Associations of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS mixtures with adipokines in midlife women

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ABSTRACT

Background: Perfluoroalkyl and polyfluoroalkyl substances (PFAS) exposure have been associated with obesity and related comorbidities, possibly through disrupting signaling pathways of adipokines. Both leptin and adiponectin can modulate metabolic processes. However, the effects of PFAS on adipokines are not well understood.
Objective: We determined if serum PFAS concentrations were associated with adipokine profiles in midlife women.
Methods: We examined 1245 women aged 45–56 years from the Study of Women's Health Across the Nation. Concentrations of 11 PFAS were quantified in baseline serum samples collected in 1999–2000. Linear and branched perfluorooctane sulfonic acid isomers (n-PFOS and Sm-PFOS) and their sum (PFOS), linear perfluorooctanoic acid (n-PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA), and 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (EtFOSAA) with detection frequencies >60% were included in the analysis. Adipokines including leptin, soluble leptin receptor (sOB-R), free leptin index (FLI, the ratio of leptin to sOB-R), total and high molecular weight (HMW) adiponectin were assessed in 2002–2003. We utilized multivariable linear regressions and Bayesian kernel machine regression (BKMR) to assess individual and overall joint effects of PFAS on adipokines with adjustment for age, race/ethnicity, study site, education, smoking status, physical activity, menopausal status, and waist circumference.
Results: A doubling of PFAS concentrations was associated with 7.8% (95% CI: 2.5%, 13.4%) higher FLI for PFOS, 9.4% (95% CI: 3.7%, 15.3%) for n-PFOA, 5.5% (95% CI: 2.2%, 9.0%) for EtFOSAA and 7.4% (95% CI: 2.8%, 12.2%) for MeFOSAA. Similar associations were found for leptin. Only EtFOSAA was associated with lower sOB-R concentrations (−1.4%, 95% CI: −2.7%, −0.1%). Results remained in women with overweight or obesity but not those with normal weight or underweight. No statistically significant associations were observed with total or HMW adiponectin, except for PFNA with total and HMW adiponectin observed in women with normal weight or underweight. In BKMR analysis, women with PFAS concentrations at the median and the 90th percentile had 30.9% (95% CI: 15.6%, 48.3%) and 52.1% (95% CI: 27.9%, 81.0%) higher FLI, respectively, compared with those with concentrations fixed at the 10th percentile.
Conclusion: Some PFAS may alter circulating levels of leptin. Understanding associations between PFAS and adipokines may help elucidate whether PFAS can influence obesity and metabolic disease.

1. Introduction

The global epidemic of overweight and obesity presents a major challenge to chronic disease prevention across the life course (Afshin et al., 2017). While the role of many modifiable risk factors for obesity including high caloric diet and sedentary lifestyle have been widely

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studied, the role of environmental factors is beginning to receive attention. One factor of interest is perfluoroalkyl and polyfluoroalkyl substances (PFAS), a family of synthetic chemicals with ubiquitous human exposure (ATSDR, 2018). PFAS have been used in many consumer products including upholstery, non-stick cookware, food packaging, personal care products, and fire-fighting foams (ATSDR, 2018). A recent nationwide survey reported that more than 200 million United States residents could have PFAS-contaminated drinking water (Andrews and Naidenko, 2020). In addition, PFAS are extremely stable in the environment and some have long elimination half-lives in humans (Ding et al., 2020a; Olsen et al., 2007).

Previous epidemiologic studies suggest that PFAS may impact metabolic function (Cardenas et al., 2018; Ding et al., 2021; Liu et al., 2018), alter glucose homeostasis (Cardenas et al, 2017, 2019; Sun et al., 2018), and affect lipid metabolism (Eriksen et al., 2013; Lin et al., 2019; Starling et al., 2014). PFAS were also found to promote murine adipogenesis and trigger adipocyte differentiation of human adipose tissues and their derived stem cells *in vitro* (Liu et al., 2019; Watkins et al., 2015; Xu et al., 2016). Growing evidence supports obesity as a disorder of energy homeostasis rather than simple accumulation of excess adiposity (Schwartz et al., 2017). To evaluate whether PFAS may increase the risk of obesity-related disorders and understand the etiology, examining the effects of PFAS on key regulatory factors of human adipose tissue is critically important.

Adipose tissue serves as an active endocrine organ and secretes multiple hormone-like adipokines such as leptin and adiponectin to modulate metabolic processes (Ouchi et al., 2011). Leptin, a key pro-inflammatory adipokine, is expressed proportionately to body fat mass and is positively associated with control of appetite and energy homeostasis (Watkins et al., 2015). Despite this, most individuals with obesity have elevated leptin concentrations in circulating and active forms, indicating a state of leptin resistance (Lee et al., 2009). Soluble leptin receptor (sOB-R) can downregulate the action of leptin through the formation of leptin-sOB-R complexes (Lammert et al., 2001). The free leptin index (FLI), the ratio of total leptin to sOB-R concentrations, has been proposed as a biomarker of leptin resistance to give information on leptin bioavailability (Kratzsch et al., 2002). As opposed to leptin, serum adiponectin correlates inversely with fat mass and insulin resistance and exerts anti-inflammatory effects (Watkins et al., 2015). The high molecular weight (HMW) isoform of adiponectin is the most biologically active, compared to its low and middle molecular weight counterparts (Ouchi et al., 2011).

The impact of PFAS on adipokines is not well understood. Among the limited number of epidemiologic studies of the association between PFAS and adipokines (Bassler et al., 2019; Cardenas et al., 2017; Lin et al., 2011; Liu et al., 2018) findings are inconsistent. Their small sample size may also limit the power to observe associations. Additionally, people are exposed to multiple PFAS on a daily basis, but all previous studies focused on individual compounds. Studies of chemicals assessed individually are subject to non-linear dose-response relationships and confounding by co-exposure (Ding et al., 2020b; Wang et al, 2018, 2019b, 2020b). Furthermore, it is largely unknown whether the menopausal transition, which is accompanied by unfavorable changes in body composition and abdominal fat deposition (Greendale et al., 2019), may be involved in the potential effects of PFAS on metabolic disorders.

Therefore, we examined associations between PFAS concentrations and leptin, sOB-R, adiponectin, and HMW adiponectin in the Study of Women's Health Across the Nation (SWAN), a large, prospective cohort of women transitioning through menopause. We also considered multiple PFAS and assessed their joint effects on adipokine profiles. Given that leptin and adiponectin may account for the associations between adipose tissue and inflammation in individuals with overweight and obesity (Conroy et al., 2011), we conducted a secondary analysis with stratification by overweight and obesity status and hypothesized that women with overweight and obesity may be more vulnerable to the effects of PFAS on adipokines. To identify the time window of susceptibility during the menopausal transition, we also examined the associations between PFAS and adipokines stratified by menopausal status.

2. Methods

2.1. Study population

The present study included a sub-cohort of participants from SWAN, a multi-site, multi-racial/ethnic, community-based prospective study designed to characterize physiological and psychosocial changes that occur during menopausal transition (Santoro et al., 2011). In 1996 to 1997, a total of 3302 premenopausal women aged 42–52 years were recruited from seven study sites, including Boston, MA; Chicago, IL; Southeast MI; Los Angeles, CA; Newark, NJ; Oakland, CA; and Pittsburgh, PA. Each site enrolled a sample of non-Hispanic White women plus women from one designated minority group, including Black (Boston, Chicago, Southeast MI, Pittsburgh), Chinese (Oakland), Japanese (Los Angeles), and Hispanic (Newark). At enrollment, eligible participants had to have an intact uterus, at least one menstrual period in the prior three months, and not have taken hormone medications within the prior three months. The Institutional Review Board at each study site approved this study, and informed consent was obtained at each study visit.

