary sampling units were adjusted in all analyses as recommended by the SAS survey procedures were used (SAS Institute, Inc., version 9.3) by applying Taylor series linearization method for the calculation of SEs. Two-tailed p-values were used for all tests, at 5% significance level.

## **Results**

### **Characteristics of Study Population**

Mean age of the study population was 42.6 (SE: 0.6) years, with no significant difference by gender (Table 1). The study population was predominantly comprised of non-Hispanic White participants. BMI was comparable by gender. Men had a higher percentage of smokers and those with ETS exposure ( $p < 0.001$ ). In men, milk consumption, and blood lead levels were significantly higher than women ( $p < 0.05$ ). Less than half of the study participants were

physically inactive (42%); there was a statistically significant difference in PA between men and women ( $p < 0.001$ ). The proportion of inactive women was higher when compared to men. High PA was almost three times more common in men compared to women

TFBMD and FNBMD was 12%, and 8% higher in men than in women, respectively (all  $p < 0.001$ ). LSBMD was also slightly higher in men than women, but the difference was not statistically significant. . Although osteoporosis diagnosis was reported in 5% of the total sample only 2% of men ( $n=17$ ), reported osteoporosis, and was reported more frequently in women ( $n=77$ ) than in men ( $p < 0.001$ ). In men, average serum PFOA, PFOS, and PFHxS concentrations were 20%, 32%, and 36% higher than in women, respectively ( $p < 0.001$  for all three). PFNA concentrations were comparable between genders ( $p=0.133$ ).

Supplemental Material Table S1 summarizes covariates and PFAS concentrations by gender. A significant relationship was noted between age categories and all PFAS in both genders except PFHxS in men. PFOS and PFNA were statistically different across race/ethnicity, in both sexes (only significant for PFHxS in men). PFAS exposure was not significantly related to BMI categories, recreational PA, and daily milk intake. No significant associations between smoking status and PFASs were noted except for PFHxS in men. In women, a significant association between mean blood lead levels with all PFASs was observed. In men only PFOS and PFHxS had a significant relationship with blood lead levels.

### **Serum PFASs and BMD**

Adjusted associations between continuous ln-transformed, and quartiles of PFAS and each BMD measure are reported separately for men and women, and for pre- and post-menopausal women, in Tables 2-4. In most cases, ln-PFAS concentrations and BMD measures were negatively associated in men, but only the association between ln-PFOS and FNBMD was

statistically significant ( $\beta = -0.013$ ; 95% CI:  $-0.024, -0.002$ ). In general, categorical PFAS exposures were not clearly associated with any of the BMD measures in men, except that compared to Q1, Q4 PFOS exposure had significant negative relationship with and FNBMD ( $\beta = -0.046$ ; 95% CI:  $-0.078, -0.015$ ).

In women, ln-PFOS was associated with significantly lower TFBMD and FNBMD, and ln-PFOA was associated with significantly lower TFBMD. *In pre-menopausal women*, no significant association between any PFASs and BMD was observed. *In postmenopausal women*, serum ln-PFOS was statistically significantly associated with TFBMD, FNBMD; PFNA was inversely related with TFBMD, FNBMD and LSBMD (all  $p < 0.05$ ).

In quartile analyses, women showed significant negative associations between TFBMD with Q2 of PFOA, Q4 of PFOS, and Q4 of PFHxS; and of FNBMD with Q4 of PFOS, relative to the lowest quartile (Q1) of each exposure, respectively. *In pre-menopausal women*, TFBMD also had significant negative associations with Q2 of PFOA and Q4 of PFHxS. *In post-menopausal women*, TFBMD was significantly lower in Q4 of PFOS. LSBMD was not significantly associated with any of the categorical PFAS exposures in men or in women, regardless of menopausal status.

