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Original Contribution

Perfluoroalkyl Substances and Incident Natural Menopause in Midlife Women: The Mediating Role of Sex Hormones

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Perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been associated with earlier natural menopause; however, the underlying mechanisms are not well understood, particularly the extent to which this relationship is mediated by sex hormones. We analyzed data (1999–2017) on 1,120 premenopausal women from the Study of Women's Health Across the Nation (SWAN). Causal mediation analysis was applied to quantify the degree to which follicle-stimulating hormone (FSH) and estradiol levels could mediate the associations between PFAS and incident natural menopause. Participants with higher PFAS concentrations had shorter times to natural menopause, with a relative survival of 0.82 (95% confidence interval (CI): 0.69, 0.96) for linear perfluorooctane sulfonate (n-PFOS), 0.84 (95% CI: 0.69, 1.00) for sum of branched-chain perfluorooctane sulfonate (Sm-PFOS), 0.79 (95% CI: 0.66, 0.93) for linear-chain perfluorooctanoate (n-PFOA), and 0.84 (95% CI: 0.71, 0.97) for perfluorononanoate (PFNA), comparing the highest tertile of PFAS concentrations with the lowest. The proportion of the effect mediated through FSH was 8.5% (95% CI: −11.7, 24.0) for n-PFOS, 13.2% (95% CI: 0.0, 24.5) for Sm-PFOS, 26.9% (95% CI: 15.6, 38.4) for n-PFOA, and 21.7% (6.8, 37.0) for PFNA. No significant mediation by estradiol was observed. The effect of PFAS on natural menopause may be partially explained by variations in FSH concentrations.

endocrine-disrupting chemicals; estrogen; follicle-stimulating hormone; hormones; mediation analysis; menopause; perfluoroalkyl substances; polyfluoroalkyl substances

Abbreviations: CDE, controlled direct effect; CI, confidence interval; FSH, follicle-stimulating hormone; MPS, Multi-Pollutant Study; NDE, natural direct effect; NIE, natural indirect effect; n-PFOA, linear-chain perfluorooctanoate; n-PFOS, linear-chain perfluorooctane sulfonate; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; Sb-PFOA, sum of branched-chain perfluorooctanoate; Sm-PFOS, sum of branched-chain perfluorooctane sulfonate; SWAN, Study of Women's Health Across the Nation.

Menopause is ascertained after 12 months of amenorrhea and represents the depletion of ovarian reserve and the near-complete cessation of estrogen secretion. Younger ages at menopause, particularly premature menopause (before age 40 years) or early menopause (before age 45 years), have been associated with increased risk of overall mortality (1–3), cardiovascular disease morbidity (4, 5) and mortality (6), low bone mineral density (7), osteoporosis (8), and other chronic conditions (9). Evidence also supports its clinical importance in the quality of life (i.e., vasomotor symptoms, sleep disturbance, and depressive symptoms) (10–12). In the Study of Women's Health Across the Nation (SWAN) (13), participants with higher serum concentrations of perfluoroalkyl and polyfluoroalkyl substances (PFAS) had an earlier onset of natural menopause. PFAS are a family of manufactured chemicals that have been widely used in consumer and industrial products such as nonstick cookware (Teflon; The Chemours Company, Wilmington, Delaware) (14, 15); food packaging materials (16–18); stain- and water-resistant coating for clothing, furniture, and carpets (Scotchgard (3M Company, St. Paul, Minnesota) and Gore-Tex (W.L. Gore & Associates, Newark, Delaware)) (19, 20); and

aqueous firefighting foam (21–24). A recent study found that at least 200 million US residents consume drinking water contaminated by these chemicals (25). Once ingested, these chemicals can build up in the human body and persist for years (26).

These compounds, especially perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), have been identified as plausible endocrine-disrupting chemicals with the potential to accelerate ovarian aging (27, 28). In animal studies, researchers have reported effects on female reproduction, including altered ovarian function, histopathological changes in the reproductive tract, and ovarian cell steroidogenesis (29–31), probably operating through the activation of various transcriptional factors (i.e., peroxisome proliferator-activated receptors) (32, 33). Several hormones in the hypothalamic-pituitary-ovarian axis are markers of ovarian aging, including follicle-stimulating hormone (FSH) and estradiol (34). FSH levels increase progressively as a woman approaches her final menstrual period, while estradiol levels start to lower several years before menopause (35–37). Thus, they are considered biomarkers of reproductive aging from active reproduction through the stages of the menopausal transition to menopause (38, 39). In recent research based on the SWAN cohort, Harlow et al. (40) observed positive associations of PFOA and PFOS with FSH levels and inverse associations of perfluorononanoate (PFNA) and PFOA with estradiol levels in midlife women during the menopausal transition. However, little is known about the mechanisms through which PFAS exert their endocrine disruption directly on endogenous sex steroids during the menopausal transition.

