

Per- and Polyfluoroalkyl Substances (PFAS) and Lipid Trajectories in Women 45–56 Years of Age: The Study of Women’s Health Across the Nation

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BACKGROUND: Per- and polyfluoroalkyl substances (PFAS) are associated with less favorable blood lipid profiles in epidemiological studies. However, little is known about the potential role of PFAS in longitudinal changes in lipids among midlife women even though women become more susceptible to metabolic alterations during the menopausal transition.

OBJECTIVES: To examine associations of serum PFAS concentrations with longitudinal trajectories of blood total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in midlife women undergoing menopausal transition.

METHODS: The sample included 1,130 women from the Study of Women’s Health Across the Nation 45–56 y of age at baseline (1999–2000). We measured serum PFAS concentrations including linear perfluorooctanoic acid (n-PFOA), perfluorononanoic acid (PFNA), linear and branched perfluorooctanesulfonic acid (n-PFOS and Sm-PFOS, respectively), and perfluorohexanesulfonic acid (PFHxS) at baseline. We used *k*-means clustering to identify subgroups with different patterns of PFAS mixture. Blood lipids were measured annually or biannually through 2016 with an average follow-up of 14.8 y. We identified longitudinal trajectories of each lipid using latent class growth models. We used multinomial log-linear models adjusted for covariates to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of lipid trajectory classes by PFAS and their mixtures.

RESULTS: Three distinct trajectories (low, middle, high) of total, LDL, and HDL cholesterol and two distinct trajectories (low and high) of triglycerides were identified. n-PFOS, Sm-PFOS, and PFHxS were positively associated with total and LDL cholesterol trajectories. n-PFOS was inversely associated with triglycerides trajectories. PFAS mixtures (high vs. low) showed positive associations with total and LDL cholesterol trajectories (high vs. low), showing ORs (95% CIs) of 1.69 (95% CI: 1.36, 2.12) and 1.79 (95% CI: 1.44, 2.22), respectively.

DISCUSSION: Concentrations of serum PFAS were positively associated with trajectories of total and LDL cholesterol, providing a line of evidence supporting adverse effects of PFAS on lipid homeostasis. <https://doi.org/10.1289/EHP12351>

Introduction

Per- and polyfluoroalkyl substances (PFAS) are manmade chemicals with a fully or partially fluorinated alkyl chain that is connected to different functional groups (e.g., carboxylate, sulfonate). PFAS have been used for various applications, including water-resistant fabrics, carpet, food packaging, and firefighting foams.^{1,2} Because of their industrial applications and chemically stable characteristics, PFAS have contaminated the global environment over decades.^{3,4} These environmentally persistent chemicals are detected in human populations^{5–8} and linked to adverse health effects such as liver injury,⁹ kidney and testicular cancer,^{2,10} and reproductive disorders.^{2,11}

PFAS exposure has been associated with less-favorable blood lipids, including higher low-density lipoprotein (LDL) cholesterol and triglycerides concentrations in epidemiological studies including cross-sectional^{6,12–18} and longitudinal studies.^{6,17,19–22} Longitudinal studies of PFAS manufacturing workers^{19,20} and residents living near PFAS manufacturing plants^{21,22} reported positive associations between serum PFAS and cholesterol or triglycerides. Several recent studies have explored adults with

prediabetes status¹⁷ and adults age 70 y⁶ and found associations of PFAS exposure with less-favorable lipids profiles (i.e., higher total cholesterol and triglycerides). The prospective epidemiologic studies on this topic to date have used a population-mean approach to analysis instead of a trajectory-based approach, which allows researchers to capture heterogeneity in lipid trajectory in the population.

Women in menopausal transition undergo important physiological changes (e.g., changes in hormones and lipids levels),^{23,24} and they have substantial heterogeneity in such changes.^{23,25} Because of the physiological changes across the menopausal transition, this life stage may be particularly vulnerable for exposure to factors that may lead to metabolic alterations. Moreover, serum PFAS concentrations increase in postmenopausal women because of cessation of menstruation.^{5,26,27} However, little is known about the potential role of PFAS in longitudinal changes in lipid profiles during this critical life stage except a study reporting a weak association of perfluorooctanoic acid (PFOA) exposure with hypercholesterolemia among women age 40–59 y.²² To address this data gap, we evaluated serum PFAS concentrations at baseline with trajectories of lipids measured for about 15 y of follow-up in a cohort of women 45–56 y of age, with a hypothesis that PFAS exposure has association with less-favorable lipid trajectories.

Methods

Study Population

The Study of Women’s Health Across the Nation (SWAN) is a multisite, multiethnic prospective cohort study designed to examine biological and psychosocial changes during menopausal transition and the association of this transition with age-related health in midlife women.²⁸ In 1996–1997, SWAN recruited a total of 3,302 women from seven study sites in the United States, including Black women (from Boston, Massachusetts, Pittsburgh, Pennsylvania, Southeast

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Michigan, Michigan, and Chicago, Illinois), Hispanic women (from Newark, New Jersey), Chinese women (from Oakland, California), Japanese women (from Los Angeles, California), and White women (from all seven sites). Women were eligible to participate when they were 42–52 y of age, did not currently use exogenous hormone affecting ovarian function, had intact uterus and both ovaries, had at least one menstrual period in the previous 3 months, and self-identified as one of the above race or ethnicity categories at a given study site. During the follow-up in 2016–2017, participants nearly annually underwent questionnaire surveys, anthropometry, and collection of biological specimens (urine and serum) for the assessment of health status and related risk factors. The institutional review board at each study site approved the study protocol; all participants provided written informed consent at each study visit.

The SWAN-Multi-Pollutant Study (SWAN-MPS) was designed to evaluate the roles of various environmental pollutants, including PFAS, in metabolic and reproductive health outcomes during the menopausal transition.⁸ The SWAN-MPS measured environmental pollutants in archived urine or serum samples from the third SWAN follow-up (Visit 3, 1999–2000, $n = 2,694$), which was set as SWAN-MPS baseline. Participants from the Chicago ($n = 368$) and Newark ($n = 278$) sites were not included in the SWAN-MPS because urine samples were not collected at these sites. Because chemicals of interest in the SWAN-MPS included urinary biomarkers, additional 648 women with insufficient serum or urine samples were excluded, yielding a base sample of 1,400 participants including 708 White women, 308 Black women, 177 Chinese women, and 207 Japanese women from five study sites (233 from Boston, 235 from Pittsburgh, 257 from Southeast Michigan, 309 from Oakland, 366 from Los Angeles). For the present study, we also excluded 270 participants having fewer than six repeated measurements of fasting blood lipids to better capture fluctuating lipid trajectories during the menopausal transition, yielding 1,130 SWAN-MPS women with at least six repeated fasting lipid measurements. These 1,130 participants had complete information on the covariates included in data analysis.