### **Serum PFAS and Osteoporosis**

There were 17 cases of osteoporosis in men (Table 1), and no significant differences in mean PFASs levels according to case status in men (Supplemental Material, Table S2). In women, mean values of all four PFASs were significantly higher in the 77 women with osteoporosis compared with the 733 women who did not report osteoporosis. aORs for PFOA and PFNA were significant both for the continuous ln-transformed exposures (aOR = 1.84; 95% CI: 1.17, 2.90 and aOR = 1.45; 95% CI: 1.02, 2.05, respectively) and for the Q4 versus Q1

comparisons (aOR = 2.59; 95% CI: 1.01, 6.67 and aOR = 3.23; 95% CI: 1.44, 7.21, respectively) (Table 5). Ln-PFHxS was significantly associated with osteoporosis both in the ln-PFAS model (aOR 1.64; 95% CI: 1.14, 2.38) and the quartile analysis, with significant associations for all three quartiles (e.g., aOR = 13.20; 95% CI: 2.72, 64.15 for Q4 versus Q1). However, there were only two women with osteoporosis in the Q1 of PFHxS, and quartile-specific aORs were very imprecise. PFOS was not significantly associated with osteoporosis in the continuous or quartile exposure models.

## **Discussion**

In this nationally representative sample of the US population, among women, PFOS and PFHxS were associated with lower TFBMD and a higher prevalence of osteoporosis. In addition, PFOS was negative associated with FNBMD, PFOA was negatively associated TFBMD, and PFNA was positively associated with osteoporosis only. In general, associations were stronger among post-menopausal women than pre-menopausal women. In men, PFOS was associated with lower FNBMD only. LSBMD was not clearly associated with any of the PFAS in men or women. Although the variance in BMD explained by PFAS was very small ( $R^2 < 1\%$ ) in this exploratory investigation, given the study sample size and nationally representative data, these results are important and support the need for further research to evaluate PFASs toxicity on bone health.

Human exposure to PFASs has gradually increased since the 1950s (Cousins 2013), although following regulatory efforts serum concentrations of some PFAS are decreasing in recent years (Kato et al. 2011). PFAS are present in many food items including meat, poultry, eggs, fish, fresh produce, and diet contributes significantly to daily human exposures to PFAS (Domingo 2012). PFAS are poorly metabolized and slowly eliminated from the human body

with half-lives of 4-8 years (Kato et al. 2011) and partition to bone tissue (Perez et al. 2013) (Bogdanska et al. 2011).

Limited data from animal models point towards PFAS toxicity on bone. Prenatal PFOS exposure in rodents was associated with fetal bone malformation (Thibodeaux et al. 2003) (OECD 2002). In mice, environmentally relevant doses of PFOS exhibited rapid deposition in bone (Bogdanska et al. 2011). Recent data from human autopsy studies suggests that PFAS are sequestered in bone; PFOA being predominant (Perez et al. 2013). Taken together these studies suggest that PFASs are deposited in bone, and may induce some osteo-toxicity.

PFASs at low doses are categorized as EDC in animals, including rats (Z Shi et al. 2009a; Z Shi et al. 2009b), mice (Zhao et al. 2010), fish (X Shi et al. 2009), and in some human studies (Knox et al. 2011b; Louis et al. 2012; White et al. 2011). Data from animal models suggest that bone tissue could be an important target for a number of EDC environmental pollutants (Agas et al. 2013; Finnila et al. 2010; Kamei et al. 2008) as EDC can disturb the complex hormonal control of bone metabolism. For example, gender-dependent associations of another EDC polychlorinated biphenyl (PCB) on bone length in female ewe fetuses were reported (Gutleb et al. 2010). In another study, pregnant ewes exposed to multiple EDC showed reduced BMD (Lind et al. 2010).