The SWAN Multi-Pollutant Study (MPS) was initiated in 2016, using the SWAN follow-up visit 03 (V03, 1999–2000) as the MPS baseline to examine the potential health effects of multiple environmental chemicals. The study designs of the SWAN MPS were previously described (Ding et al., 2020a; Park et al., 2019; Wang et al., 2019a, 2020a, 2021). In brief, SWAN cohort at visit 03 had 2694 women eligible to be included in the SWAN MPS cohort. Women from Chicago (n = 368) and Newark (n = 278) were excluded due to a lack of urine samples. After further excluding 648 women with no or insufficient urine or serum samples for chemical assessment, 1400 women were included in the SWAN MPS. PFAS concentrations were quantified in 1400 participants at the SWAN MPS baseline (1999–2000), using serum samples from the SWAN Repository. After excluding 92 women with missing information on adipokines and 63 missing key covariates of interest, data from 1245 were available for the current analyses. The study design is displayed in the Supplemental Materials Figure S1.

2.2. PFAS assessment

Serum samples were sent to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC) for analysis. The CDC laboratory's involvement did not constitute engagement in human-subjects research. We measured perfluorohexane sulfonic acid (PFHxS), linear perfluorooctane sulfonic acid (n-PFOS), sum of branched isomers of PFOS (Sm-PFOS), linear perfluorooctanoic acid (n-PFOA), sum of branched isomers of PFOA (Sb-PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA), and 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (EtFOSAA) in 0.1 mL of serum by online solid phase extraction-high performance liquid chromatography-isotope dilution-tandem mass spectrometry (Kato et al., 2011). Total PFOS (PFOS) was computed as the sum of n-PFOS and Sm-PFOS. Most of the total PFOA (PFOA) is n-PFOA because of low detection of Sb-PFOA (<20%), and thus we did not calculate total PFOA and used instead n-PFOA in the analysis. The limit of detection (LOD) was 0.1 ng/mL for all PFAS examined. Measurements below the LODs were replaced by $LOD/\sqrt{2}$ (Hornung and Reed, 1990). The coefficients of variation of low- and high-concentration quality control (QC) pools were 5.9–12.1% (low QC) and 5.9–10.6% (high QC), depending on the analyte. PFAS were

significantly and positively correlated with each other, as shown in the Supplemental Materials Figure S2.

2.3. Adipokine assessment

Assays were run on stored serum samples collected from the SWAN follow-up visit 6, corresponding to the MPS follow-up (2002–2003). At collection, a 12-h fasting blood draw was performed within days 2–5 of the menstrual cycle if a woman was still menstruating. For women not regularly menstruating or if a blood sample was not obtainable in the day 2–5 window, a random fasting blood draw was obtained. For women who were postmenopausal, a blood sample was drawn at their clinic visit, scheduled to be on the anniversary of their previous visit. Leptin, sOB-R, adiponectin and HMW adiponectin were determined at the University of Michigan in duplicate, using commercially available colorimetric enzyme immunoassay kits according to the manufacturer's instructions (adiponectin, HMW adiponectin, resistin, and leptin, Millipore, St. Charles, MO and soluble leptin receptor, R& D systems, Minneapolis, MN). The mean coefficient of variation percent for duplicate samples for each subject and lower limit of detection, respectively, were: adiponectin: 4%, 0.78 ng/mL; HMW adiponectin: 8.1%, 0.5 ng/ml; resistin: 5%, 0.16 ng/mL; leptin: 4%, 0.5 ng/mL; and soluble leptin receptor: 3.7%, 0.31 ng/ml.

2.4. Covariates

A comprehensive set of confounders were selected *a priori* (Khan et al., 2012; Park et al., 2019). Information on covariates was obtained from standardized interviews. Covariates selected in this analysis are from SWAN follow-up visit 3, to coincide with the SWAN MPS baseline. Sociodemographic variables included age (in years), self-identified race/ethnicity, study site, and education (high school or less, some college, and college degree or higher). Self-reported lifestyle factors consisted of smoking status (never smoked, past smoker, and current smoker) (Ferris, 1978) and physical activity (Sternfeld et al., 1999). Menopausal status was obtained from self-reported bleeding patterns in the prior year, categorized as follows: premenopausal (menses in past 3 months with no change in bleeding pattern), early perimenopausal (menses in past 3 months but decreasing predictability between menses), late perimenopausal (no menses in past 3–11 months), natural menopause (no menses in past 12 or more months), surgical menopause (history of hysterectomy or had two ovaries removed), and unknown (past-year use of HT before the documentation of a final menstrual period). Height and weight were measured without shoes, and in light indoor clothing. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Waist circumference was measured to the nearest 0.1 cm at the level of the natural waist, defined as the narrowest part of the torso as seen from the anterior aspect.

2.5. Statistical analysis

Descriptive statistics were calculated for baseline PFAS concentrations, participant characteristics and adipokine concentrations. PFAS concentrations were log-transformed with base 2, and leptin, sOB-R, total and HMW adiponectin concentrations were log-transformed to ensure normality. Associations between PFAS and adipokines were estimated using linear regression models with PFAS assessed individually. Effect estimates were back transformed and interpreted as percent changes (95% confidence intervals, 95% CIs) in adipokine concentrations per doubling increase in PFAS concentrations. Percent changes should, therefore, be interpreted as relative differences in adipokine concentrations. Models were initially adjusted for age, race/ethnicity, study site, education, smoking status, physical activity, and menopausal status. Because adipokines are secreted by adipose tissues, adiposity could be an intermediate on the causal pathway from PFAS to adipokines. On the other hand, obesity status is an independent determinant

of PFAS concentrations (Park et al., 2019), and thus adiposity could also be a confounder. Thus, we compared models with and without adjustment of waist circumference as a surrogate measure of adiposity. Sensitivity analyses considered BMI instead of waist circumference as the marker of adiposity used as a covariate in the regressions. To relax the assumption of linearity of the associations between PFAS and adipokines, we categorized PFAS concentrations based on quartiles of their distributions and examined the associations between PFAS quartiles and adipokines.

We evaluated the joint effects of n-PFOS, Sm-PFOS, n-PFOA, PFHxS, PFNA, EtFOSAA and MeFOSAA on adipokine profiles, using Bayesian kernel machine regression (BKMR), while accounting for non-linear dose-response relationships, correlations among chemicals, and confounding by correlated co-pollutants (Bobb et al., 2015). BKMR is a semi-parametric machine learning method that can be used to evaluate the effects of individual PFAS, the overall effects, and interactions between different PFAS. The general modeling framework can be represented by the equation, $Y_i = h(Z_i) + X_i^T \beta + \varepsilon_i$, where Y_i is the outcome, h is a kernel function of the predictor variables ., and X_i is a vector of covariates with the corresponding vector of coefficients β. Kernel machine regression provides flexibility to model non-linear relations between a number of variables and a particular response variable, and to estimate the overall cumulative effects of PFAS mixtures. A Gaussian kernel was chosen to support flexible exposure-response shapes. Posterior inclusion probabilities (PIPs) were derived to estimate the importance of variables for the associations between PFAS mixtures and adipokines (Bobb et al., 2015). We also ran our BKMR analysis controlling for waist circumference and BMI in separate analyses.

