Negative Association Between Serum Perfluorooctane Sulfate Concentration and Bone Mineral Density in US Premenopausal Women: NHANES, 2005–2008

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Context: Perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS) are used in a variety of products worldwide. However, the relationship among serum PFOA, PFOS concentration, bone mineral density (BMD), and the risk of fractures has never been addressed.

Objectives: The study examined the association among serum PFOA, PFOS concentration, and lumbar spine and total hip BMD in the general US population.

Design and Participants: We analyzed data on 2339 adults (aged ≧20 y) from the National Health and Nutrition Examination Survey conducted in 2005–2006 and 2007–2008 to determine the relationship among serum PFOA, PFOS concentration, and total lumbar spine and total hip BMD measured by dual-energy x-ray absorptiometry and history of fractures cross-sectionally.

Results: After weighting for sampling strategy, a 1-U increase in the natural log-transformed serum PFOS level was associated with a decrease in total lumbar spine BMD by 0.022 g/cm^2 (95% confidence interval −0.038, −0.007; $P = .006$) in women not in menopause. There was no association among PFOA, PFOS concentration, and self-reported fracture in adults.

Conclusion: Serum PFOS concentration is associated with decreased total lumbar spine BMD in women not in menopause. However, the potential biological significance of this effect is marginal and subclinical in the general US population. Further studies are warranted to clarify the causal relationship between perfluorinated chemical exposure and BMD. **(*J Clin Endocrinol Metab* 99: 2173–2180, 2014)**

Osteoporotic fractures are an important cause of disability (1). One report has shown that hip fracture is associated with a 20% increase in mortality in the year after fracture (2). Given the economic and social costs of osteoporotic fractures, strategies to identify and manage osteoporosis in the primary care setting are important.

Bone mineral density (BMD) is one of the most important parameters for the measurement of bone quality. BMD results are highly predictive of fracture risk (3). There are various risk factors associated with low BMD; among these, endocrine disorders such as hypogonadism, hyperthyroidism, and hyperparathyroidism have gained much attention recently. Moreover, recent studies have shown a correlation among increased oxidative stress, inflammatory cytokines, and increased risk of fracture (4). Currently humans and wildlife are exposed to various en-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received September 8, 2013. Accepted February 18, 2014.
First Published Online February 28, 2014

Abbreviations: AhR, aryl hydrocarbon receptor; BMD, bone mineral density; BMI, body mass index; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; EDC, endocrine-disrupting chemical; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; PFC, perfluorinated chemical; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfate.

vironmental endocrine-disrupting chemicals (EDCs), typically at low doses. Various EDCs such as organic tin compounds, alkylphenols, dioxin, and dioxin-like compounds can alter the systemic hormonal regulation of the bone remodeling process and the skeletal formation (5). Although many of the evidence are from in vitro or animal experiments, the possibility that some EDCs might contribute to bone disorders in humans is supported by a range of epidemic studies in humans (6–9).

Perfluorinated chemicals (PFCs) that act as EDCs are exclusively manmade chemicals that are widely used to manufacture popular consumer products and are used in the nonstick and stain-resistant coatings of cookware, food containers, furniture, and carpets. PFCs feature strong carbon-fluorine bonds, resist environmental degradation, and have become widespread, persistent environmental pollutants (10). The two most widely known PFCs are perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS), which belong to the 8-carbon backbone subgroup. The public health relevance of exposure to PFCs is still being examined because of their biological persistence and the lack of information on their possible long-term health implications (11). Toxicological studies have shown that exposure to PFOS and PFOA alters the biosynthesis of thyroid hormone levels and sex-specific steroid hormones (12–16) and causes increased oxidative stress (17, 18) in exposed animals and the cell system. In epidemiological studies, reports have provided evidence that exposure to PFOS and PFOA have been linked to endocrine disorders (19–21). However, investigations of the effects of PFCs on BMD in both animals and humans are unfortunately scant.

Given the findings on the relationship among PFOA, PFOS, and the reproductive system, thyroid function, and oxidative stress, it is reasonable to ask whether PFOA or PFOS affects BMD or is associated with a risk of fractures. We hypothesized that PFOA or PFOS might have adverse effects on BMD and may be related to fracture history in US adults. To test this hypothesis, we used a large-scale data set of serum PFOA, PFOS level, total lumbar spine and total hip BMD, and history of fractures released by the US National Health and Nutrition Examination Survey (NHANES).