Epidemiological research supports the EDC hypothesis for some form of PFAS toxicity. PFAS exposure was related with later onset of puberty (Lopez-Espinosa et al. 2011). Older age at menarche has been associated with an increased risk of fractures in women at approximately 20 years of age (Chevalley et al. 2012), with lower BMD in perimenopausal women (Tuppurainen et al. 1995) and with an increased risk of hip fractures in older women (Paganini-Hill et al. 2005). Moreover, serum PFOA and PFOS (specifically) were associated with earlier

age at menopause in the C8 Health Project (Knox et al. 2011b) and NHANES (Taylor et al. 2014). Knox et al. (2011b) also reported that serum PFOS was negatively associated with serum estradiol concentration (Knox et al. 2011b). Another potential pathway that may link PFAS and BMD is through thyroid hormone modulation. Thyroid hormones play a crucial role in bone health and remodeling (Lee et al. 2010). PFAS exposures were associated with serum thyroxine (T4) and triiodothyronine (T3) levels in two cross-sectional studies (Knox et al. 2011a; Wen et al. 2013) and with altered responses to T3 in a T3-dependent cell line *in vitro* (Long et al. 2013). Based on a cross-sectional study of adult NHANES participants, Wen et al. (2013) reported that serum PFHxS was positively associated with subclinical hyperthyroidism (defined as TSH <0.24 mIU/mL) in women, which is a risk factor for osteoporosis (El Hadidy et al. 2011).

In the current analysis, while men had higher serum levels of PFASs; women experienced a greater BMD deficit, and osteoporosis risk than men. This corroborates recently published findings of Lin et al. (2014) where PFOS exposure was related to a BMD deficit only in women (Lin et al. 2014). The gender difference in PFAS and BMD association suggests that reproductive hormones may play a role. Females may be more sensitive to PFASs toxicity or, as animal studies suggest, PFASs may be differently eliminated in males and females (Betts 2007).

We observed higher prevalence of osteoporosis with PFOA, PFNA and PFHxS in women. The odds ratio and 95% CI of osteoporosis were large but imprecise for PFHxS in the quartile analysis due to the small number of observations. We are unable to explain this strong association, and because of the low precision of the estimates the associations should be interpreted with caution. PFHxS has the longest half-life (8.5 years) of the four PFASs and has recently been associated with impaired thyroid function and earlier menopause in epidemiological studies (Taylor et al. 2014; Wen et al. 2013).

One of the study limitations was the cross sectional design; we cannot confirm that the exposures preceded the outcomes of interest, or rule out the possibility of reverse causation (Taylor et al. 2014). The strengths of this study include a large sample representative of the US population. Four PFASs commonly detected in US residents were assayed. To our knowledge this is the first report assessing the relationship of four PFAS with BMD at three bone sites; as the only such study by Lin et al.(2014) was limited to two PFAS (PFOA, PFOS) and BMD at lumbar spine and total hip.

We observed significant negative associations between PFOS, PFNA, PFHxS and TFBMD and FNBMD, as well as with osteoporosis in women. Lin et al. (2014) reported a significant negative association between PFOS and LSBMD in pre-menopausal women only while we did not observe any significant association between LSBMD and PFOS in our sample of pre-menopausal women.

Some potential reasons for discrepant relationships between PFOS and LSBMD in the present study and Lin et al. paper could be attributable to the differences in NHANES survey cycles examined; sample size, age range, and covariates included. For example, in comparison with two combined NHANES 2005-2006 and 2007-2008 surveys in Lin et al. study, our sample comprised of one NHANES survey conducted in 2009-2010. Furthermore, decreasing mean serum PFOS concentration in US population could have masked an association with LSBMD in our study. As evidenced in the NHANES surveys, geometric mean (GM) serum PFOS levels in US general population including women decreased over 2005-2006 (overall:17.1 ng/ml, women:14.4 ng/ml), 2007-2008 (overall:13.2 ng/ml, women:10.7 ng/ml) and 2009-2010 surveys (overall:9.3 ng/ml, women:7.7 ng/ml). (CDC 2015b).

In conclusion, our findings indicate that some PFAS are associated with lower BMD and a higher prevalence of osteoporosis in US women. However, findings must be interpreted with caution given the cross-sectional study design, the large number of comparisons made, and the small numbers of osteoporosis cases in the study population.

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**Table 1.** Characteristics of 2009-2010 NHANES study participants, distribution of serum perfluoroalkyl substances, bone mineral density, overall, and by sex.