Measurement of Serum PFAS Concentrations

Quantification of PFAS in serum was conducted at the Division of Laboratory Sciences at the U.S. Centers for Disease Control and Prevention (U.S. CDC). The U.S. CDC laboratory's involvement did not constitute engagement in human-subjects research. We used a modification of a previously described method.²⁹ In brief, online solid-phase extraction coupled with high-performance liquid chromatography–isotope dilution tandem mass spectrometry was used to quantify serum concentrations of linear PFOA (n-PFOA), sum of branched PFOA isomers (Sb-PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorohexane sulfonic acid (PFHxS), linear perfluorooctanesulfonic acid (n-PFOS), sum of perfluoromethylheptane sulfonic acid isomers (Sm-PFOS), 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA), and 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid (EtFOSAA). Internal standards, including ¹³C₄-PFOA, ¹³C₅-PFNA, ¹⁸O₂-PFHxS, ¹³C₄-PFOS, ¹³C₂-PFDA, ¹³C₂-PFDoDA, D₃-MeFOSAA, were used for quality assurance purposes. The coefficients of variation of quality control samples of low and high concentrations ranged from 5.9% to 12.1%, depending on the analyte. The limit of detection (LOD) was 0.1 ng/mL for all analytes. Concentrations below the LOD of PFAS with detection frequencies >90% (n-PFOA, PFNA, PFHxS, n-PFOS, Sm-PFOS, MeFOSAA, EtFOSAA; Table S1) were imputed with LOD divided by square root of 2 for analysis.³⁰ We did not include Sb-PFOA and PFDoDA in data analysis because of low detection frequencies (<20%). PFDA and PFUnDA with detection frequencies of 40.4%

and 31.2%, respectively, were included in data analysis as categorical variables (“detected” and “nondetected”). Previous studies have mainly focused on PFOA, PFNA, n-PFOS, Sm-PFOS, and PFHxS. Therefore, results of these PFAS were shown in the main text, and results for the other PFAS (i.e., PFDA, PFUnDA, MeFOSAA, EtFOSAA) were shown in the Supplemental Material.

Measurement of Blood Plasma Lipids

Methods for lipids measurements were previously described in detail.^{24,25} In brief, concentrations of lipids were determined in ethylenediamine tetraacetic acid–treated plasma. Total cholesterol and triglycerides were measured by enzymatic methods. Concentration of HDL cholesterol was determined by heparin-manganese precipitation procedure or the method of Izawa et al.³¹ LDL cholesterol was calculated by the Friedewald equation.³² Measurement of lipids were available only at visit 3 (SWAN-MPS baseline; 1999–2001), visit 4 (2000–2002), visit 5 (2001–2003), visit 6 (2002–2004), visit 7 (2003–2005), visit 9 (2005–2007), visit 12 (2009–2011), visit 13 (2011–2013), and visit 15 (2015–2016) because of fiscal limitations (Table S2).

Covariates

Sociodemographic factors including race or ethnicity, age (continuous, years), education [$\leq$ high school, some college (college attended but no degree obtained; associate degree), college graduate, post-graduate] were ascertained by self-administered questionnaire at SWAN baseline. Because race and ethnicity and study site may have interaction effects on PFAS concentrations or lipids, we combined race and ethnicity and study site into a 10-level variable (White participants from five sites; Black participants from three sites, Chinese and Japanese participants from one site each).⁸ Menopausal status (pre- or early perimenopause, late peri- or postmenopause, unknown due to hormone therapy, surgical menopause) was based on bleeding patterns, hormone use, and surgical history (bilateral salpingo-oophorectomy or hysterectomy). Pre- or early perimenopause was defined as having menses within the past 3 months. Late peri- or postmenopause was defined as 3 consecutive months of amenorrhea without other causes such as hormone therapy or surgical menopause. Smoking status (never, former, current smoker), alcohol consumption (0, >0 drinks/wk), and lipid-lowering medication use (no use, statins use, nonstatin medication use, both statin and nonstatin medication use) were collected using standardized questionnaires at the visits when blood lipids were measured. Body mass index (BMI) was calculated as measured weight (kilograms per square meter) at visit 3. Physical activity score, which ranged from 3 to 15 (higher scores indicating higher activity), was assessed using a modified version of the Kaiser Physical Activity Survey.^{33,34} Physical activity included sports, exercise, household, childcare, and daily routine. Total energy intake was obtained from intake of each food item based on a modified Block food frequency questionnaire.³⁵ For trajectory analyses, we included lipid-lowering medication use obtained at the visits when blood lipids were measured. For association analyses, we used covariates measured at visit 3 (SWAN-MPS baseline) but total energy intake measured at SWAN baseline (1996–1997), because dietary assessment was not conducted at visit 3 (Table S2). We did not consider time-varying BMI or menopausal status because serum PFAS concentrations were associated with longitudinal changes in body size/composition³⁶ and age at menopause³⁷ in the same cohort, suggesting that these factors could be mediators.

Statistical Analyses

All statistical analysis was performed using R (version 4.1.1; R Development Core Team). Multiple comparison was addressed

by calculating adjusted *p*-values using the Benjamini–Hochberg Method³⁸ based on false discovery rate of 0.05. PFAS concentrations showed right-skewed distributions and were log-transformed with base 2 before analyses. Triglycerides were also log-transformed to normalize the distribution, but other lipids that were normally distributed were not log-transformed. PFAS concentrations were also categorized into tertiles to be associated with lipids trajectories.