Understanding the mediation mechanisms between PFAS exposure and natural menopause can provide evidence of associations, identify risk factors for ovarian aging, and inform precision health studies that seek to target specific biological pathways for interventions. However, it remains unknown whether and to what extent the association between PFAS and natural menopause is mediated by FSH or estradiol. Our goal in the present study was to examine and quantify the mediating roles of FSH and estradiol in the association between PFAS exposure and incident natural menopause using SWAN data from the period 1999–2017. Specifically, we used a causal mediation approach to decompose the natural direct effect (NDE) and the natural indirect effect (NIE) in the survival setting (41).

METHODS

Study population

Data for this study were from the participants in SWAN, a multisite, multiethnic, longitudinal cohort study of a community-based group of midlife women in the United States. SWAN was designed to characterize physiological and psychosocial changes that occur during the menopausal transition and observe their effects on subsequent risk factors for chronic disease (42). Between 1996 and 1997, a total of 16,065 participants were screened for eligibility, and 3,320 were eligible to participate in the cohort study. Eligibility criteria for entry into the longitudinal cohort study included age 42–52 years, an intact uterus and at least 1 ovary, at least 1 menstrual period and no use of hormone medications within the 3 months before screening, the ability to speak English or another designated language (including Spanish, Cantonese, or Japanese), and self-identification as a member of one of the 5 eligible racial/ethnic groups (Black, Chinese, Japanese, Hispanic, or White). Investigators at each study site recruited White women and women with one of the other prespecified racial/ethnic identities based on a 1:1 ratio. Black women were enrolled in Pittsburgh, Pennsylvania; Boston, Massachusetts; Chicago, Illinois; and Detroit, Michigan; Japanese women were enrolled in Los Angeles, California; Chinese women were enrolled in Oakland, California; and Hispanic women were enrolled in Newark, New Jersey. Data and specimens were collected at annual follow-up visits from 1996/1997 through 2016/2017. The institutional review board at each participating site approved the study protocol.

The SWAN Multi-Pollutant Study (MPS) was a substudy of SWAN initiated in 2016 to examine the associations of multiple environmental pollutants, including PFAS, polychlorinated biphenyls, organochlorine pesticides, polybrominated diphenyl ethers, metals, phenols, phthalates, and organophosphate pesticides, with reproductive and cardiometabolic outcomes among midlife women (26, 43–49). We used repository samples from the third SWAN visit (visit 3, 1999–2000) as the MPS baseline for the environmental exposure assessment. Of 2,694 participants enrolled at MPS baseline, we additionally excluded participants from Chicago ($n = 368$) and Newark ($n = 278$) because urine samples were not available at these 2 sites and 648 participants with insufficient volumes of serum or urine samples, resulting in 1,400 participants in the MPS cohort. Therefore, only White, Black, Chinese, and Japanese women were included in the sample. Of 1,400 participants with serum samples available at MPS baseline, we excluded 232 participants who had already reached natural menopause and 48 participants who had had a hysterectomy or oophorectomy prior to visit 3; this resulted in a final sample size of 1,120 premenopausal women eligible for this study. The study design is displayed in Web Figure 1 (available at https://doi.org/10.1093/aje/kwac052).

PFAS measurement

Serum PFAS concentrations were assessed at the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (Atlanta, Georgia). Serum samples were analyzed using an online solid-phase extraction–high-performance liquid chromatography–isotope dilution–tandem mass spectrometry method (50). The analytical techniques and quality control procedures employed have been described in detail elsewhere (26). We measured 9 PFAS homologs, including perfluorohexane sulfonate (PFHxS), linear-chain PFOS (n-PFOS), the sum of branched-chain PFOS (Sm-PFOS), linear-chain PFOA (n-PFOA), the sum of branched-chain PFOA (Sb-PFOA), PFNA, perfluorodecanoate, perfluoroundecanoate, and perfluorododecanoate. The limit of detection was 0.1