Characteristic Variable	Overall		Male		Female		p-value <sup>a</sup>
	No.	Mean ± SE or %	No.	Mean ± SE or %	No.	Mean ± SE or %	
Age (Year)	1914	42.6 ± 0.64	956	42.0±0.69	959	43.1 ± 0.68	0.139
Age groups							
12-20	368	15	204	15.6	164	14.5	
21-50	802	50	375	50.5	427	50.4	
>50	744	35	377	34.0	367	35.1	0.674
BMI (Kg/m <sup>2</sup> )	1908	27.4 ± 0.20	953	26.8 ± 0.34	955	27.2 ± 0.24	0.277
Smoking status	1913						
Smoker	410	22	237	26.0	173	18.2	
ETS	86	4.4	55	5.7	31	3.2	
Non-Smokers	1417	73.5	663	68.3	754	78.6	<.001 <sup>b</sup>
Race/ethnicity	1914						
NH White	883	68	453	67.9	430	68.1	
NH Black	314	10.5	164	10.5	150	10.4	
Mexican-American	411	10.0	198	10.9	213	9.1	
Other Hispanic	205	5.1	97	5.4	108	4.7	
Other Multiracial	101	6.5	44	5.3	57	7.7	0.083
Regular drink milk 5 times/week	1575						
Yes	1224	80	618	83	606	76	
No	351	20	146	17	205	24	0.002
Recreational activity	1905						
Inactive	896	42	411	39	485	45	
Low activity	290	17	120	14	170	21	
Moderate activity	230	13	112	13	118	13	
High activity	489	28	307	34	182	11	<.001
Blood lead (ug/dL)	1914	1.43 ± 0.06	956	1.67± 0.08	958	1.18± 0.03	<.001
BMD Total femur(g/cm <sup>2</sup> )	1914	0.97 ± 0.01	956	1.03 ± 0.01	958	0.91 ± 0.01	0.001
BMD Femoral neck (g/cm <sup>2</sup> )	1914	0.84 ± 0.01	956	0.88 ± 0.01	958	0.81 ± 0.01	0.001
BMD Lumbar spine(g/cm <sup>2</sup> )	1505	1.02 ± 0.01	741	1.03 ± 0.01	764	1.01 ± 0.01	0.051
Osteoporosis	1575						
Yes	94	5	17	2	77	8	
No	1481	95	748	98	733	92	0.001
PFOA (ng/ml) <sup>c</sup>	1914	3.7 ± 0.18	956	4.1 ± 0.21	958	3.3± 0.15	0.001
PFOS (ng/ml) <sup>c</sup>	1914	12.7 ± 1.20	956	15.1 ± 1.6	958	10.3 ± 0.75	0.001
PFHxS (ng/ml) <sup>c</sup>	1914	2.50 ± 0.10	956	3.1 ± 0.18	958	1.9 ± 0.09	0.001
PFNA (ng/ml) <sup>c</sup>	1914	1.9 ± 0.20	956	2.0 ± 0.28	958	1.8 ± 0.13	0.134

<sup>a</sup> p-values for differences between males and females: continuous variables: t-test; categorical variables: Rao-Scott chi-square

<sup>b</sup> Smoking categories based on serum cotinine concentration, ETS: environmental tobacco smoke

<sup>c</sup> Untransformed Serum Perfluoroalkyl Substances

**Table 2.** Multivariate<sup>a</sup> adjusted linear regression coefficients for perfluoroalkyl substances and total femur bone mineral density (TNBMD).