Latent class growth model (LCGM) was used to identify trajectories of each lipid. LCGM is a group-based trajectory model, which allow us to cluster the individuals with similar trajectories of a given variable.^{39,40} Functions “stepFlexmix” and “FLXMRglmfix” in R package “flexmix” (version 2.3-17)³⁹ was used for LCGM. We assumed a different number of latent classes, ranging from two to four. The data were randomly assigned to the classes, and a model was estimated for each class based on maximum likelihood and expectation-maximization algorithm with at most 100 iterations. To prevent local maxima, this process was repeated 100 times with different starting values.³⁹ In the models, time since SWAN-MPS baseline was included as an independent variable and blood levels of each lipid as dependent variables. To account for the effect of lipid-lowering medications, we used two approaches. Wu et al. (2007)⁴¹ reviewed published clinical trials and proposed constant values to impute the original lipid levels without lipid-lowering medication. As a primary approach, we added the sensible constant values suggested by Wu et al. (2007) to the measured lipid levels of those with lipid-lowering medication (“Adding constant method”; Table S3). Then, the imputed lipid levels were included in the models as dependent variables. The secondary approach was to fit measured lipids levels as dependent variables and lipid-lowering medication use (no use, use of statins, use of nonstatin medication, use of both medications at each visit) as a covariate (“Covariate method”).⁴² To determine the best fitting LCGM models, we considered multiple models with different numbers of classes from two to four and different time terms (a linear and/or quadratic term or restricted cubic spline with 2–4 knots placed at quantile or equidistant points). Among the multiple models (total 27 models for each lipid; see Tables S4–S7), only models with posterior probability >0.7 and a class size >10% of the population remained.⁴³ Among the remaining models, the best fitting model was selected based on Bayesian information criterion (BIC).⁴³

Univariate statistics were calculated by lipid trajectory classification; participants having at least one of the less-favorable lipid trajectories (high total cholesterol trajectory, high LDL cholesterol trajectory, low HDL cholesterol trajectory, or high triglycerides trajectory) were classified as “less favorable lipid profile,” and those not having any of the less-favorable lipid trajectories were classified as “more favorable lipid profile.” For comparison, chi-square and Wilcoxon rank-sum tests were used for categorical and continuous variables, respectively. We calculated Spearman’s correlation coefficients between the serum concentrations of PFAS.

To examine association between PFAS serum concentrations and trajectories of each lipid, we estimated odds ratios (ORs) of latent class membership (the low class as the reference) using multinomial log-linear models with log₂-transformed PFAS as exposure. To examine nonlinear association of PFAS, we categorized PFAS concentrations into tertiles. ORs and 95% confidence intervals (95% CIs) of latent class membership (medium and high) of lipids were calculated for a doubling of increase or tertiles of PFAS concentrations. The models were adjusted for a combination of race or ethnicity and study site (10 categories) to consider confounding by race or ethnicity and site effects as well as their interaction. Baseline characteristics including age, education, menopausal status, smoking status, alcohol consumption, BMI, physical activity, and total energy intake were also included

as covariates in the models. These covariates were selected based on prior knowledge. Because BMI may mediate the associations between PFAS exposure and metabolic outcomes,⁴⁴ we conducted a sensitivity analysis without BMI as a covariate to check the impact of BMI adjustment.

Given the high correlations between serum PFAS concentrations (Figure S1), we assessed effects of PFAS mixture in association with lipid trajectories using *k*-means clustering. As we examined multiple outcome variables (trajectories of four lipids), supervised methods such as weighted quantile sum regression or quantile g-computation are not appropriate in this study because the created chemical mixture would vary depending on the lipids. We chose *k*-means clustering, which is an unsupervised method and therefore creates a single mixture variable independent of outcome variables. *K*-means clustering is a nonparametric classification method that creates a categorical variable representing *k* clusters as minimizing within-cluster variances. *K*-means clustering was conducted for the PFAS with detection frequencies >90% (n-PFOA, PFNA, n-PFOS, Sm-PFOS, PFHxS, MeFOSAA, EtFOSAA) after log-transformation and standardization of their concentrations. We used “kmeans” function in R package “stats.” The maximum number of iterations was set as 10, and Hartigan–Wong algorithm was used. The number of clusters (*k*) was chosen based on cubic clustering criterion, pseudo *F* statistics, elbow method, and interpretability. Associations of the clusters with lipid trajectories were examined by multinomial log-linear models adjusted for the same set of covariates.

We additionally assessed cross-sectional associations between serum PFAS concentrations with baseline blood lipid levels (Visit 3) or rate of change in lipid levels, and the results are provided as Supplemental Tables. Lipid levels imputed by “Adding constant method” were used for these analyses. Rate of change was calculated by the following equation:

$$\text{Rate of change in lipid level (mg/dL/y)} = \frac{\text{lipid level at follow-up (mg/dL)} - \text{Baseline lipid level (mg/dL)}}{\text{Interval between the measurements (y)}}, \quad (1)$$

where we considered the change in lipids from baseline (Visit 3) to Visit 6, Visit 9, and Visit 12, which, respectively, corresponded on average to 3.01, 5.98, and 10.69 y of follow-up. Linear regression was used to associate the blood lipid levels or rate of change in lipid levels with serum PFAS concentrations. The models were adjusted for the same sets of covariates included in the multinomial log-linear models. The threshold for statistical significance was set at 2-sided *p* < 0.05.

Results

Lipid Trajectories and Participants’ Characteristics

Three trajectories of total, LDL, and HDL cholesterol (denoted “Low,” “Middle,” and “High”), and two trajectories of triglycerides (denoted “Low” and “High”) were identified by latent class growth models with “adding constant method” (Figure 1). No trajectories overlapped during the follow-up. For total and LDL cholesterol, the high trajectory groups (19%) were characterized by an increasing trend at the beginning followed by decreasing and constant trajectories (Figure 1A,B). The middle (48%–49%) and low (32%–33%) trajectory groups started with an increasing trend and then remained constant. It is noteworthy that the high trajectory groups showed peak concentrations at the early period of the follow-up (~5 and ~3 y since baseline in total and LDL cholesterol trajectories, respectively), whereas there was no such peak in other groups. HDL cholesterol trajectories showed