Small adipocytes in individuals with normal weight promote metabolic homeostasis; the enlarged adipocytes of individuals with obesity recruit macrophages and promote inflammation. Therefore, as a secondary analysis, we assessed effect modification by overweight and obesity status, to evaluate potential heterogeneity in the magnitude of the associations. To examine this, we stratified our linear regression models by overweight/obesity status. Overweight/obesity was defined as BMI≥25 kg/m^2 for White and Black women, and BMI≥23 kg/m^2 for Chinese and Japanese women. Furthermore, it is unclear whether menopausal status may play a role in the potential effects of PFAS on adipokines. Thus, we evaluated the associations between PFAS and adipokines stratified by menopausal status. Due to small sample sizes and uncertainties in surgical menopause and hormone therapy, menopausal status was classified into pre-/early perimenopause and late peri-/postmenopause. All analyses were conducted using SAS version 9.4 (SAS Institute Inc), except for BKMR, which we implemented with R package 'bkmr' using R version 3.6.0 (R Foundation For Statistical Computing).

3. Results

3.1. Participant characteristics

Table 1 shows participant characteristics and adipokine concentrations at SWAN-MPS baseline. Women had a median age of 49.5 (interquartile range, IQR: 47.3, 51.5) years. Nearly half of the sample (51.1%) was White, and 20.9% were African American, 12.6% Chinese, and 15.4% Japanese. Approximately half of women (51%) attended some college or more, and most (62.9%) were never smokers. Most women were pre- (11.4%), early peri- (50.7%), or late perimenopausal (8.4%), whereas only 14.8% were postmenopausal and nearly 15% could not be determined due to use of hormone therapy. Women had a median BMI of 25.9 (IQR: 22.5, 31.4) kg/m^2, and a median waist circumference of 81.7 (IQR: 73.4, 94.1) cm.

The median (IQR) of MPS baseline serum concentrations was 1.5 (1.0–2.4) ng/mL for PFHxS, 24.7 (17.6–36.0) ng/mL for PFOS, 4.1 (2.9–5.8) ng/mL for n-PFOA, 0.6 (0.4–0.8) ng/mL for PFNA, 1.2

Table 1

Characteristics of study participants from the Study of Women's Health Across the Nation Multi-Pollutant Study (SWAN MPS) baseline (1999–2000) and adipokine concentrations at SWAN MPS follow-up (2002–2003).

Characteristics	Total (N = 1245)
	Median (IQR) or N (%)
Age, years	49.5 (47.3, 51.5)
Race/ethnicity	
White	636 (51.1%)
Black	260 (20.9%)
Chinese	157 (12.6%)
Japanese	192 (15.4%)
Education	
≤High school	219 (17.6%)
High school	391 (31.4%)
College	317 (25.5%)
Post college	318 (25.5%)
Smoking status	
Never smoker	783 (62.9%)
Past smoker	340 (27.3%)
Current smoker	122 (9.8%)
Menopausal status	
Surgical postmenopause	39 (3.1%)
Natural postmenopause	145 (11.7%)
Late perimenopause	105 (8.4%)
Early perimenopause	631 (50.7%)
Premenopause	142 (11.4%)
Unknown due to hormone therapy	183 (14.7%)
Physical activity score	7.9 (6.6, 9.0)
Body mass index, kg/m^2	25.9 (22.5, 31.4)
Waist circumference, cm	81.7 (73.4, 94.1)
Adipokines	
Leptin, ng/mL	16.3 (9.0, 29.1)
sOB-R, ng/mL	29.9 (24.0, 37.4)
Free leptin index	0.54 (0.27, 1.07)
Adiponectin, μg/mL	11.2 (7.7, 15.7)
HMW adiponectin, μg/mL	6.8 (4.5, 10.5)

(0.7–2.2) ng/mL for EtFOSAA, and 1.5 (0.9–2.3) ng/mL for MeFOSAA (Table S1). Sb-PFOA, PFDA, PFUnDA and PFDoDA with detection frequencies <60% were not included in the analysis.

3.2. Individual associations of PFAS with adipokine profiles: linear models

Associations between serum PFAS concentrations and adipokines are presented in Table 2. Both leptin and FLI had a statistically significant positive relationship with baseline PFAS concentrations, with the exception of PFNA and PFHxS. Controlling for age, race/ethnicity, study site, education, smoking status, physical activity and menopausal status, FLI was associated with PFOS (percent change: 14.7%, (95% CI: 7.7%, 22.3%) per doubling in concentrations), n-PFOS (12.4%, 95% CI: 5.4%, 19.9%), Sm-PFOS (15.3%, 95% CI: 9.3%, 21.6%), n-PFOA (16.0%, 95% CI: 8.5%, 24.0%), EtFOSAA (9.8%, 95% CI: 5.4%, 14.3%), and MeFO-SAA (8.9%, 95% CI: 3.1%, 15.1%). Similar results were also observed for leptin. The magnitude of the associations between PFAS and leptin and FLI were attenuated by approximately 50% in models further adjusted for waist circumference. Further, after adjustment for waist circumference, PFNA was now statistically significantly associated with both leptin (5.4%, 95% CI: 1.2%, 9.9%) and FLI (7.3%, 95% CI: 2.1%, 12.8%). Baseline concentrations of n-PFOA and EtFOSAA, however, were significantly inversely associated with sOB-R concentrations (percent change: 2.5%, 95% CI: 4.7%, −0.3% for n-PFOA; −2.1%, 95% CI: 3.4%, −0.7% for EtFOSAA). These associations were also attenuated following further adjustment for waist circumference, and the association between n-PFOA and sOB-R was no longer statistically significant. There were no statistically significant associations with any of PFAS compounds and adiponectin or HMW adiponectin. When modeling PFAS quartiles, PFOS, and n-PFOA were positively associated with leptin and FLI (Table 3). Additionally, we observed potential non-linear

Table 2

Percent change and 95% confidence interval (95% CI) of free leptin index, leptin, sOB-R, adiponectin, and HMW adiponectin per doubling increase in serum concentrations of PFOS, n-PFOS, Sm-PFOS, n-PFOA, PFNA, PFHxS, EtFOSAA and MeFOSAA.

	Free leptin index	Leptin, ng/mL	sOB-R, ng/mL	Adiponectin, μg/mL	HMW adiponectin, μg/mL
	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %
PFOS					
Model 1	14.7 (7.7, 22.3)***	13.6 (7.8, 19.7)***	−1.0 (−3.1, 1.1)	0.9 (−2.5, 4.3)	0.2 (−3.5, 4.1)
Model 2	7.8 (2.5, 13.4)**	8.0 (3.5, 12.6)**	0.1 (−1.9, 2.2)	2.7 (−0.5, 6.0)	2.1 (−1.6, 5.9)
n-PFOS					
Model 1	12.4 (5.4, 19.9)**	11.6 (5.9, 17.7)***	−0.7 (−2.8, 1.5)	1.1 (−2.3, 4.6)	0.8 (−3.0, 4.7)
Model 2	7.3 (2.0, 13.0)**	7.5 (3.0, 12.2)**	0.2 (−1.9, 2.3)	2.4 (−0.8, 5.8)	2.1 (−1.5, 6.0)
Sm-PFOS					
Model 1	15.3 (9.3, 21.6)***	13.8 (8.9, 18.9)***	−1.3 (−3.1, 0.5)	0.4 (−2.4, 3.3)	−0.5 (−3.7, 2.7)
Model 2	6.9 (0.2, 11.5)**	7.0 (3.2, 10.9)**	0.1 (−1.6, 1.8)	2.7 (−0.03, 5.5)	1.8 (−1.3, 4.9)
n-PFOA					
Model 1	16.0 (8.5, 24.0)***	13.1 (7.0, 19.5)***	−2.5 (−4.7, −0.3)*	−1.9 (−5.3, 1.6)	−3.2 (−7.0, 0.8)
Model 2	9.4 (3.7, 15.3)**	7.8 (3.2, 12.7)**	−1.4 (−3.5, 0.7)	−0.2 (−3.5, 3.2)	−1.5 (−5.2, 2.3)
PFNA					
Model 1	4.6 (−1.8, 11.4)	3.1 (−2.1, 8.3)	−1.3 (−3.4, 0.8)	0.2 (−3.1, 3.6)	−1.1 (−4.7, 2.7)
Model 2	7.3 (2.1, 12.8)**	5.4 (1.2, 9.9)**	−1.8 (−3.7, 0.2)	−0.6 (−3.7, 2.5)	−1.9 (−5.3, 1.7)
PFHxS					
Model 1	−1.2 (−5.8, 3.7)	0.0 (−3.9, 4.0)	1.2 (−0.4, 2.8)	0.7 (−1.8, 3.3)	1.9 (−0.9, 4.9)
Model 2	0.8 (−3.0, 4.7)	1.6 (−1.5, 4.9)	0.8 (−0.7, 2.4)	0.1 (−2.2, 2.5)	1.3 (−1.4, 4.1)
EtFOSAA					
Model 1	9.8 (5.4, 14.3)***	7.5 (3.9, 11.1)***	−2.1 (−3.4, −0.7)**	−0.5 (−2.6, 1.7)	−1.5 (−3.9, 0.9)
Model 2	5.5 (2.2, 9.0)**	4.1 (1.3, 6.9)**	−1.4 (−2.7, −0.1)*	0.6 (−1.4, 2.7)	−0.4 (−2.7, 1.9)
MeFOSAA					
Model 1	8.9 (3.1, 15.1)**	7.9 (3.1, 13.0)**	−0.9 (−2.8, 0.9)	−1.0 (−3.8, 2.0)	0.5 (−2.8, 3.8)
Model 2	7.4 (2.8, 12.2)**	6.7 (2.9, 10.6)**	−0.7 (−2.4, 1.1)	−0.6 (−3.3, 2.2)	0.9 (−2.3, 4.1)