<b>N=1566</b>	<b>Men (n=956)</b>	<b>All women (n=958)</b>	<b>Pre-menopausal women (n=590)</b>	<b>Post-menopausal women (n=368)</b>
	<b>β (95%CI)</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>
<b>PFAS</b>				
<b>PFOA</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.010 (-0.034, 0.055)	-0.020 (-0.040, -0.001)	-0.026 (-0.051, -0.001)	-0.011 (-0.059, 0.037)
Q3	-0.012 (-0.056, 0.033)	-0.002 (-0.038, 0.034)	0.006 (-0.041, 0.052)	-0.002 (-0.049, 0.045)
Q4	-0.001 (-0.042, 0.041)	-0.030 (-0.063, 0.003)	-0.029 (-0.068, 0.010)	-0.024 (-0.072, 0.024)
Ln-PFOA	-0.007 (-0.028, 0.014)	-0.017 (-0.038, 0.003)	-0.017 (0.038, 0.004)	-0.012 (-0.043, 0.019)
<b>PFOS</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.029 (-0.074, 0.016)	-0.007 (-0.038, 0.023)	-0.013 (-0.050, 0.023)	-0.001 (-0.072, 0.069)
Q3	-0.029 (-0.063, 0.006)	-0.009 (-0.037, 0.019)	-0.017 (-0.048, 0.014)	0.002 (-0.065, 0.070)
Q4	-0.032 (-0.072, 0.008)	-0.044 (-0.074, -0.014)	-0.013 (-0.046, 0.021)	-0.059 (-0.115, -0.002)
Ln-PFOS	-0.010 (-0.027, 0.006)	-0.018 (-0.034, -0.002)	-0.004 (-0.020, 0.012)	-0.033 (-0.049, -0.015)
<b>PFHxS</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.004 (-0.046, 0.038)	-0.007 (-0.038, 0.023)	-0.013 (-0.050, 0.023)	-0.001 (-0.072, 0.069)
Q3	-0.004 (-0.043, 0.036)	-0.009 (-0.037, 0.019)	-0.017 (-0.048, 0.014)	0.002 (-0.065, 0.070)
Q4	-0.026 (-0.065, 0.013)	-0.044 (-0.074, -0.014)	-0.013 (-0.046, 0.021)	-0.059 (-0.115, -0.002)
Ln- PFHxS	-0.010 (-0.025, 0.004)	-0.012 (-0.026, 0.002)	-0.009 (-0.026, 0.007)	-0.009 (-0.029, 0.011)
<b>PFNA</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.003 (-0.053, 0.047)	-0.017(-0.050, 0.016)	-0.017 (-0.054, 0.020)	-0.008 (-0.070, 0.053)
Q3	-0.006 (-0.039, 0.026)	-0.008 (-0.041, 0.026)	-0.004 (-0.046, 0.038)	0.001 (-0.060, 0.060)
Q4	0.007 (-0.031, 0.045)	-0.040 (-0.077, -0.003)	-0.039 (-0.071, -0.006)	-0.023 (-0.083, 0.042)
Ln- PFNA	-0.006 (-0.030, 0.018)	-0.017 (-0.038, 0.003)	-0.009 (-0.028, 0.010)	-0.027 (-0.053, -0.002)

<sup>a</sup>Adjusted for age (continuous),and age categories (12-20, 21-50, >50), ethnicity, BMI, serum cotinine , physical activity, milk consumption, and blood lead concentration.

**Table 3.** Multivariate<sup>a</sup> adjusted linear regression coefficients for perfluoroalkyl substances and total femur neck mineral density (FNBMND).