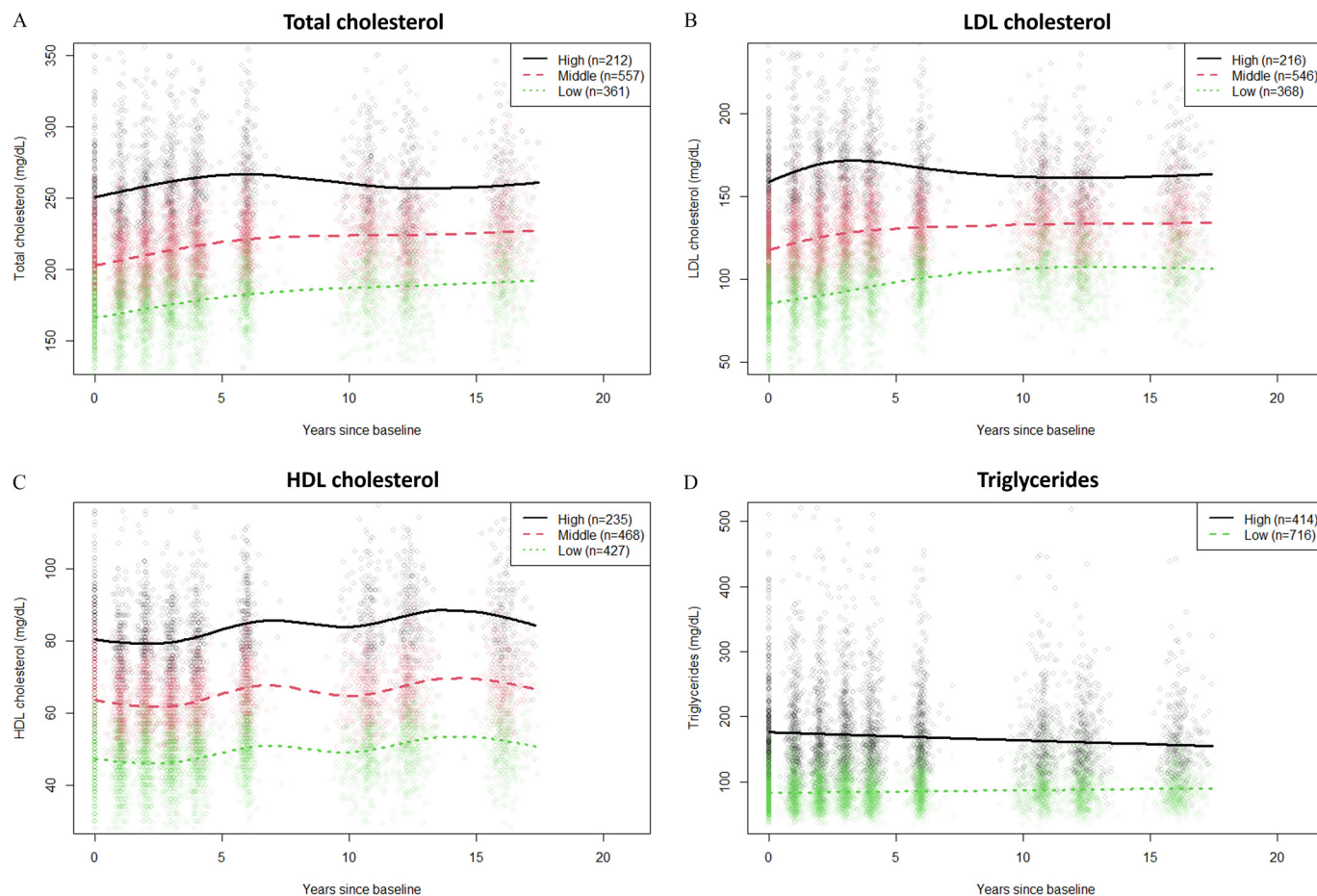


Figure 1. Trajectories of (A) total cholesterol, (B) LDL cholesterol, (C) HDL cholesterol, and (D) triglycerides identified by latent class growth models in the SWAN-MPS cohort (1999–2016, $n = 1,130$). Lipid concentrations of participants taking lipid-lowering medication were imputed by the “adding constant method” described in the main text. Note: HDL, high-density lipoprotein; LDL, low-density lipoprotein; SWAN-MPS, SWAN-Multi-Pollutant Study.

fluctuating trends over time (Figure 1C). The high trajectory group of triglycerides (37%) showed a decreasing trend, whereas the low trajectory group (63%) remained constant (Figure 1D). Similar trajectories were obtained using the “covariate method.” About 95% of the participants were classified into same group memberships when using these two different methods (Figure S2 and Table S8).

Baseline characteristics of the study population are summarized in Table 1. Site and race/ethnicity were statistically significantly associated with less/more favorable lipid profile categorization. Women from the Oakland site and of Chinese race/ethnicity were more likely to have more favorable lipid profiles, whereas women from the Southeast Michigan site and of Black race/ethnicity were more likely to have less-favorable lipid profiles. Participants with more favorable lipid profiles tended to be of younger age, have higher education levels, have lower BMI, and reported no alcohol consumption in comparison with women with less-favorable lipid profiles.

Associations of Lipid Trajectories with Serum Concentrations of Individual PFAS

When serum PFAS concentrations were considered continuously, ORs and their 95% CIs for high vs. low LDL cholesterol trajectories per doubling of PFAS serum concentrations were 1.28 (95% CI: 1.04, 1.57) for n-PFOS and 1.25 (95% CI: 1.05, 1.49) for Sm-PFOS (Table 2). Sm-PFOS and PFHxS concentrations were associated with total cholesterol trajectories [ORs and CIs for high vs. low triglycerides trajectories per doubling: 1.20 (95%

CI: 1.00, 1.44) for Sm-PFOS; and 1.17 (95% CI: 1.00, 1.36) for PFHxS], but these associations became nonsignificant after controlling for multiple comparisons. On the contrary, a doubling of PFNA concentration was inversely associated, with OR = 0.84 (95% CI: 0.72, 0.98) for high vs. low triglycerides trajectories, though this association also became nonsignificant with multiple comparison corrections. Detection of PFDA and PFUnDA was positively associated with trajectories of total or LDL cholesterol, whereas MeFOSAA was positively associated with middle vs. low LDL cholesterol trajectories (Table S9). The detection of PFUnDA was also positively associated with middle vs. low HDL cholesterol trajectories. The detection of PFDA and doubling of EtFOSAA were positively associated with triglycerides trajectory.

When PFAS concentrations were categorized into tertiles, n-PFOS, Sm-PFOS, PFHxS, and MeFOSAA were positively associated with trajectories of total and LDL cholesterol, whereas EtFOSAA showed an inverse association with trajectories of total cholesterol (Figure 2A,B; Tables S10 and S11). The ORs (95% CIs) for high vs. low trajectories of total cholesterol were: 1.61 (1.27, 2.06) for n-PFOS, 1.65 (1.29, 2.11) for Sm-PFOS, 1.63 (1.29, 2.05) for PFHxS, 1.50 (1.18, 1.91) for MeFOSAA, and 0.76 (0.59, 0.97) for EtFOSAA. The OR (95% CI) for high vs. low trajectories of LDL cholesterol were: 1.89 (1.50, 2.38) for n-PFOS, 1.92 (1.52, 2.42) for Sm-PFOS, 1.41 (1.12, 1.78) for PFHxS, and 1.43 (1.13, 1.81) for MeFOSAA. PFNA showed nonmonotonous associations with total and LDL cholesterol trajectories (Figure 2A,B). Sm-PFOS was inversely associated with trajectories of HDL cholesterol with OR for high vs. low

Table 1. Baseline characteristics of study participants in the SWAN-MPS cohort (1999–2016, *n* = 1,130).