Model 1 was adjusted for age, race/ethnicity, study site, education, smoking status, physical activity and menopausal status.
Model 2: Model 1 + waist circumference.
***$P < 0.0001$, **$P < 0.01$, *$P < 0.05$.

Table 3

Percent change and 95% confidence interval (95% CI) of free leptin index, leptin, sOB-R, adiponectin, and HMW adiponectin by quartiles of serum concentrations of PFOS, n-PFOS, Sm-PFOS, n-PFOA, PFNA, PFHxS, EtFOSAA and MeFOSAA.

Concentrations by PFAS quartiles, range (ng/mL)	Free leptin index	Leptin, ng/mL	sOB-R, ng/mL	Adiponectin, µg/mL	HMW adiponectin, µg/mL
	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %
PFOS					
Model 1					
Q1 (2.0–17.5)	Ref	Ref	Ref	Ref	Ref
Q2 (17.6–24.6)	1.38 (−12.39, 17.31)	4.23 (−7.60, 17.57)	2.81 (−2.17, 8.04)	3.66 (−4.06, 12.00)	2.65 (−5.96, 12.05)
Q3 (24.7–36.0)	25.29 (8.13, 45.17)**	22.17 (8.19, 37.95)**	−2.49 (−7.25, 2.52)	1.62 (−6.02, 9.88)	−2.44 (−10.69, 6.58)
Q4 (36.1–376.0)	36.53 (17.16, 59.09)***	32.67 (16.94, 50.52)***	−2.82 (−7.75, 2.37)	0.48 (−7.35, 8.97)	−0.04 (−8.81, 9.57)
Model 2					
Q1 (2.0–17.5)	Ref	Ref	Ref	Ref	Ref
Q2 (17.6–24.6)	0.77 (−10.32, 13.22)	3.58 (−5.97, 14.09)	2.79 (−1.93, 7.73)	4.07 (−3.26, 11.95)	2.97 (−5.30, 11.96)
Q3 (24.7–36.0)	8.97 (−3.16, 22.61)	9.05 (−1.12, 20.26)	0.08 (−4.57, 4.95)	5.82 (−1.72, 13.94)	1.67 (−6.59, 10.66)
Q4 (36.1–376.0)	20.05 (6.22, 35.68)**	19.46 (7.93, 32.22)**	−0.49 (−5.28, 4.54)	4.34 (−3.37, 12.65)	3.85 (−4.88, 13.40)
n-PFOS					
Model 1					
Q1 (1.4–12.3)	Ref	Ref	Ref	Ref	Ref
Q2 (12.4–17.4)	7.99 (−6.66, 24.93)	10.58 (−1.94, 24.70)	2.40 (−2.54, 7.59)	4.72 (−3.04, 13.10)	4.51 (−4.22, 14.03)
Q3 (17.5–25.0)	17.00 (0.91, 35.66)*	16.88 (3.46, 32.05)*	−0.10 (−4.99, 5.04)	5.78 (−2.18, 14.38)	4.58 (−4.28, 14.27)
Q4 (25.1–250.0)	34.89 (15.72, 57.24)**	32.09 (16.40, 49.88)***	−2.08 (−7.04, 3.15)	0.29 (−7.51, 8.75)	0.83 (−8.01, 10.51)
Model 2					
Q1 (1.4–12.3)	Ref	Ref	Ref	Ref	Ref
Q2 (12.4–17.4)	4.65 (−6.81, 17.52)	7.66 (−2.21, 18.52)	2.87 (−1.83, 7.79)	5.89 (−1.52, 13.87)	5.61 (−2.83, 14.77)
Q3 (17.5–25.0)	7.31 (−4.62, 20.73)	8.97 (−1.18, 20.15)	1.54 (−3.17, 6.48)	8.41 (0.70, 16.70)	7.27 (−1.43, 16.74)
Q4 (25.1–250.0)	21.82 (7.81, 37.64)**	21.56 (9.86, 34.51)**	−0.21 (−5.00, 4.83)	3.29 (−4.31, 11.49)	3.91 (−4.80, 13.43)
Sm-PFOS					
Model 1					
Q1 (<LOD-4.7)	Ref	Ref	Ref	Ref	Ref
Q2 (4.8–7.2)	3.31 (−10.98, 19.90)	5.48 (−6.72, 19.26)	2.10 (−2.95, 7.41)	−0.14 (−7.74, 8.08)	0.56 (−8.06, 9.99)
Q3 (7.3–11.2)	20.33 (3.51, 39.89)*	20.38 (6.32, 36.30)**	0.04 (−4.96, 5.30)	2.46 (−5.42, 11.00)	0.03 (−8.63, 9.52)
Q4 (11.3–126.0)	47.23 (25.96, 72.09)***	40.22 (23.28, 59.48)***	−4.76 (−9.69, 0.43)	−2.56 (−10.31, 5.87)	−3.69 (−12.32, 5.79)
Model 2					
Q1 (<LOD-4.7)	Ref	Ref	Ref	Ref	Ref
Q2 (4.8–7.2)	−4.36 (−15.12, 7.77)	−1.09 (−10.42, 9.21)	3.41 (−1.44, 8.51)	2.39 (−4.98, 10.34)	3.05 (−5.41, 12.27)
Q3 (7.3–11.2)	4.29 (−7.58, 17.69)	7.11 (−3.11, 18.42)	2.70 (−2.18, 7.83)	6.92 (−0.87, 15.34)	4.44 (−4.24, 13.92)
Q4 (11.3–126.0)	16.46 (2.67, 32.09)*	15.81 (4.31, 28.58)**	−0.56 (−5.48, 4.62)	4.47 (−3.46, 13.05)	3.35 (−5.59, 13.14)
n-PFOA					
Model 1					
Q1 (<LOD-2.8)	Ref	Ref	Ref	Ref	Ref
Q2 (2.9–4.1)	5.52 (−9.18, 22.59)	5.39 (−6.90, 19.30)	−0.12 (−5.08, 5.09)	0.27 (−7.39, 8.57)	1.10 (−7.59, 10.61)
Q3 (4.2–5.8)	19.46 (2.06, 39.83)*	15.33 (1.26, 31.36)*	−3.46 (−8.48, 1.84)	−3.24 (−10.99, 5.19)	−5.96 (−14.43, 3.35)
Q4 (5.9–56.5)	45.11 (23.66, 70.29)***	33.92 (17.33, 52.86)***	−7.71 (−12.59, −2.56)**	−6.09 (−13.73, 2.22)	−8.90 (−17.23, 0.27)
Model 2					
Q1 (<LOD-2.8)	Ref	Ref	Ref	Ref	Ref
Q2 (2.9–4.1)	3.66 (−8.03, 16.84)	3.71 (−6.11, 14.56)	0.05 (−4.66, 4.99)	1.05 (−6.27,8.94)	1.79 (−6.61, 10.93)
Q3 (4.2–5.8)	4.91 (−7.49, 18.98)	3.68 (−6.62, 15.12)	−1.17 (−6.06, 3.97)	0.51 (−7.13, 8.78)	−2.30 (−10.74, 6.96)
Q4 (5.9–56.5)	24.51 (9.55, 41.52)**	18.11 (6.19, 31.37)**	−5.14 (−9.91, −0.12)*	−1.77 (−9.36, 6.45)	−4.69 (−13.07, 4.50)
PFNA					
Model 1					
Q1 (<LOD-0.4)	Ref	Ref	Ref	Ref	Ref
Q2 (0.5)	3.55 (−12.15, 22.06)	5.75 (−7.67, 21.11)	2.12 (−3.39, 7.94)	−4.98 (−12.85, 3.61)	−6.85 (−15.53, 2.73)
Q3 (0.6–0.7)	12.95 (−1.73, 29.83)	14.85 (2.39, 28.84)*	1.68 (−2.98, 6.57)	−0.54 (−7.56, 7.02)	0.82 (−7.19, 9.53)
Q4 (0.8–5.0)	12.24 (−2.57, 29.31)	7.69 (−4.18, 21.02)	−4.06 (−8.53, 0.63)	−0.34 (−7.49, 7.36)	−3.03 (−10.87, 5.48)
Model 2					
Q1 (<LOD-0.4)	Ref	Ref	Ref	Ref	Ref
Q2 (0.5)	5.67 (−7.23, 20.35)	7.60 (−3.42, 19.89)	1.83 (−3.35, 7.30)	−5.67 (−13.05, 2.35)	−7.48 (−15.72, 1.57)
Q3 (0.6–0.7)	10.97 (−0.62, 23.91)	13.36 (3.44, 24.24)**	2.16 (−2.27, 6.78)	−0.24 (−6.90, 6.90)	1.23 (−6.47, 9.56)
Q4 (0.8–5.0)	20.87 (8.03, 35.22)**	14.56 (4.36, 25.75)**	−5.22 (−9.39, −0.85)*	−2.67 (−9.28, 4.43)	−5.26 (−12.59, 2.68)
PFHxS					
Model 1					
Q1 (<LOD-0.9)	Ref	Ref	Ref	Ref	Ref
Q2 (1.0–1.4)	−0.33 (−14.40, 16.06)	3.87 (−8.39, 17.77)	4.21 (−1.03, 9.73)	7.51 (−0.78, 16.50)	10.57 (0.97, 21.08)*
Q3 (1.5–2.4)	20.22 (3.76, 39.29)*	20.73 (6.93, 36.32)**	0.42 (−4.46, 5.56)	−0.06 (−7.52, 8.01)	1.74 (−6.81, 11.07)
Q4 (2.5–46.5)	−5.46 (−19.24, 10.65)	−2.13 (−14.05, 11.44)	3.53 (−1.85, 9.20)	3.34 (−4.89, 12.29)	7.38 (−2.25, 17.94)
Model 2					
Q1 (<LOD-0.9)	Ref	Ref	Ref	Ref	Ref
Q2 (1.0–1.4)	1.28 (−10.29, 14.33)	5.38 (−4.70, 16.53)	4.05 (−0.90, 9.25)	6.80 (−0.99, 15.21)	9.94 (0.80, 19.90)*
Q3 (1.5–2.4)	11.83 (−0.57, 25.76)	13.98 (3.41, 25.64)*	1.93 (−2.77, 6.86)	1.82 (−5.39, 9.57)	3.80 (−4.56, 12.90)
Q4 (2.5–46.5)	0.17 (−11.64, 11.56)	2.76 (−7.40, 14.03)	2.59 (−2.46, 7.89)	1.41 (−6.24, 9.68)	5.45 (−3.61, 15.35)