<b>N=1566</b>	<b>Men (n=956)</b>	<b>All women (n=958)</b>	<b>Pre-menopausal women (n=590)</b>	<b>Post-menopausal women (n=368)</b>
<b>PFAS</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>
<b>PFOA</b>				
Q1	Referent	Referent	Referent	Referent
Q2	0.011 (-0.021, 0.043)	-0.025 (-0.052, 0.002)	-0.028 (-0.060, 0.003)	-0.022 (-0.077, 0.033)
Q3	-0.013 (-0.053, 0.028)	-0.002 (-0.039, 0.034)	0.014 (-0.031, 0.060)	-0.024 (-0.083, 0.035)
Q4	0.004 (-0.035, 0.043)	-0.028 (-0.058, 0.001)	-0.019 (-0.056, 0.018)	-0.041 (-0.098, 0.016)
Ln-PFOA	0.001 (-0.025, 0.022)	-0.017 (-0.033, -0.001)	-0.012 (-0.030, 0.007)	0.020 (-0.049, 0.010)
<b>PFOS</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.036 (-0.077, 0.006)	0.001(-0.019, 0.019)	-0.005 (-0.028, 0.018)	-0.005 (0.087, 0.077)
Q3	-0.027 (-0.063, 0.009)	-0.001 (-0.025, 0.025)	-0.005 (-0.028, 0.017)	-0.001 (-0.082, 0.080)
Q4	-0.046 (-0.078, -0.015)	-0.034 (-0.059, -0.009)	-0.001 (-0.029, 0.029)	-0.062 (0.134, 0.009)
Ln-PFOS	-0.013 (-0.024, -0.002)	-0.016(-0.029, -0.002)	-0.001 (-0.015, 0.015)	-0.033 (-0.049, -0.017)
<b>PFHxS</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.002 (-0.042, 0.038)	-0.008 (-0.039, 0.023)	-0.004 (-0.042, 0.033)	-0.025 (-0.100, 0.050)
Q3	-0.004 (-0.031, 0.023)	0.001 (-0.025, 0.027)	0.010 (-0.018, 0.038)	-0.017 (-0.092, 0.058)
Q4	-0.013 (-0.052, 0.025)	-0.018 (-0.051, 0.016)	-0.010 (-0.039, 0.018)	-0.026 (-0.104, 0.051)
Ln- PFHxS	-0.009 (-0.024, 0.006)	-0.005 ( -0.018, 0.008)	-0.001 (-0.015, 0.013)	-0.005 (-0.024, 0.013)
<b>PFNA</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.010 (-0.058, 0.037)	-0.020 (-0.066, 0.027)	-0.023 (-0.073, 0.027)	-0.013 (-0.087, 0.060)
Q3	-0.004 (-0.035, 0.028)	-0.001 (-0.029, 0.029)	0.001 (-0.035, 0.038)	-0.009 (-0.078, 0.060)
Q4	0.009 (-0.022, 0.039)	-0.023 (-0.051, 0.005)	-0.005 (-0.049, 0.040)	-0.046 (-0.103, 0.012)
Ln- PFNA	-0.005 (-0.021, 0.018)	-0.014 (-0.032, 0.003)	-0.005 (-0.025, 0.016)	-0.025 (-0.049, -0.001)

<sup>a</sup>Adjusted for age (continuous),and age categories (12-20, 21-50, >50), ethnicity, BMI, serum cotinine , physical activity, milk consumption, and blood lead concentration.

**Table 4.** Multivariate<sup>a</sup> adjusted linear regression coefficients for perfluoroalkyl substances and lumbar spine bone mineral density.