Characteristics	<i>n</i> (%) or median (interquartile range)			<i>p</i> -Value ^c
	Total (<i>n</i> = 1,130)	More-favorable lipid profile (<i>n</i> = 481) ^a	Less-favorable lipid profile (<i>n</i> = 647) ^b	
Site				0.0008
Los Angeles, CA	320 (28)	140 (29)	180 (28)	
Oakland, CA	249 (22)	123 (26)	126 (19)	
Southeast Michigan	202 (18)	64 (13)	138 (21)	
Pittsburgh, PA	188 (17)	71 (15)	117 (18)	
Boston, MA	171 (15)	83 (17)	88 (14)	
Race/ethnicity				0.007
White	579 (51)	247 (51)	332 (51)	
Black	231 (20)	81 (17)	150 (23)	
Chinese	138 (12)	74 (15)	64 (10)	
Japanese	182 (16)	79 (16)	103 (16)	
Education				0.01
≤High school diploma	186 (16)	69 (14)	117 (18)	
Some college	355 (31)	134 (28)	221 (34)	
College	287 (25)	138 (29)	149 (23)	
Postgraduate	302 (27)	140 (29)	162 (25)	
Menopausal status				0.65
Pre-/early perimenopause	717 (63)	312 (65)	405 (62)	
Late peri-/postmenopause	214 (19)	92 (19)	122 (19)	
Unknown (hormone therapy)	160 (14)	63 (13)	97 (15)	
Surgical menopause	39 (3)	14 (3)	25 (4)	
Smoking				0.08
Never smoker	721 (64)	311 (65)	410 (63)	
Former smoker	307 (27)	137 (28)	170 (26)	
Current smoker	102 (9)	33 (7)	69 (11)	
Alcohol consumption				<0.0001
No (0 drinks/wk)	600 (53)	216 (45)	384 (59)	
Yes (>0 drinks/wk)	530 (47)	265 (55)	265 (41)	
Lipid-lowering medication				<0.0001
No	1,098 (97)	479 (100)	619 (95)	
Yes	32 (3)	2 (0)	30 (5)	
Age (y)	49.5 (47.4–51.5)	49.2 (47.3–51.0)	49.7 (47.4–51.8)	0.009
BMI (kg/m ²)	26.0 (22.4–31.5)	23.6 (21.1–27.6)	28.0 (24.0–33.2)	<0.0001
Total energy intake (kcal)	1,691 (1,341–2,170)	1,666 (1,332–2,105)	1,722 (1,358–2,228)	0.07
Total cholesterol (mg/dL)	195 (174–219)	184 (168–201)	205 (184–233)	<0.0001
LDL cholesterol (mg/dL)	112 (93–131)	100 (83–115)	123 (104–144)	<0.0001
HDL cholesterol (mg/dL)	60 (50–71)	68 (60–78)	52 (46–61)	<0.0001
Triglycerides (mg/dL)	98 (73–142)	76 (62–94)	127 (94–180)	<0.0001
n-PFOA (ng/mL)	4.1 (2.8–5.8)	4.0 (2.9–5.5)	4.3 (2.8–6.2)	0.02
PFNA (ng/mL)	0.6 (0.4–0.8)	0.6 (0.4–0.8)	0.6 (0.4–0.8)	0.45
n-PFOS (ng/mL)	17.4 (12.5–24.4)	16.8 (12.2–23.2)	17.9 (12.7–25.4)	0.07
Sm-PFOS (ng/mL)	7.4 (4.7–9.2)	6.6 (4.5–9.7)	7.7 (5.1–11.5)	0.0008
PFHxS (ng/mL)	1.5 (1.0–2.4)	1.5 (1.0–2.3)	1.5 (1.0–2.5)	0.38

Note: *n* (%) for categorical variables; median (interquartile range) for continuous variables. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; n-PFOA, linear perfluorooctanoic acid; n-PFOS, linear perfluorooctanesulfonic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; Sm-PFOS, sum of perfluoromethylheptane sulfonic acid isomers; SWAN-MPS, SWAN-Multi-Pollutant Study.

^aNot having in any of the less-favorable trajectories (high total cholesterol, high LDL cholesterol, low HDL cholesterol, or high triglycerides).

^bHaving in at least one of the less-favorable trajectories (high total cholesterol, high LDL cholesterol, low HDL cholesterol, or high triglycerides).

^cChi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables were used.

trajectories of 0.57 (95% CI: 0.44, 0.75) (Figure 2C; Table S12). n-PFOS was inversely associated with trajectories of triglycerides with OR for high vs. low trajectories of 0.71 (95% CI: 0.51, 0.99), but this association became nonsignificant after controlling for multiple comparisons (Figure 2D; Table S13). The sensitivity analysis without BMI as a covariate generally showed minuscule effects on the estimates, but the inverse associations of n-PFOS (tertile) and Sm-PFOS (continuous) with HDL cholesterol became significant after removing BMI from the models (Figure S3; Table S14).

Mixture Effects of PFAS on Lipid Trajectories

K-means clustering identified three PFAS mixture clusters representing low (*n* = 291), medium (*n* = 569), and high concentrations (*n* = 270) of individual PFAS, respectively (Figure S4). PFAS mixture was associated with higher trajectories of total and LDL cholesterol (Figure 2A,B). Comparing high PFAS concentrations

with low PFAS concentrations, OR for high vs. low trajectories of total cholesterol was 1.69 (95% CI: 1.36, 2.12), and OR for high vs. low trajectories of LDL cholesterol was 1.79 (95% CI: 1.44, 2.22). The OR for high vs. low trajectories of HDL cholesterol was significant only when BMI was not included in the model [OR = 0.63 (95% CI: 0.49, 0.81)] (Figure 2C; Figure S2C). Associations between PFAS mixture and triglycerides trajectories were not significant (Figure 2D).

Associations of Serum Concentrations of PFAS with Baseline Lipids and Rate of Change in Lipids

In the cross-sectional analysis at baseline (Visit 3), serum PFAS concentrations were not associated with total, LDL, or HDL cholesterol, whereas n-PFOA, PFNA, and n-PFOS were inversely associated with triglycerides (Table S15). Serum PFAS concentrations were not associated with rate of change in total or LDL cholesterol during follow-up, whereas n-PFOS was inversely associated with

Table 2. Associations of serum PFAS concentrations with trajectories of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in the SWAN-MPS cohort (1999–2016, *n* = 1130).