Table 3 (*continued*)

Concentrations by PFAS quartiles, range (ng/mL)	Free leptin index	Leptin, ng/mL	sOB-R, ng/mL	Adiponectin, μg/mL	HMW adiponectin, μg/mL
	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %	Percent change (95% CI), %
EtFOSAA					
Model 1					
Q1 (<LOD-0.6)	Ref	Ref	Ref	Ref	Ref
Q2 (0.7–1.1)	32.64 (14.25, 53.98)**	24.01 (9.61, 40.29)**	−6.51 (−11.12, −1.65)**	1.03 (−6.66, 9.35)	−2.84 (−11.17, 6.27)
Q3 (1.2–2.2)	41.24 (21.46, 64.24)***	29.04 (13.91, 41.19)***	−8.64 (−13.20, −3.84)**	−6.62 (−13.80, 1.16)	−7.40 (−15.42, 1.38)
Q4 (2.3–112.5)	43.71 (23.14, 67.71)***	32.24 (16.39, 50.26)***	−7.98 (−12.68, −3.03)**	0.43 (−7.47, 9.00)	−4.68 (−13.12, 4.59)
Model 2					
Q1 (<LOD-0.6)	Ref	Ref	Ref	Ref	Ref
Q2 (0.7–1.1)	18.32 (5.00, 33.32)**	12.78 (2.12, 24.55)*	−4.68 (−9.16, 0.00)*	4.74 (−2.81, 12.87)	0.66 (−7.63, 9.68)
Q3 (1.2–2.2)	24.99 (10.78, 41.02)**	16.72 (5.58, 29.04)**	−6.61 (−11.04, −1.96)**	−3.15 (−10.20, 4.45)	−3.95 (−11.93, 4.76)
Q4 (2.3–112.5)	21.56 (7.41, 37.58)**	15.29 (4.01, 27.79)**	−5.16 (−9.77, −0.31)*	5.53 (−2.34, 14.04)	0.20 (−8.34, 9.53)
MeFOSAA					
Model 1					
Q1 (<LOD-0.9)	Ref	Ref	Ref	Ref	Ref
Q2 (1.0–1.4)	43.99 (24.03, 67.18)***	35.76 (20.03, 53.55)***	−5.72 (−10.39, −0.81)*	−7.50 (−14.53, 0.12)	−11.37 (−18.95, −3.09)**
Q3 (1.5–2.3)	29.19 (11.30, 49.95)**	25.91 (11.34, 42.38)**	−2.54 (−7.35, 2.53)	−0.95 (−8.47, 7.19)	−1.66 (−10.05, 7.51)
Q4 (2.4–14.4)	31.78 (12.69, 54.10)**	27.20 (11.79, 44.73)**	−3.48 (−8.48, 1.80)	−1.92 (−9.72, 6.56)	0.64 (−8.36, 10.52)
Model 2					
Q1 (<LOD-0.9)	Ref	Ref	Ref	Ref	Ref
Q2 (1.0–1.4)	21.66 (7.93, 37.13)**	18.45 (7.24, 30.82)**	−2.64 (−7.23, 2.18)	−3.04 (−10.06, 4.52)	−6.99 (−14.65, 1.35)
Q3 (1.5–2.3)	13.92 (1.12, 28.35)*	13.74 (3.03, 25.57)*	−0.16 (−4.85, 4.76)	2.58 (−4.82, 10.54)	1.94 (−6.42, 11.04)
Q4 (2.4–14.4)	22.48 (8.09, 38.77)**	19.94 (8.13, 33.04)**	−2.07 (−6.88, 3.00)	0.02 (−7.52, 8.18)	2.72 (−6.08, 12.35)