Materials and Methods

Study design and population

Data were adopted from the 2005–2006 and 2007–2008 NHANES. NHANES is a population-based survey designed to collect information on the health and nutrition of US households and to obtain a representative sample of the noninstitutionalized civilian US population. Survey data are published biannually. Detailed contents of the 2005–2006 and 2007–2008 NHANES are available on the NHANES web site.

In the 2005–2006 and 2007–2008 NHANES, serum PFC concentrations were measured in a one-third subsample of individuals aged 12 years and older. A dual-energy x-ray absorptiometry (DXA) examination over the lumbar spine and hip was performed for eligible survey participants aged 8 years and older. Subjects who were pregnant, had a history of radiographic contrast material use in the past 7 days, had a history of nuclear medicine studies in the past 3 days, or weighed more than 300 lb were ineligible for a DXA examination. In total, 2339 subjects 20 years of age or older, provided data on PFC concentrations and DXA examination in the final analysis.

Assessment of PFC concentrations

Twelve types of PFCs are described in NHANES. We included PFOA and PFOS serum sample results for analysis based on our hypothesis. A brief summary of the assessment of PFCs (22) is as follows. After dilution with formic acid, 1 aliquot of serum (100 μL) was injected into a commercial column switching system for concentration and chromatographic separation of analytes. Detection and quantification were performed by tandem mass spectrometry. The limit of detection was 0.1 ng/mL for PFOA and 0.2 ng/mL for PFOS. For concentrations less than the limit of detection (0.2% for PFOA, 0.2% for PFOS), a value equal to the limit of detection divided by the square root of 2 was used (23).

Dual-energy x-ray absorptiometry

The inclusion of DXA measurements of the lumbar spine in NHANES 2005–2008 provides the first nationally representative data for this skeletal site for the US population. Measurements of the femur from the same sample can provide information about the current femur skeletal status for the US population. As described previously (24), lumbar spine and hip BMD was measured with Hologic QDR 4500A fan-beam densitometers (Hologic, Inc) using Discovery version 12.4 software. Scanning was performed in the fast mode. Rigorous quality control programs were used in both surveys, which included the use of anthropomorphic phantoms and a review of each quality control and respondent scan at a central site (Department of Radiology, University of California, San Francisco, San Francisco, California, in NHANES (2005–2006, 2007–2008). Total lumbar spine and total hip BMD were chosen as the outcomes of this study because a number of clinical guidelines are based on these measurements (25, 26).

Other covariates

According to the statements on the NHANES web site, data were collected at all study sites by trained personnel using standardized procedures. Sociodemographic information such as age, sex, and race/ethnicity was collected during the household interview. Smoking status was categorized as active smoker, former/passive smoker, or nonsmoker by the smoking questionnaire and serum cotinine levels as described previously (27). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. In NHANES 2005–2008, a questionnaire about alcohol intake, history of osteoporosis, and fractures was available for subjects aged 20 years and older. Alcohol intake was determined by the questionnaire ("In any one year, have you had at least 12 drinks of any type of alcoholic beverage?") and was dichotomized. Osteoarthritis was deter-

mined from the questionnaire ("Has a doctor or other health professional ever told you that you had arthritis?" and "Which type of arthritis was it?"). Osteoporosis was determined from the questionnaire ("Has a doctor ever told you that you had osteoporosis, sometimes called thin or brittle bones?"). Prior osteoporosis treatment was determined from the questionnaire ("Were you ever treated for osteoporosis?"), as was prior use of prednisone or cortisone medication ("Have you ever taken any prednisone or cortisone pills nearly every day for a month or longer?"). History of hip, wrist, or spine fracture was determined from the questionnaire ("Has a doctor ever told you that you had a broken or fractured hip, wrist, or spine?"). All types of fracture were determined by the sum of hip, wrist, or spine fractures and other fractures from the questionnaire ("Has a doctor ever told you that you had broken or fractured any other bone after 20 years of age?"). Menopause was assessed by women choosing menopause/hysterectomy as an answer ("What is the reason that you have not had a period in the past 12 months?") in the questionnaire.