ng/mL for all analytes. Measurements below the limit of detection were assigned a value of limit of detection/$\sqrt{2}$. Comprehensive quality assurance/quality control was conducted (50). Low-concentration (approximately 1.6–8.5 ng/mL) and high-concentration (approximately 5.4–27.8 ng/mL) quality control materials were prepared from a base calf serum pool and then analyzed with reagent blank, serum blank, and SWAN samples using the procedure described above. The quality control pools were characterized to define the mean values and 95% and 99% control limits of PFAS concentrations by 48 measurements in a 3-week period. The coefficients of variation were 6%–12% for low-concentration and high-concentration quality controls. PFHxS, n-PFOS, Sm-PFOS, n-PFOA, and PFNA with detection rates greater than 70% were included in the statistical analyses.

FSH and estradiol assessment

Collection of biological specimens was scheduled for each participant before 10:00 A.M. on days 2–5 of a follicular phase occurring within 60 days of recruitment at the baseline visit and annually after that. If a follicular phase sample could not be obtained, a random fasting sample was taken within 90 days of the anniversary of the baseline visit. Serum FSH measurements were conducted with a 2-site chemiluminometric immunoassay using an ACS:180 automated analyzer (Bayer Diagnostics Corporation, Norwood, Massachusetts). The limit of detection was 1.1 mIU/mL. FSH was detected in all serum samples at MPS baseline. The inter- and intraassay coefficients of variation were 12% and 6%, respectively. The estradiol assay modifies the rabbit anti-estradiol-6 ACS:180 immunoassay to increase sensitivity, with a limit of detection of 1.0 pg/mL. Duplicate estradiol assays were conducted, and results are reported as the arithmetic mean value for each subject; coefficients of variation were 3%–12%. We used serum concentrations of FSH and estradiol measured at visit 3 (MPS baseline) in 1999–2000 because there is currently no well-established method for handling multiple, correlated, time-varying mediators in the context of censored survival outcomes.

Incidence of natural menopause

Ascertainment of natural menopause was based on self-reported bleeding patterns during the annual follow-up visits (13, 51). Natural menopause was defined as 12 months of amenorrhea (not due to hysterectomy, bilateral oophorectomy, or hormone therapy) since the last menstrual period. If a participant missed at least 3 consecutive visits before the first postmenopausal visit, the date of the final menstrual period was set to missing.

Covariates

Covariates were chosen on the basis of prior literature (43, 52–56). Data on study site, race/ethnicity, level of education, and parity (number of live births) were collected at the SWAN enrollment interview. Race/ethnicity was classified as self-identified Black, Chinese, Japanese, or White. We categorized educational attainment as high school or less, some college, a college degree, or postgraduate study. Information on prior hormone use was collected at MPS baseline. Data on other covariates were also obtained at MPS baseline. Age was based on the date of birth and the date of the visit and centered at 45 years. Weight and height were measured using a stadiometer and calibrated scales, respectively. Body mass index was calculated as weight (kg) divided by squared height (m^2) at MPS baseline. Self-reported smoking status was classified as never smoker, former smoker only, or current smoker based on 7 smoking questions adapted from the American Thoracic Society standard questions (56). Physical activity was assessed using an adaptation of the Kaiser Physical Activity Survey (57), which consists of 38 questions with primarily Likert-scale responses about physical activity in various domains, including sports/exercise, household/caregiving, and daily routine (defined as walking or bicycling for transportation and hours of television-watching, which are reverse-coded). Domain-specific indices were derived by averaging the ordinal responses to questions in each domain, resulting in values from 1 to 5. Thus, the total physical activity score ranged from 3 to 15, with 15 indicating the highest activity level.

Statistical analyses

Univariate statistics were calculated for participant characteristics and PFAS serum concentrations at MPS baseline by racial/ethnic group. We computed χ^2 or Fisher's exact statistics for categorical variables and used analysis of variance or the Kruskal-Wallis test for continuous variables. We censored a participant's data 1) if she reported initiating hormone therapy, 2) if no subsequent hormone-therapy–free bleeding occurred at the date of hysterectomy or bilateral oophorectomy, 3) on the date of the last menstrual period at the end of data collection if it happened before 12 months of amenorrhea, or 4) because data collection ended.