Model 1 was adjusted for age, race/ethnicity, study site, education, smoking status, physical activity and menopausal status.
Model 2: Model 1 + waist circumference.
***$P < 0.0001$, **$P < 0.01$, *$P < 0.05$.

relationships of PFHxS, EtFOSAA and MeFOSAA with leptin and FLI. EtFOSAA also showed non-linear associations with sOB-R. For instance, EtFOSAA (Q3 vs. Q1) was associated with higher leptin (16.72%, 95% CI: 5.58%, 29.04%), lower sOB-R (−6.61%, 95% CI: 11.04%, −1.96%), and higher FLI (24.99%, 95% CI: 10.78%, 41.02%); while other PFAS quartiles (Q2 or Q4 vs. Q1) were associated with smaller changes. Adjustment for BMI instead of waist circumference did not change the results (Tables S2-S3).

### 3.3. Associations of PFAS mixtures with adipokines: bayesian kernel machine regression

Fig. 1 shows the dose-response relationships between PFAS and FLI estimated using BKMR with PFAS as a mixture, after adjusting for all covariates except for waist circumference and holding all other PFAS at their median concentrations. Based on the estimated posterior inclusion probabilities, BKMR detected all 7 PFAS including n-PFOS (PIP = 0.79), Sm-PFOS (PIP = 1.00), n-PFOA (PIP = 0.85), PFHxS (PIP = 0.98), PFNA (PIP = 0.68), EtFOSAA (PIP = 0.89), and MeFOSAA (PIP = 0.87) as important contributors to the overall association. We observed non-linear associations of log-transformed FLI with log-transformed concentrations of Sm-PFOS, EtFOSAA and MeFOSAA, as well as fairly linear associations with n-PFOS, n-PFOA, PFHxS and PFNA. However, only 3 PFAS remained with additional adjustment for waist circumference, namely Sm-PFOS (PIP = 0.50), n-PFOA (PIP = 0.44), and EtFOSAA (PIP = 0.98) as important predictors, but not n-PFOS (PIP = 0.11), PFHxS (PIP = 0.03), PFNA (PIP = 0.13), or MeFOSAA (PIP = 0.03) (Fig. 2).

Fig. 3 represents the overall joint effects of PFAS mixtures as the estimated percent change in FLI comparing concentrations of all PFAS together at different percentiles of their distributions (e.g. 20th, 40th, 60th) to all PFAS at their 10th percentiles, after controlling for all covariates without (Fig. 3A) and with adjustment for waist circumference (Fig. 3B). Cumulative PFAS mixtures were positively associated with FLI with and without adjustment for waist circumference. After

controlling for all covariates including waist circumference, compared to those with PFAS concentrations fixed at the 10th percentiles, the overall joint effect of PFAS for women with PFAS concentrations at the medians was a 30.9% (95% CI: 15.6%, 48.3%) higher FLI; and those with PFAS concentrations at the 90th percentiles had 52.1% (95% CI: 27.9%, 81.0%) higher FLI. We did not observe any evidence for interactions among PFAS (data not shown). Leptin showed similar results to FLI (Figures S3-S4). We did not detect any associations of PFAS mixtures with total or HMW adiponectin (data not shown). In the sensitivity analysis, including BMI instead of waist circumference did not change the results (Figures S5-S6).

3.4. Stratification by overweight/obesity and menopausal status

Stratified models revealed that PFOS, n-PFOS, Sm-PFOS, n-PFOA and EtFOSAA was significantly associated with higher leptin concentrations and larger FLI in BMI ≥25 kg/m² but not among women with a BMI <25 kg/m² or 23 kg/m² for Chinese and Japanese (Table S4). However, EtFOSAA concentrations were positively associated with both leptin and FLI in both groups, and the magnitude was similar. On the other hand, MeFOSAA was only statistically significantly associated with leptin and FLI among women with underweight or normal weight, and the magnitude of this association was more than double that among women with overweight/obesity and women with obesity. Interestingly, these compounds had different directions of associations with total and HMW adiponectin. While PFOS and Sm-PFOS were associated with higher total adiponectin in women with overweight/obesity and women with obesity, PFNA was related to lower total and HMW adiponectin in women with normal weight or underweight. Stratification by menopausal status also detected significant associations of PFOS, PFOA, EtFOSAA and MeFOSAA with leptin and FLI in pre-/early perimenopause (Table S5). Higher EtFOSAA was associated with lower sOB-R in pre-/early perimenopause. Higher n-PFOA was also associated with lower total adiponectin and HMW adiponectin in pre-/early
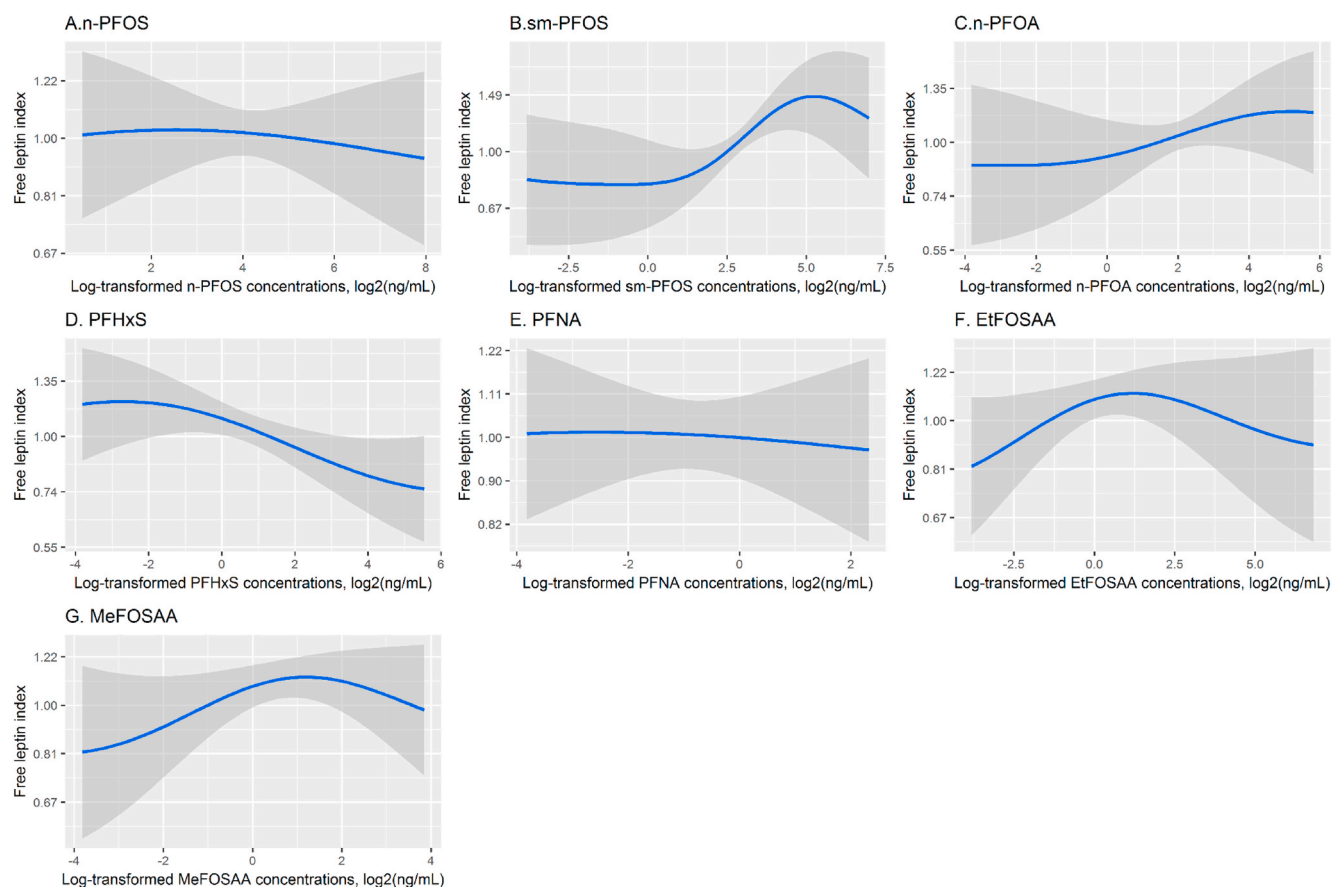
Fig. 1. Dose-response relationships and 95% confidence interval (95% CI) bands for A. n-PFOS, B. Sm-PFOS, C. n-PFOA, D. PFHxS, E. PFNA, F. EtFOSAA, and G. MeFOSAA with free leptin index holding all other PFAS at median serum concentrations, estimated by Bayesian kernel machine regression. The model was adjusted for age, race/ethnicity, study site, education, Smoking status, physical activity and menopausal status.
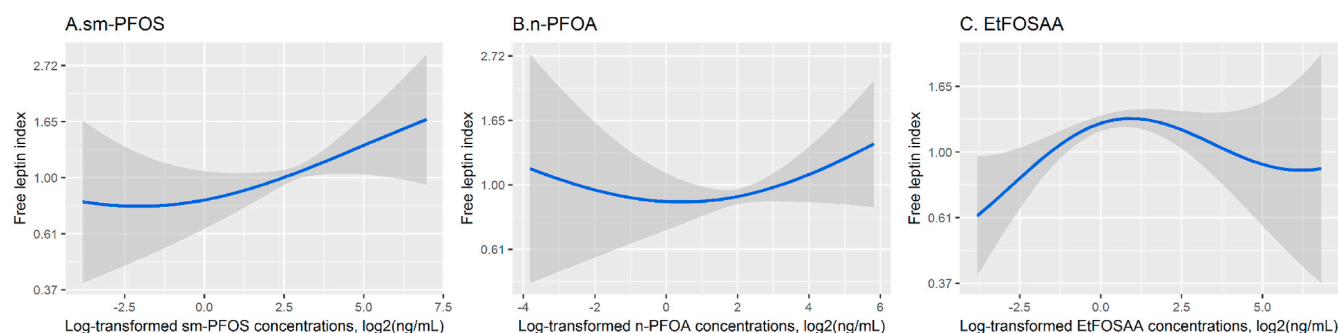