PFAS	Total cholesterol				LDL cholesterol				HDL cholesterol				Triglycerides			
	Trajectory		OR (95% CI) ^a		Trajectory		OR (95% CI) ^a		Trajectory		OR (95% CI) ^a		Trajectory		OR (95% CI) ^a	
	Low (<i>n</i> = 361)	Middle (<i>n</i> = 557)	High (<i>n</i> = 212)	<i>p</i> -Value ^b	Low (<i>n</i> = 368)	Middle (<i>n</i> = 546)	High (<i>n</i> = 216)	<i>p</i> -Value ^b	Low (<i>n</i> = 427)	Middle (<i>n</i> = 468)	High (<i>n</i> = 235)	<i>p</i> -Value ^b	Low (<i>n</i> = 716)	High (<i>n</i> = 414)	<i>p</i> -Value ^b	<i>p</i> -Value ^b
n-PFOA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
PFNA	Low (<i>n</i> = 361)	Low (<i>n</i> = 557)	Low (<i>n</i> = 212)	0.95	Low (<i>n</i> = 368)	Low (<i>n</i> = 546)	Low (<i>n</i> = 216)	0.74	Low (<i>n</i> = 427)	Low (<i>n</i> = 468)	Low (<i>n</i> = 235)	0.96	Low (<i>n</i> = 716)	Low (<i>n</i> = 414)	0.96	0.16
	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 212)	0.70	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 216)	0.99	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 235)	0.95	Middle (<i>n</i> = 414)	Middle (<i>n</i> = 414)	0.95	—
	High (<i>n</i> = 212)	High (<i>n</i> = 557)	High (<i>n</i> = 212)	0.58	High (<i>n</i> = 216)	High (<i>n</i> = 546)	High (<i>n</i> = 216)	0.27	High (<i>n</i> = 235)	High (<i>n</i> = 468)	High (<i>n</i> = 235)	0.65	High (<i>n</i> = 414)	High (<i>n</i> = 414)	0.62	0.12
n-PFOS	Low (<i>n</i> = 361)	Low (<i>n</i> = 557)	Low (<i>n</i> = 212)	0.11	Low (<i>n</i> = 368)	Low (<i>n</i> = 546)	Low (<i>n</i> = 216)	0.17	Low (<i>n</i> = 427)	Low (<i>n</i> = 468)	Low (<i>n</i> = 235)	0.96	Low (<i>n</i> = 716)	Low (<i>n</i> = 414)	0.96	0.16
	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 212)	0.57	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 216)	0.04	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 235)	0.95	Middle (<i>n</i> = 414)	Middle (<i>n</i> = 414)	0.95	—
	High (<i>n</i> = 212)	High (<i>n</i> = 557)	High (<i>n</i> = 212)	0.11	High (<i>n</i> = 216)	High (<i>n</i> = 546)	High (<i>n</i> = 216)	0.15	High (<i>n</i> = 235)	High (<i>n</i> = 468)	High (<i>n</i> = 235)	0.91	High (<i>n</i> = 414)	High (<i>n</i> = 414)	0.91	0.72
Sm-PFOS	Low (<i>n</i> = 361)	Low (<i>n</i> = 557)	Low (<i>n</i> = 212)	0.12	Low (<i>n</i> = 368)	Low (<i>n</i> = 546)	Low (<i>n</i> = 216)	0.20	Low (<i>n</i> = 427)	Low (<i>n</i> = 468)	Low (<i>n</i> = 235)	0.85	Low (<i>n</i> = 716)	Low (<i>n</i> = 414)	0.85	0.72
	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 212)	0.11	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 216)	0.15	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 235)	0.45	Middle (<i>n</i> = 414)	Middle (<i>n</i> = 414)	0.45	—
	High (<i>n</i> = 212)	High (<i>n</i> = 557)	High (<i>n</i> = 212)	0.11	High (<i>n</i> = 216)	High (<i>n</i> = 546)	High (<i>n</i> = 216)	0.20	High (<i>n</i> = 235)	High (<i>n</i> = 468)	High (<i>n</i> = 235)	0.45	High (<i>n</i> = 414)	High (<i>n</i> = 414)	0.45	—
PFHxS	Low (<i>n</i> = 361)	Low (<i>n</i> = 557)	Low (<i>n</i> = 212)	0.12	Low (<i>n</i> = 368)	Low (<i>n</i> = 546)	Low (<i>n</i> = 216)	0.15	Low (<i>n</i> = 427)	Low (<i>n</i> = 468)	Low (<i>n</i> = 235)	0.85	Low (<i>n</i> = 716)	Low (<i>n</i> = 414)	0.85	0.72
	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 557)	Middle (<i>n</i> = 212)	0.12	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 546)	Middle (<i>n</i> = 216)	0.15	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 468)	Middle (<i>n</i> = 235)	0.85	Middle (<i>n</i> = 414)	Middle (<i>n</i> = 414)	0.85	0.72
	High (<i>n</i> = 212)	High (<i>n</i> = 557)	High (<i>n</i> = 212)	0.11	High (<i>n</i> = 216)	High (<i>n</i> = 546)	High (<i>n</i> = 216)	0.20	High (<i>n</i> = 235)	High (<i>n</i> = 468)	High (<i>n</i> = 235)	0.45	High (<i>n</i> = 414)	High (<i>n</i> = 414)	0.45	—

Note: —, no data; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; n-PFOA, linear perfluorooctanoic acid; n-PFOS, linear perfluorooctanesulfonic acid; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; Sm-PFOS, sum of perfluoromethylheptane sulfonic acid isomers; SWAN-MPS, SWAN-Multi-Pollutant Study.
^aORs and their 95% CIs per doubling of each PFAS concentration.
^bAdjusted *p*-value based on false discovery rate. The models were adjusted for site x race/ethnicity, age, education, menopausal status, smoking status, alcohol consumption, BMI, physical activity, and total energy intake.

rate of change in HDL cholesterol from baseline to Visit 6 (Table S16). n-PFOS was also positively associated with rate of change in triglycerides from baseline to Visit 12.

Discussion

In this prospective study of a population of women age 45–56 y, we found serum concentrations of several PFAS (including PFAS mixture groups clustered by *k*-means algorithm) was predictive of higher total and LDL cholesterol trajectories, which were characterized by an early high peak in the trajectories. We also found that Sm-PFOS were inversely associated with HDL cholesterol trajectory.