Statistical analysis

Concentrations of PFCs are expressed as the geometric mean with a 95% confidence interval (CI) in different subgroups and were tested by Student's two-tailed *t* test and one-way ANOVA. A natural log transformation was performed for PFC concentrations with a significant deviation from a normal distribution before further analyses. We calculated separate multivariable linear regression models for men, women not in menopause, and women in menopause. We also used an extended model approach for covariates to adjust for potential confounds in multiple linear regression models to study the association between PFOA, PFOS and total lumbar spine and total hip BMD. Model 1 adjusted for age (continuous variable) and race/ethnicity (categorical). Model 2 adjusted for model 1, BMI (continuous variable), smoking status (categorical), alcohol use (categorical), prior osteoporosis treatment (categorical), and prior daily use of prednisone or cortisone (categorical). To avoid a model-dependent association, an association was considered significant only when it was statistically significant in both models. To evaluate dose response effects across the population, the PFCs were also stratified across the population in quartiles. Each PFC was modeled separately in analyses.

Logistic regression analyses were conducted to examine the odds ratios (ORs) of self-reported fractures with a unit increase in natural log-transformed PFCs concentrations. Analyses were performed with sample weights to examine the effects of weighting. Sampling weights were calculated using procedures that follow the National Center for Health Statistics Analytic Guidelines and properly account for the complex survey design employed in NHANES 2005–2008 by the Complex Sample Survey module of SPSS 20.0 f (SPSS Inc). A mobile examination center-weight variable was created by assigning half of the 2-year weight for 2005 to 2006 and assigning half of the 2-year weight for 2007 to 2008. A value of $P < .05$ was considered significant.

Results

The geometric means (SD) of PFOA and PFOS are 3.96 (3.86) ng/mL and 15.32 (17.58) ng/mL, respectively. The basic demographics of the sample population are outlined in Table 1. The study sample consisted of 1192 men and 1147 women. The results indicate that the men had a higher average concentration of PFC compounds than women. Advanced age (60 y and older) was associated with a higher serum PFC concentration. Mexican-American subjects had a lower mean serum concentration of these compounds than Hispanic-American subjects and non-Hispanic African-American subjects. In addition, PFOA was found in a higher concentration in subjects who were active smokers.

Among the 1147 women (842 premenopausal, 305 menopausal) in this study, the prevalence rates of osteoarthritis and osteoporosis in premenopausal women were 5.6% and 4.9%, respectively, and 16.4% and 20.7% in menopausal women, respectively. The data analysis with

Table 1. Basic Demographics and Geometric Mean With 95% CI of Serum PFC Concentrations

	n	PFOA, ng/mL	*P* Value	PFOS, ng/mL	*P* Value
Sex			<.001		<.001
Male	1192	4.70 (4.54, 4.87)		19.23 (18.46, 20.03)	
Female	1147	3.31 (3.19, 3.45)		12.09 (11.56, 12.64)	
Age, y			<.001		<.001
<40	839	3.60 (3.43, 3.77)		11.95 (11.37, 12.56)	
<60	836	3.89 (3.72, 4.06)		15.22 (14.44, 16.04)	
≧60	664	4.58 (4.37, 4.79)		21.13 (20.00, 22.32)	
Race			<.001		<.001
Non-Hispanic-American	1128	4.46 (4.31, 4.62)		16.57 (15.88, 17.28)	
Non-Hispanic African-American	464	3.98 (3.72, 4.25)		19.32 (17.90, 20.85)	
Mexican-American	456	3.14 (2.96, 3.34)		11.66 (10.91, 12.47)	
Others	291	3.57 (3.30, 3.87)		11.95 (10.92, 13.09)	
Smoking status			<.001		.883
Never smoked	1010	3.75 (3.59, 3.92)		15.21 (14.49, 15.97)	
Passive smoker	636	3.92 (3.72, 4.13)		15.56 (14.65, 16.53)	
Active smoker	693	4.33 (4.13, 4.54)		15.24 (14.40, 16.13)	

Tested by a Student's two-tailed *t* test and a one-way ANOVA in different subgroups.

Table 2. Adjusted Regression Coefficients (95% CIs) for Changes in Total Lumbar Spine BMD Relative to a Unit Increase in Natural Log-Transformed PFC Concentrations, With Results Weighted for Sampling Strategy

	Total Lumbar Spine BMD, g/cm^2		
		Women	
Analytes	Men	Not in Menopause	In Menopause
PFOA			
Model 1	0.009 (−0.011, 0.029)	0.000 (−0.023, 0.021)	0.021 (−0.012, 0.054)
Model 2	0.006 (−0.014, 0.026)	0.001 (−0.020, 0.022)	0.018 (−0.014, 0.049)
PFOS			
Model 1	0.005 (−0.009, 0.019)	−0.027 (−0.043, −0.010)[a]	0.005 (−0.024, 0.034)
Model 2	0.000 (−0.013, 0.013)	−0.022 (−0.038, −0.007)[a]	−0.004 (−0.026, 0.034)

Model 1 was adjusted for age, race/ethnicity. Model 2 was adjusted for model 1 plus BMI, smoking, drinking, treatment for osteoporosis, and use of prednisone or cortisone daily.