Fig. 2. Dose-response relationships and 95% confidence interval (95% CI) bands for A. Sm-PFOS, B. n-PFOA, and C. EtFOSAA with free leptin index, holding all other PFAS at median serum concentrations, estimated by Bayesian kernel machine regression. The model was adjusted for age, race/ethnicity, study site, education, Smoking status, physical activity, menopausal status, and waist circumference.

perimenopause.

4. Discussion

We found that leptin concentrations and FLI at a 3-year follow-up were significantly higher in women with higher baseline concentrations of PFOS, n-PFOA, PFNA, EtFOSAA and MeFOSAA. However, PFAS concentrations were not associated with sOB-R, total adiponectin, or HMW adiponectin. Thus, the association of PFAS with FLI is likely driven by the association of PFAS and leptin, not any differences in sOB-

R. Further, the present study is the first to report the overall joint effects of PFAS mixtures and nonlinear associations between PFAS and adipokines. A cumulative mixture of PFAS, driven mainly by Sm-PFOS, n-PFOA and EtFOSAA, was associated with higher leptin and FLI. These findings suggest that potential metabolic effects of PFAS may be through promoting the leptin pathways. PFAS concentrations in SWAN are quite comparable to those in the U.S. 1999–2000 National Health and Nutrition Examination Survey (CDC 2019). So, any effects observed are at background exposure levels. Taken together, these findings extend existing understanding of the potential effects of PFAS in weight gain

A. Without adjustment for waist circumference
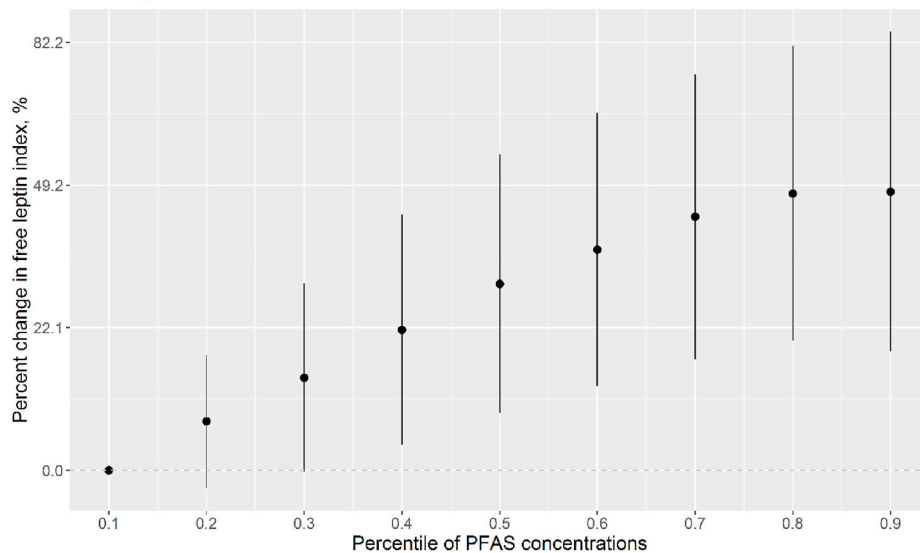
B. With adjustment for waist circumference
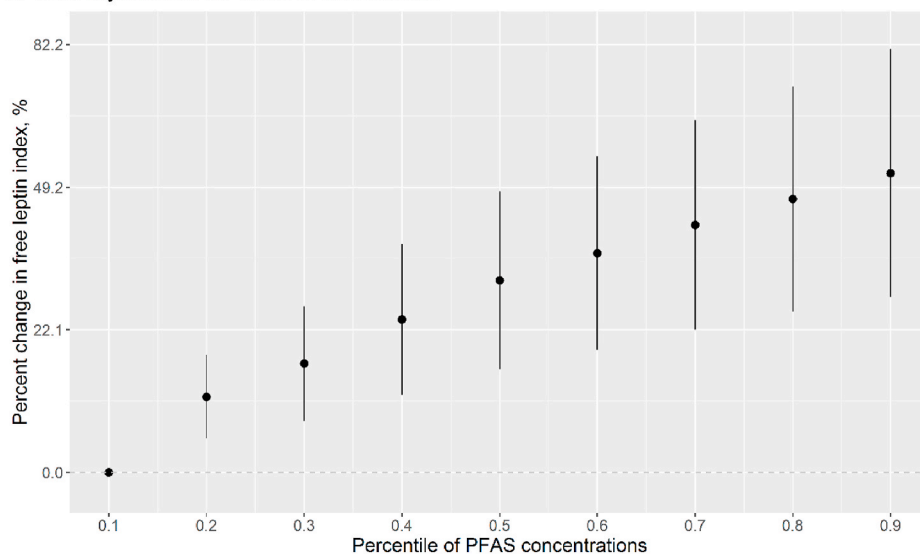
Fig. 3. Cumulative effects of perfluoroalkyl and polyfluoroalkyl substances (PFAS) mixtures on free leptin index, estimated by Bayesian kernel machine regression (A. Without adjustment for waist circumference; B. With adjustment for waist circumference). This plot shows the estimated exposure-response relations and 95% confidence intervals (95% CIs) when all PFAS concentrations are held at a certain percentile, compared to when PFAS concentrations are held at the 10th percentile. The models were adjusted for age, race, study site, education, smoking status, physical activity and menopausal status.