The primary analytical objective was to estimate the extent to which FSH or estradiol mediated associations between PFAS exposure and natural menopause incidence while adjusting for levels of confounders of the PFAS–natural menopause and FSH/estradiol–natural menopause associations (age, race/ethnicity, study site, education, parity, body mass index, physical activity, smoking status, and prior hormone use) at MPS baseline. Time to natural menopause was modeled using accelerated failure time models with a Weibull distribution. Accelerated failure time models were chosen because they can produce coefficients that can estimate causal effects (41, 58). The Weibull distribution was selected by comparing Akaike information criterion values.

The outcome accelerated failure time model initially took the following general form (using FSH as an example; estradiol had the same model):

$$\log(T|\text{PFAS}, \text{FSH}, \text{covariates}) = \theta_0 + \theta_1\text{PFAS} + \theta_2\text{FSH} + \theta_3\text{PFAS} \times \text{FSH} + \theta_4\text{covariates} + \sigma\varepsilon,$$

where "PFAS" corresponds to tertiles of serum PFAS concentrations, "FSH" is log-transformed serum FSH concentrations at visit 3, "PFAS × FSH" is the interaction between PFAS and FSH, "covariates" are confounders at MPS baseline, σ describes the Weibull distribution scale and shape parameters, and ε symbolizes the errors, which are independently and identically distributed. After evaluation of the interaction term, there was no significant exposure-mediator interaction; thus, the model was reduced to

$$\text{Log}(T|\text{PFAS}, \text{FSH}, \text{covariates}) = \theta_0 + \theta_1\text{PFAS} + \theta_2\text{FSH} + \theta_3\text{covariates} + \sigma\varepsilon. \quad (1)$$

The NDE described above is calculated as $\exp(\theta_1)$, which is interpreted as relative survival (i.e., a ratio of time to natural menopause) comparing the second and third tertiles of PFAS concentrations with the lowest tertile. If $\exp(\theta_1) = 1$, then there is a null association between PFAS and natural menopause; if $\exp(\theta_1) < 1$, PFAS exposure is associated with earlier time to natural menopause; and if $\exp(\theta_1) > 1$, PFAS exposure is associated with the postponed onset of natural menopause. We then fitted a linear regression model for the mediator, FSH:

$$\text{E}(\text{FSH}|\text{PFAS}, \text{covariates}) = \beta_0 + \beta_1\text{PFAS} + \beta_2\text{covariates} + \varepsilon. \quad (2)$$

Within the causal framework, we investigated the NDE and NIE with survival data. Considering the average time to natural menopause (T), the NDE refers to the difference in the mean event time associated with a defined change in PFAS exposure (A) from the exposed level (a) to the reference level (a^*) that would exist if someone were to intervene on the pathway from A to M to deactivate it and set the covariates at the reference level. The NIE represents the difference in the mean time to natural menopause that would exist if someone were to intervene on the pathway of A to Y to deactivate it and maintained the A-to-M and M-to-Y pathways. The causal pathways are illustrated in Figure 1.

The mediation analysis described above assumes that the measured covariates control for confounding of 1) the exposure-outcome association, 2) the mediator-outcome association, and 3) the exposure-mediator relationship and that 4) none of the mediator-outcome confounders are influenced by the exposure. If model 1 holds for the outcome and model 2 holds for the mediator, these models yield the NDE and NIE of PFAS exposure on natural menopause as follows:

$$\text{NDE} = \exp(\theta_1)$$

and

$$\text{NIE} = \exp\left[(\theta_2\beta_1)\left(a - a^*\right)\right]$$

for changes in PFAS exposure from a to a^* in a counterfactual setting. The statistical significance was determined using 95% confidence intervals (CIs) calculated using the bootstrap method with 1,000 resamplings.

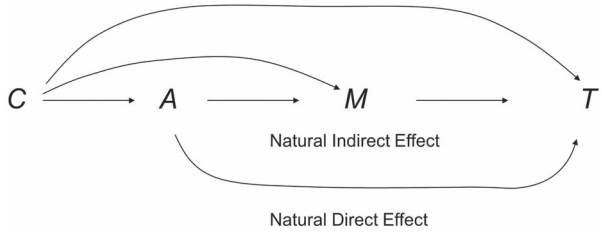

Figure 1. Conceptual model for an analysis of mediation of the PFAS–natural menopause association by sex hormones in the Study of Women's Health Across the Nation, 1999–2017. Confounders (*C*) include age at Multi-Pollutant Study (MPS) baseline, race/ethnicity, study site, education, body mass index (weight (kg)/height (m)^2) at MPS baseline, parity at MPS baseline, non–work-related physical activity at MPS baseline, smoking status at MPS baseline, and prior oral contraceptive use at MPS baseline; *A* represents serum concentrations of PFAS at MPS baseline; *M* represents follicle-stimulating hormone and estradiol levels at MPS baseline; and *T* represents time to natural menopause. The natural direct effect is the effect of PFAS on natural menopause not mediated by hormones, while the natural indirect effect is the effect of PFAS on natural menopause through hormonal changes. PFAS, perfluoroalkyl and polyfluoroalkyl substances.