[a] $P < .01$.

results weighted for sampling is presented in Tables 2 and 3. In women not in menopause, a 1-U increase in natural log-transformed serum PFOS level was associated with a decrease in total lumbar spine BMD of 0.022 g/cm^2 (95% CI −0.038, −0.007; $P = .006$) in the final model. There was no association among serum PFOA, PFOS concentration, and femoral neck BMD.

A summary of the association among serum quartiles of PFCs, total lumbar spine, and total hip BMD after adjusting for potential covariates in men and women (both premenopausal and menopausal) is provided in Table 4. When serum PFOS concentrations were entered into the weighted model (controlling for age, race/ethnicity, BMI, smoking, drinking, prior osteoporosis treatment, and prior daily use of prednisone or cortisone), the mean total lumbar spine BMD decreased significantly with increasing quartiles of PFOS levels in women who were not in menopause (P for trend <0.001).

In logistic regression models adjusting for age, race/ethnicity, BMI, smoking, drinking, prior osteoporosis treatment, and prior daily prednisone or cortisone use (Table 5), there was no association between natural log-transformed serum PFCs concentration and self-reported fracture in both women and men.

Discussion

To our knowledge, there is no previous epidemiological study on exposure to PFCs and BMD. In the present cross-sectional analysis of the general US population, PFOS was associated with decreased total lumbar spine BMD in women not in menopause. This study shows that the relationship between PFCs and BMD may be much more complex than expected.

Experimental studies with laboratory rodents in vivo and with bone tissues in vitro suggest that bone tissue could be an important target for a number of EDCs such as organic tin compounds (28), alkylphenols (29), dioxin, and dioxin-like compounds (5, 30). For example, tributyltin, an organic tin compounds mainly exposed via seafood, was associated with reduced ossification in the fetuses of Sprague Dawley rat after the treatment of tributyltin from 0 to 19 gestation days with doses of 10 or

Table 3. Adjusted Regression Coefficients (95% CIs) for Changes in Total Hip BMD Relative to a Unit Increase in Natural Log-Transformed PFC Concentrations, With Results Weighted for Sampling Strategy

	Total Hip BMD, g/cm^2		
		Women	
Analytes	Men	Not in Menopause	In Menopause
PFOA			
Model 1	0.006 (−0.017, 0.028)	0.006 (−0.014, 0.025)	0.029 (−0.003, 0.061)
Model 2	−0.002 (−0.023, 0.019)	0.008 (−0.010, 0.027)	0.022 (−0.011, 0.055)
PFOS			
Model 1	0.007 (−0.008, 0.022)	−0.008 (−0.027, 0.011)	0.023 (−0.009, 0.054)
Model 2	−0.003 (−0.016, 0.010)	0.000 (−0.017, 0.017)	0.017 (−0.012, 0.047)

Model 1 adjusted for age, race/ethnicity. Model 2 adjusted for model 1 plus BMI, smoking, drinking, treatment for osteoporosis, and use of prednisone or cortisone daily.

Table 4. Mean ± SE of BMD Across Quartiles of Serum PFOS and PFNA in Linear Regression Models in Women, With Results Weighted for Sampling Strategy