(Cardenas et al., 2018; Ding et al., 2021; Liu et al., 2018), insulin resistance (Cardenas et al, 2017, 2019; Sun et al., 2018), and other metabolic disorders (Eriksen et al., 2013; Lin et al., 2019; Starling et al., 2014).

Adipose tissue has a central role in the production of leptin (Ouchi et al., 2011). PFAS exposure may affect adipocyte proliferation and differentiation in toxicologic studies. Some PFAS are structural analogous of fatty acids and both can activate peroxisome proliferator-activated receptors (PPARs) and further induce endocrine disruption (Kraugerud et al., 2011; Pedersen et al., 2016), as well as disturbance of lipid and glucose metabolism, inflammation and adipocyte differentiation (Berger et al., 2005; Staels and Fruchart, 2005). Experimental studies showed that treatment of human visceral pre-adipocytes *in vitro* with PFOS at 5 and 50 μM induced adipogenesis and increased cellular lipid accumulation (Xu et al., 2016). PFOS and PFOA exposure also promoted adipogenesis in human mesenchymal stem cells (Liu et al., 2019) and mouse 3T3-L1 preadipocytes *in vitro* (Watkins et al., 2015). Epidemiologic studies have reported associations of PFAS exposure with larger body size, more body fat, and increased risks of obesity (Cardenas et al., 2017; Ding et al., 2021; Liu et al., 2018). In SWAN, we previously observed that exposure to PFOS, PFOA, EtFOSAA

and MeFOSAA was associated with increased BMI, waist circumference, fat mass and proportion fat mass, as well as higher rates of increases during menopausal transition in midlife women (Ding et al., 2021). These current findings extend our work by demonstrating that PFOS, PFOA, EtFOSAA and MeFOSAA are associated with both leptin and FLI. These findings are novel and contribute to advancing our understanding of the biological mechanisms by which PFAS act on leptin to influence adipose tissue, and how adipose tissue metabolism is affected by these chemicals. Thus, while the association between PFAS and adiposity is widely reported (Cardenas et al., 2018; Ding et al., 2021; Liu et al., 2018), few studies have examined the underlying mechanisms for these associations. The limited available evidence suggests that PFAS-induced alterations in leptin may be one mechanism linking PFAS, adiposity, and metabolic disorders. In a murine model, low doses of PFOA (0.01 mg/kg) during young adulthood was associated with midlife body weight gain and high leptin concentrations (Hines et al., 2009). Our findings of a strong and consistent association between baseline PFAS concentrations and leptin and FLI three years later is consistent with this hypothesis.

There have been few previous reports examining adipokines in relation to PFAS and the results are mixed. Lin et al. (2011) found that

plasma PFNA concentrations were positively associated with adiponectin concentrations (8.82, 8.86, 9.14, and 9.41 ng/mL across quartiles of PFNA concentrations, *P* for trend = 0.03) among 81 adults aged 20–30 years; no relationships were observed for PFOA, PFOS or PFUnDA. Bassler et al. (2019) examined leptin in 200 adults from the C8 Health Project and found higher leptin and higher adiponectin concentrations associated with serum concentrations of PFHxS and PFNA but not with PFOS, PFOA, and PFDA. Liu et al. (2018) examined 562 overweight and obese participants aged 30–70 years from the POUNDS Lost trial, and found associations of higher leptin concentrations with PFOA at baseline (Partial Spearman correlation = 0.09, P < 0.05) and between changes in leptin concentrations and PFNA during weight regain period right after a clinical intervention on weight loss (Partial Spearman correlation = 0.10, P < 0.05); while no association was detected for other compounds or sOB-R. Cardenas et al. (2018) found PFOA and EtFOSAA (but not PFOS, PFNA, PFHxS or MeFOSAA) related to lower adiponectin concentrations in a Diabetes Prevention Program of people at high risk of developing type 2 diabetes. The results from Liu et al. (2018) and Cardenas et al. (2018) may be subject to selection bias by restricting the study sample to those with overweight and obesity, or at high risks of developing diabetes, which may underestimate the true effects of PFAS on adipokine profiles.

When restricting our study population to women with overweight/obesity and women with obesity, the slight increase of total and HMW adiponectin was associated with higher PFOS concentrations. It has been suggested that adiponectin resistance with elevated serum adiponectin concentrations is a compensatory response to a lack of adipocyte-specific insulin sensitivity or adiponectin responsiveness in persons with obesity (Blüher et al., 2002; Engin, 2017; Kim et al., 2006). In contrast, PFNA was associated with lower total and HMW adiponectin in women with normal weight or underweight. It is unknown if adiponectin responses to PFAS depend on the adiposity status. Future studies should explore the exact physiological role of PFAS in adiponectin pathways.

Our findings also supported pre- and early perimenopause as time window of susceptibility to PFAS toxicity. It is possible that women who were pre- and early perimenopausal were more susceptible to PFAS toxicity and further developed metabolic diseases in their later life. Declines in estradiol during the menopausal transition affect energy homeostasis and metabolic processes. Toxicology research *in vitro* and *in vivo* and epidemiologic studies have suggested that PFAS may alter biosynthesis of sex hormones (Ding et al., 2020c). Higher leptin and lower adiponectin concentrations were significantly associated with higher estradiol, regardless of obesity status (Karim et al., 2015). This study underscores a critical need to uncover the mechanistic actions of PFAS on chronic inflammation and metabolic disturbances in women.

Strengths of our study include a large, community-based cohort of midlife women from four racial/ethnic groups, which provided comparisons in a single study with consistent questions and methodology across race/ethnicity. Further, a sophisticated method (i.e., BKMR) was implemented to examine the non-linear dose-response relationships and account for correlation structures and confounding by co-exposures. It also enabled us to evaluate the cumulative effects of multiple PFAS compounds. Finally, the extensive covariate information was available, allowing for adjustment for numerous indicators of adiposity. Because we were able to examine adjustment by both BMI and waist circumference, we could examine differences in adipokine concentrations while considering their adiposity.

However, some important limitations must be noted. Although the design of our study allowed us to evaluate the relationship between PFAS concentrations and subsequent adipokine data, we did not have adipokine data at the time of PFAS measurement. Thus, we do not know the timing of the relationship between PFAS and adipokine concentrations. Further, women in our study sample were in the mid-life and data collection occurred during the menopausal transition. Previous studies have reported lower adiponectin and higher leptin concentrations as

women transitioned from their pre-to postmenopause stage; and the observed changes in adiponectin and leptin were significantly correlated with increases in intraabdominal fat during the menopausal transition (r = −0.37 for adiponectin and 0.41 for leptin) (Lee et al., 2009; Sowers et al., 2008). Thus, we do not know if the differences in adipokines observed are unique to this life stage or extend across the life course.

In this cohort of midlife women, we found a statistically significant and positive association of baseline serum concentrations of PFOS, n-PFOA, PFNA, EtFOSAA and MeFOSAA with FLI and leptin concentrations. These findings suggest that exposure to certain PFAS may affect circulating leptin levels in midlife women, with potential implications for subsequent metabolic health outcomes during menopausal transition.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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