<b>N=1566</b>	<b>Men (n=956)</b>	<b>All women (n=958)</b>	<b>Pre-menopausal women (n=590)</b>	<b>Post-menopausal women (n=368)</b>
<b>PFAS</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>	<b>β (95%CI)</b>
<b>PFOA</b>				
Q1	Referent	Referent	Referent	Referent
Q2	0.013 (-0.042, 0.068)	-0.008 (-0.035, 0.019)	-0.008 (-0.041, 0.025)	-0.001 (-0.089, 0.088)
Q3	-0.023 (-0.083, 0.037)	0.015 (-0.019, 0.049)	0.020 (-0.020, 0.060)	0.011 (-0.090, 0.113)
Q4	-0.005 (-0.058, 0.049)	-0.020 (-0.049, 0.009)	-0.010 (-0.042, 0.021)	-0.017 (-0.111, 0.077)
Ln-PFOA	-0.011 (-0.039, 0.017)	-0.009 (-0.029, 0.011)	0.001 (-0.020, 0.021)	-0.017 (-0.058, 0.024)
<b>PFOS</b>				
Q1	Referent	Referent	Referent	Referent
Q2	-0.023 (-0.064, 0.018)	0.001 (-0.038, 0.040)	0.001 (-0.044, 0.045)	-0.040 (-0.165, 0.085)
Q3	-0.026 (-0.066, 0.014)	0.008 (-0.024, 0.039)	0.009 (-0.026, 0.045)	-0.023 (-0.144, 0.097)
Q4	-0.023 (-0.064, 0.017)	-0.011 (-0.053, 0.032)	0.015 (-0.022, 0.052)	0.058 (-0.192, 0.075)
Ln-PFOS	-0.011 (-0.028, 0.006)	-0.003 (-0.022, 0.017)	0.010 (-0.008, 0.027)	-0.019 (-0.047, 0.009)
<b>PFHxS</b>				
Q1	Referent	Referent	Referent	Referent
Q2	0.015 (-0.021, 0.050)	0.017 (-0.019, 0.053)	0.026 (-0.017, 0.069)	-0.017 (-0.103, 0.069)
Q3	0.021 (-0.015, 0.057)	0.026 (-0.013, 0.065)	0.028 (-0.017, 0.073)	0.035 (-0.067, 0.137)
Q4	0.005 (-0.022, 0.033)	-0.015 (-0.046, 0.016)	-0.014 (-0.043, 0.015)	0.001 (-0.089, 0.091)
Ln- PFHxS	0.001 (-0.011, 0.012)	-0.003 (-0.015, 0.009)	0.003 (-0.013, 0.019)	-0.001 (-0.021, 0.020)
<b>PFNA</b>				
Q1	Referent	Referent	Referent	Referent
Q2	0.004 (-0.046, 0.054)	-0.013 (-0.078, 0.052)	-0.009 (-0.069, 0.050)	-0.016 (-0.145, 0.113)
Q3	-0.013 (-0.056, 0.029)	0.005 (-0.034, 0.043)	0.009 (-0.021, 0.039)	-0.022 (-0.134, 0.098)
Q4	0.009 (-0.026, 0.044)	-0.023 (-0.057, 0.012)	0.004 (-0.035, 0.043)	-0.061 (-0.170, 0.048)
Ln- PFNA	-0.006 (-0.029, 0.017)	-0.016 (-0.032, 0.001)	-0.016 (-0.032, 0.001)	-0.043 (-0.073, -0.013)

<sup>a</sup>Adjusted for age (continuous), and age categories (12-20, 21-50, >50), ethnicity, BMI, serum cotinine, physical activity, milk consumption, and blood lead concentration.

**Table 5.** Multivariate<sup>a</sup> logistic regression of perfluoroalkyl substances and osteoporosis in women.

PFAS	Osteoporosis (n)	No osteoporosis (n)	Odds Ratio (95% CI)	P-value <sup>b</sup>
<b>PFOA</b>				
Q1	8	175	Referent	--
Q2	16	186	1.25 (0.38, 4.06)	0.713
Q3	17	178	1.23 (0.37, 4.05)	0.734
Q4	36	194	2.59 (1.01, 6.67)	0.049
Ln-PFOA	77	733	1.84 (1.17, 2.90)	0.008
<b>PFOS</b>				
Q1	11	175	Referent	--
Q2	8	186	0.42 (0.13, 1.32)	0.137
Q3	22	190	0.83 (0.45, 1.51)	0.540
Q4	36	184	1.07 (0.36, 3.19)	0.908
Ln-PFOS	77	733	1.14 (0.68, 1.94)	0.619
<b>PFHxS</b>				
Q1	11	175	Referent	--
Q2	8	184	9.29 (1.81, 47.62)	0.008
Q3	22	190	8.06 (1.84, 35.25)	0.006
Q4	36	184	13.20 (2.72, 64.15)	0.001
Ln-PFHxS	77	733	1.64 (1.14, 2.38)	0.008
<b>PFNA</b>				
Q1	11	175	Referent	--
Q2	8	184	1.93 (0.72, 5.10)	0.191
Q3	22	190	0.82 (0.25, 2.64)	0.735
Q4	36	184	3.23 (1.44, 7.21)	0.004
Ln-PFNA	77	733	1.45 (1.02, 2.05)	0.001

<sup>a</sup>Adjusted for age (continuous) age categories (12-20, 21-50, >50), ethnicity, BMI, serum cotinine, physical activity, milk consumption, and blood lead concentration.

<sup>b</sup>Wald Chi-square p-values