	Total Lumbar Spine BMD, g/cm²			Total Hip BMD, g/cm²		
		Women			Women	
	Men	Not in Menopause	In Menopause	Men	Not in Menopause	In Menopause
PFOA, ng/mL						
≤25th	0.983 ± 0.035	1.022 ± 0.024	0.876 ± 0.020	0.966 ± 0.022	0.928 ± 0.017	0.805 ± 0.021
25th to 50th	0.985 ± 0.036	1.020 ± 0.019	0.886 ± 0.034	0.964 ± 0.025	0.934 ± 0.015	0.801 ± 0.024
50th to 75th	0.984 ± 0.037	1.035 ± 0.023	0.859 ± 0.017	0.958 ± 0.023	0.924 ± 0.016	0.809 ± 0.019
>75th	0.992 ± 0.036	1.022 ± 0.023	0.928 ± 0.016	0.962 ± 0.024	0.943 ± 0.013	0.885 ± 0.018
P value for trend	.933	.688	.062	.097	.374	.072
PFOS, ng/mL						
≤25th	0.978 ± 0.033	1.027 ± 0.020	0.894 ± 0.024	0.959 ± 0.021	0.923 ± 0.015	0.802 ± 0.021
25th to 50th	0.995 ± 0.038	1.040 ± 0.021	0.882 ± 0.025	0.973 ± 0.025	0.920 ± 0.015	0.803 ± 0.022
50th to 75th	0.991 ± 0.037	1.034 ± 0.023	0.892 ± 0.015	0.968 ± 0.024	0.950 ± 0.015	0.829 ± 0.020
>75th	0.990 ± 0.036	0.995 ± 0.023	0.904 ± 0.022	0.957 ± 0.022	0.924 ± 0.015	0.848 ± 0.017
P value for trend	.620	<.001	.689	.503	.141	.245

Adjusted for age, race/ethnicity, BMI, smoking, drinking, treatment for osteoporosis, and use of prednisone or cortisone daily.

20 mg/kg (28). The mechanisms were proposed to be associated with the disturbances in maternal thyroid hormone homeostasis (28).

Another notable example is alkylphenols, which are nonionic surfactants used in the manufacture of plastics, detergents, paints and pesticides (31). 4-Tert-octylphenol, a major degradation product of alkylphenol, was demonstrated to reduce bone formation in offspring mice when adding in drinking water with a low (1 μg/mL) or high (10 μg/mL) dose of the maternal mice from gestational day 10 lactation period to the pups (29). In vitro studies have proved that 4-tert-octylphenol and other alkylphenols have the potential to affect the intracellular signaling processes indispensable for bone remodeling and homeostasis, such as the gene expression regulated via the estrogen receptors and the conversion of T into estrogen by aromatase, and the function of aryl hydrocarbon receptor (AhR) (32). Overall, the molecular mechanisms of the EDC action on bone tissue are complex and exerted upon multiple targets. EDCs have been described to act as selected modulators of estrogen, androgen, thyroid, and other receptors by activating several signaling cascades, in particular those related to the AhR, a receptor involved in the metabolism of many xenobiotic substances (5, 33). High-affinity AhR ligands such as biphenyl exposure, a dioxin-like compound, has been shown to be associated with decreased BMD in human epidemiological study (34).

The effects of PFCs on BMD in both animals and humans have never been reported. At present, the mechanisms by which PFCs might affect normal bone metabolism are not known. There are several potential pathways that might link PFCs to reduced BMD. First, PFCs, like other EDCs, have been demonstrated to be associated with thyroid and sex hormone dysfunction, and both are important determinant factors for BMD (1). For thyroid function, chronic exposure to PFCs has been shown to suppress serum T_4 and T_3 in human studies (19, 20, 35–37), probably through increased conjugation of total T_4 in

Table 5. Associations Between Bone Fractures and Unit Increase in Natural Log-Transformed PFCs in Logistic Regression Models in Women and Men, With Results Weighted for Sampling Strategy

	All Types of Fracture OR (95% CI)	Hip, Wrist and Spine Fracture OR (95% CI)	Hip Fracture OR (95% CI)	Wrist Fracture OR (95% CI)	Spine Fracture OR (95% CI)
Women not in menopause					
PFOA	0.98 (0.75–1.28)	1.09 (0.66–1.79)	1.59 (0.57–4.46)	1.07 (0.65–1.77)	1.83 (0.59–5.61)
PFOS	0.97 (0.75–1.24)	1.05 (0.64–1.73)	1.12 (0.62–2.03)	1.04 (0.63–1.72)	0.52 (0.15–1.86)
Women in menopause					
PFOA	1.53 (0.63–3.74)	1.12 (0.38–3.29)	0.48 (0.06–4.16)	1.21 (0.46–3.13)	0.84 (0.46–1.53)
PFOS	1.59 (0.88–2.86)	1.21 (0.61–2.38)	0.83 (0.23–3.00)	1.22 (0.61–2.45)	1.12 (0.26–4.78)
Men					
PFOA	0.84 (0.67–1.07)	1.04 (0.71–1.54)	0.64 (0.39–1.06)	1.12 (0.75–1.70)	1.54 (0.85–2.79)
PFOS	0.92 (0.73–1.16)	1.04 (0.74–1.47)	1.07 (0.76–1.52)	1.09 (0.72–1.66)	1.27 (0.67–2.42)