The associations of several individual PFAS and PFAS mixture with total and LDL cholesterol trajectories observed in this study are generally in line with previous epidemiological findings (Table S17),^{2,10} providing a line of evidence that supports harmful effects of PFAS on blood cholesterol profile. Positive associations of blood concentrations of PFAS (e.g., PFOA, PFOS) with blood total or LDL cholesterol levels have been consistently reported in cross-sectional studies of general populations.^{12–14,16,45,46} Similar observations have been reported in longitudinal studies. Occupational exposure to PFOA was positively associated with higher level of total cholesterol.^{19,20} PFOA/PFOS exposure has been associated with higher levels of total and/or LDL cholesterol among residents living in a PFAS-contaminated area.^{21,22} Associations of PFAS exposure with higher cholesterol have been reported in recent longitudinal studies with general populations such as U.S. adults with prediabetes status (*n* = 888) or Swedish older adults (*n* = 864),^{6,17} although insignificant associations have been also reported in Swedish adults (*n* = 187).⁴⁷

Our findings on total and LDL cholesterol are supported by results from relevant experimental studies. Alterations in gene or protein expression regarding lipid metabolism was observed along with lipid accumulation in 3T3-L1 preadipocytes exposed to PFAS such as PFOA, PFOS, and PFHxS during their adipocyte differentiation.^{48,49} Although rodents studies generally showed lower serum cholesterol levels after PFAS exposure,⁵⁰ results from mouse studies with human-relevant diet (i.e., high fat diet) were generally aligned with our observations, showing increased serum cholesterol levels after the exposure.^{51,52} Several mechanisms for PFAS-induced cholesterol dysregulation have been suggested. PFAS have been reported to be associated with liver injury, and PFAS-induced hepatic lipid accumulations have been shown in experiments with mice,⁵³ including peroxisome proliferator-activated receptor α (PPAR α)-null or humanized PPAR α mice.^{54,55} Because of the link between PFAS and liver injury,⁹ researchers have suggested that liver injury may be mediating the relationship between PFAS and increased cholesterol levels. Other possible mechanisms include epigenetic control of lipid metabolism^{56,57} and activation of nuclear receptors such as constitutive androstane receptor, pregnane X receptor, and liver X receptor.^{52,58}

Sex hormone disrupting effects of PFAS may be another potential mechanism underlying their effects on lipid regulation. It is known that estrogen decreases circulating LDL cholesterol and increases HDL cholesterol.⁵⁹ Ovariectomized rats showed increased cholesterol blood levels, which were decreased by estrogen treatment.^{60,61} Stimulation of hepatic LDL receptor⁶² and hepatic lipase⁶³ and enhanced cholesterol efflux from peripheral tissues⁶⁴ are proposed mechanisms for the effects of estrogen on cholesterol. Total and LDL cholesterol levels increase as estrogen decreases during the menopausal transition.^{23,24} Estrogen therapy in postmenopausal women increased their LDL cholesterol levels and decreased HDL cholesterol levels.⁶⁵ On the other hand, PFOS has been inversely associated with estrogen levels in female populations.¹¹ In a previous analysis of the SWAN-MPS PFAS data,