Adjusted for age, race/ethnicity, BMI, smoking, drinking, treatment for osteoporosis, and use of prednisone or cortisone daily.

the liver (12) and directly interfering the AhR function (38). For sex hormones, in vitro studies have proved that, like other EDCs, PFCs might interfere directly with estrogen and androgen receptors (39). In vivo, numerous studies have demonstrated that PFCs are capable of disrupting the biological effects of sex hormones in animals (14–16), whereas the data are limited in human beings. Recent studies have suggested that exposure to PFOA and PFOS is associated with a reduced fecundity in women (40, 41). One survey investigated PFCs and endocrine disruption in women living near a chemical plant (the C8 Health Project), and there was a significant inverse association between PFOS and estradiol in the perimenopausal and menopausal age groups (42). The C8 Health Project also found that PFOS was associated with delayed puberty in boys, and both PFOS and PFOA were associated with reduced postmenarche for girls (21). A delay in puberty is associated with a higher risk of osteopenia in men (43) and with BMD in women, which may be related to osteoporosis (44). Second, toxicological studies in animals and cell systems have shown that PFOS and PFOA increase oxidative stress (17, 18), which is a predisposing factor for reduced BMD (4).

One interesting finding of the current analyses is that a higher serum PFOS concentration is associated with decreased total lumbar spine BMD only in women who are not in menopause. The reasons are unclear, but one could hypothesize that the antiestrogen effect of PFOS might be estrogen dependent. In one in vitro study, PFOS was demonstrated to have certain estrogenic activities, but exposure to a combination of estrogen and PFOS produced antiestrogenic effects in primary cultured hepatocytes from fish (45). Indeed, the association between serum PFOS concentration and BMD is not observed among postmenopausal women, who have no endogenous estrogen production from the ovaries. Another finding is that the lumbar spine seems to be more sensitive than the hip to PFOS exposure in women. There are several potential explanations for this observation. First, the lumbar region of the bone might have a higher metabolic rate than the hip region because of a higher content of trabecular bone. Trabecular bone has a greater surface area in comparison with cortical bone; consequently, it is ideal for metabolic activity. Second, hormones influence skeletal morphology and physiology, but not all parts of a bone show similar responses. For example, the trabecular part of the long bones is used as an easily accessible calcium storage source for physiological functions, whereas other skeletal elements are not used as readily (46).

Finally, our results showed no association between serum PFC concentration and self-reported fractures in women. Clinical trials of bisphosphonates that found differences in clinical outcomes such as fracture rates reported corresponding differences in BMD of 5% or greater in women with postmenopausal osteoporosis (47). The difference in the mean BMD between the low quartile and high quartile of PFOS was only 5.8% in premenopausal women; these subjects had a higher average BMD than postmenopausal women. This effect of PFOS on lumbar spine BMD may be too small to discern a significant difference in clinical outcomes in this group of women. Additionally, not all osteoporotic lumbar vertebral fractures are symptomatic.

There were several limitations to the present analysis. First, because of the observational and cross-sectional study design, we are unable to make any conclusions regarding causation in the relationship between serum PFOS concentration and BMD. Second, a common physiology could influence serum PFOS concentration and BMD independent of exposure. Third, exercise is known to increase bone density and improve bone health (48). However, there were different subsamples of subjects between the measurement of serum PFC level and the questionnaire on physical activity, so we did not account for this as a covariate in this study. Fourth, an increase in oxidative stress is a possible cause of the reduced bone mass measured in subjects with higher PFC levels. However, no antioxidant enzyme assay has been performed in the study population, and we cannot include it as a covariate in the analysis. Finally, data on thyroid levels were not available in the 2005–2006 NHANES database, and data on parathyroid hormone levels were not available in 2007–2008, so we could not use these as covariates in this survey.

In conclusion, using the NHANES data from the US population, we found that a higher serum concentration of PFOS is associated with a decrease in total lumbar spine BMD in women not in menopause. These findings suggest an effect of low-dose PFOS in humans, although the potential biological significance of this effect is small and subclinical in the general US population.

Acknowledgments

We thank the many people who have contributed to the National Health and Nutrition Examination Survey data, including all of the anonymous participants in the study.

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Disclosure Summary: The authors have nothing to disclose.

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