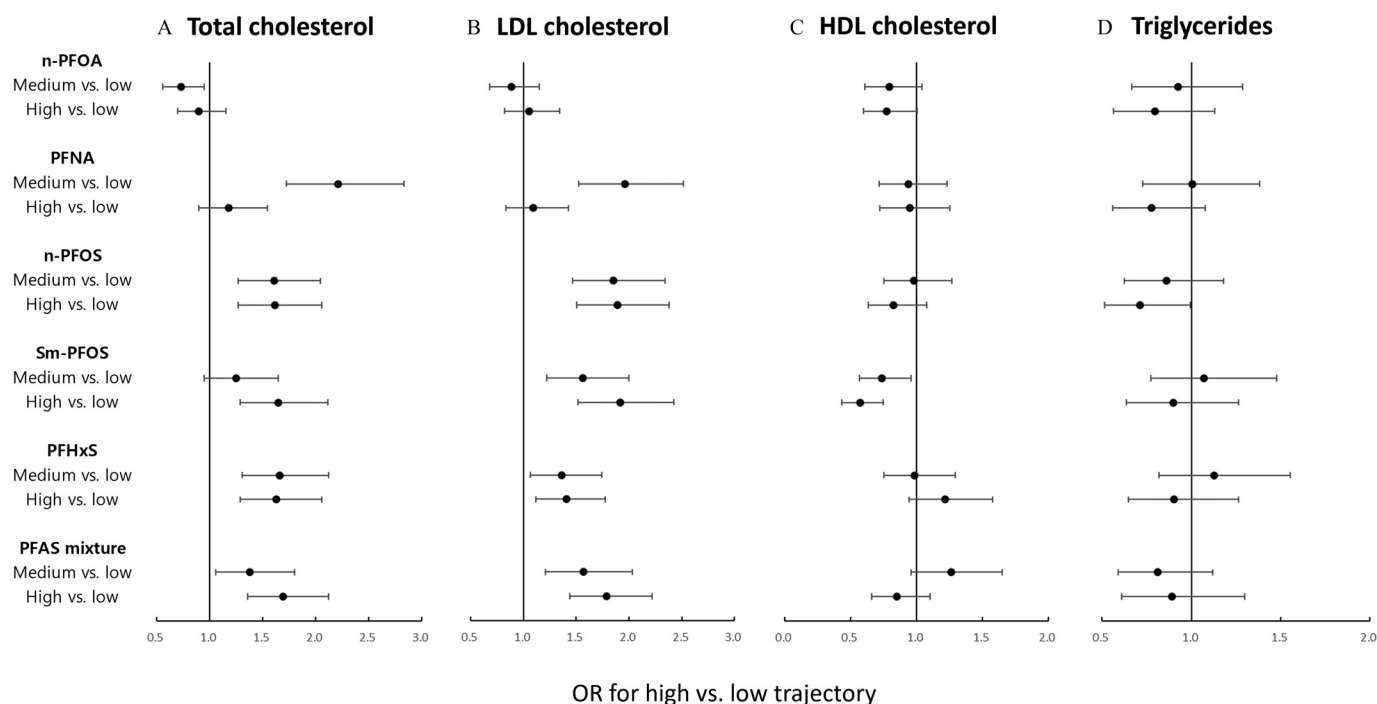


Figure 2. ORs (dot) and their 95% CIs (bar) for associations of tertiles of PFAS concentrations (vs. tertile 1) or PFAS mixture (vs. low concentration) with trajectories (high vs. low trajectories) of (A) total cholesterol, (B) LDL cholesterol, (C) HDL cholesterol, and (D) triglycerides in the SWAN-MPS cohort (1999–2016, $n = 1,130$). The models were adjusted for site $\times$ race/ethnicity, age, education, menopausal status, smoking status, alcohol consumption, BMI, physical activity, and total energy intake. Cutoff points for PFAS tertile groups: 3.4 and 5.3 ng/mL for n-PFOA, 0.6 and 0.8 ng/mL for PFNA, 14.2 and 21.4 ng/mL for n-PFOS, 5.7 and 9.4 ng/mL for Sm-PFOS, and 1.2 and 2.0 ng/mL for PFHxS. The ORs and their 95% CIs can also be found in Supplemental Tables S10, S11, S12, and S13. Note: CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; n-PFOA, linear perfluorooctanoic acid; n-PFOS, linear perfluorooctane sulfonic acid; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctanesulfonic acid; Sm-PFOS, sum of perfluoromethylheptane sulfonic acid isomers; SWAN-MPS, SWAN-Multi-Pollutant Study.

PFOA and PFNA were inversely associated with estradiol, although the association between PFOS and estradiol was not significant.⁶⁶ Given the association of PFAS exposure with estrogen and the effects of estrogen on cholesterol regulation, we hypothesize that PFAS exposure increases blood total and LDL cholesterol levels mediated by a PFAS-induced decrease in estrogen. However, exposure to PFOA or PFOS increased estrogen levels in rats⁶⁷ and a human adrenal carcinoma cell line.⁶⁸ Species difference in PFAS-induced estrogen change may be another reason for the discrepant associations of PFAS with cholesterol between humans and animals, but further investigation is needed.

The direction of the associations of PFAS with HDL cholesterol or triglycerides in previous epidemiological studies has been mixed (Table S17),^{2,10} but longitudinal studies mostly have shown primarily associations of serum PFAS with higher triglycerides levels. PFOA, PFNA, PFOS, and PFHxS were associated with hypertriglyceridemia among U.S. adults with prediabetes status ($n = 888$).¹⁷ Similar positive associations of serum PFAS and triglycerides were observed in longitudinal studies of Swedish older adults ($n = 864$),⁶ U.S. adults exposed to PFOA contaminated drinking water ($n = 560$),²¹ and occupationally exposed workers ($n = 174$),¹⁹ whereas a study of Swedish adults ($n = 187$) reported inverse associations of PFNA and PFOS with triglycerides.⁴⁷ It is unclear whether our observation of the inverse association of Sm-PFOS with HDL cholesterol trajectory was by chance or a result of a causal relationship. Further investigation may help to understand the reasons behind this observation.

It was an unexpected finding that the second tertile of n-PFOA (vs. first tertile) was inversely associated with the odds of high trajectory of total cholesterol. We do not have clear reasons for this observation. This inverse association with total cholesterol trajectory

might be partly attributed to the inverse association of PFOA with HDL cholesterol, although it is known that the majority of total cholesterol consists of LDL cholesterol rather than HDL cholesterol.⁶⁹ This might also be a result of chance, given that the association was nonlinear (only significant in the second tertile, not in the third tertile).

This study has numerous strengths. First, to the best of our knowledge, this is the first study investigating associations of PFAS exposure with lipids trajectories in midlife women. The trajectory analysis of this study allowed us to incorporate not only the levels of lipids but also the change patterns in lipids with PFAS exposure. For example, we identified that the high LDL cholesterol trajectory had an early rapid increase and a subsequent decrease, whereas the low trajectory had a slow increase. Because of such fluctuations in lipids in midlife women and individual differences in the patterns, a conventional population-mean approach with rate of changes in lipids levels during a certain period of time, as done in several previous studies, may not fully capture complex patterns in lipid change, which may vary between individuals. Our cross-sectional analyses using the population-mean approach did not show any significant associations of PFAS concentrations with baseline LDL cholesterol or the rate of change in LDL cholesterol during follow-up (Tables S15 and S16). These results emphasize the importance of using group-based trajectory analysis to secure a comprehensive understanding of the relationship between PFAS exposure and longitudinal changes in lipids.

Second, we accounted for lipid-lowering medication. Studies with lipid outcomes often exclude participants with lipid-lowering medication, which can, however, lead to selection bias.⁷⁰ We accounted for the effects of lipid-lowering medication using two

different methods, which yielded similar classifications of lipids trajectories. Third, we evaluated multiple PFAS compounds as a mixture. We used *k*-means clustering to identify three clusters of PFAS mixtures (low, medium, high). The observed monotonous positive associations between PFAS mixture clusters and total and LDL cholesterol trajectories further our understanding of the combined effects of PFAS on lipid disruption. In addition to previous studies using *k*-means clustering for PFAS mixture,^{37,71} our results suggest that *k*-means clustering is a useful method to cluster the participants with similar exposure status. Fourth, the present study was based on a multiethnic population with about half of the participants from minority race or ethnicity groups. There are limitations to this study. First, PFAS were measured only once at baseline. Although most PFAS considered in this study are biologically persistent with half-lives of the order of several years, our previous analysis of a subpopulation of SWAN-MPS (*n* = 75) showed relatively low intraclass correlation coefficients among four repeated measurements of PFAS in serum collected 1999 through 2011, indicating relatively high within-subject variability over time.⁵ Therefore, a single measurement of PFAS at baseline might not be sufficient to represent overall PFAS exposure through follow-up. Second, serum PFAS concentrations can be affected by kidney function,²⁷ which may lead to exposure assessment measurement error and associations toward null. However, even though we lacked information on kidney function, prevalence of kidney disease among women age 45–56 y is expected to be small. Third, although the present study was conducted in a racially/ethnically diverse female population, the results of this study may not be generalizable to males and other racial/ethnic groups, in particular Hispanic populations. Finally, we cannot rule out the possibility of unmeasured residual confounding.

In this first study examining longitudinal associations of PFAS with lipids trajectories among midlife women, we showed that PFAS serum concentrations were associated with less-favorable lipids trajectories, particularly total and LDL cholesterol trajectories. These findings suggest that PFAS exposure is a potential modifiable risk factor for lipid metabolic disorders, even though the underlying mechanisms of action are still poorly understood. Further studies investigating potential mechanisms of PFAS-induced lipid alteration (e.g., estrogen-mediated pathway) would expand the scientific knowledge in this area.

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