Assumptions and sensitivity analyses

For assumptions 1–4, which cannot be assessed directly, we conducted sensitivity analyses to estimate the potential bias in NDE and NIE estimates due to unmeasured confounding (59–61). The directed acyclic graph in Web Figure 2 illustrates potential confounding on the causal pathway from PFAS to natural menopause. Assuming a binary unknown confounder (e.g., exposure to other endocrine-disrupting chemicals), we used the bias formula to correct for unmeasured confounding (61),

$$\text{Bias(NDE)} = \frac{1 + (\gamma - 1)\,\pi_a}{1 + (\gamma - 1)\,\pi_{a^*}} \text{ and}$$

$$\text{Bias(NIE)} = \frac{1 + (\gamma - 1)\,\pi_{a^*}}{1 + (\gamma - 1)\,\pi_a},$$

where γ is the effect of the hypothesized unadjusted confounder, U, on natural menopause; π_a is the prevalence of U among participants in the highest tertile of PFAS concentrations given covariates at MPS baseline; and π_{a^*} is the prevalence of U among participants in the lowest tertile of PFAS concentrations given covariates at MPS baseline.

We conducted sensitivity analyses by testing interaction terms in the regression models. Assuming the existence of interaction effects between exposure and mediator, the controlled direct effect (CDE) is defined as the difference in the mean event time associated with a defined change in PFAS exposure (A) from the exposed level (a) to the reference level (a^*) that would exist if the mediator were set to a fixed value (m). In this case, CDE, NDE, and NIE are

defined as follows:

$$\mathrm{CDE} = \exp\left[(\theta_1 + \theta_3 m)\left(a - a^*\right)\right];$$

$$\mathrm{NDE} = \exp\left[\theta_1 + \theta_3\left(\beta_0 + \beta_1 a^* + \beta_2 \mathrm{covariates} + \theta_2\sigma^2\right)\left(a - a^*\right) + 0.5\theta_3{}^2\sigma^2\left(a^2 - a^{*2}\right)\right];$$

and

$$\mathrm{NIE} = \exp\left[(\theta_2\beta_1 + \theta_3\beta_1 a)\left(a - a^*\right)\right],$$

where PFAS exposure levels changed from a to a^* (from the highest tertile of serum PFAS concentrations to the lowest) in a counterfactual setting. Without a term for interaction between PFAS and FSH, CDE should equal NDE.

To ensure the temporality between the exposure and the mediator, we conducted further analyses using FSH and estradiol levels measured at SWAN visit 4 in 2000–2001. We excluded 101 participants who had reached their final menstrual period or were censored by visit 4 and an additional 53 participants with missing values for FSH levels, after which 966 were available for the sensitivity analyses. We excluded 54 participants with missing estradiol values and had an analytical sample of 965 for the sensitivity analyses. All of the analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Participant characteristics

The analytical sample consisted of 1,120 premenopausal women with a median age of 48.9 years (interquartile range, 47.0–50.8) at MPS baseline. Participant characteristics, detection frequencies, and median serum PFAS concentrations are displayed in Tables 1 and 2. Other descriptive data, including geometric mean values and selected percentiles, are shown in Web Table 1. A total of 577 women (51.5%) were White, 235 (21.0%) were Black, 142 (12.7%) were Chinese, and 166 (14.8%) were Japanese. More than half had received a college education and had never smoked. Most participants had given birth to at least 1 child, and 22.1% had a history of prior hormone use. The median physical activity score was 7.9 (interquartile range, 6.6–9.0), indicating moderate physical activity. The median body mass index was 26.1 (interquartile range, 22.7–31.5).

Mediation by FSH or estradiol

Population median time to natural menopause was 6.5 years (95% CI: 6.1, 6.8). As expected, participants with higher FSH concentrations at visit 3 tended to have an earlier onset of natural menopause. After adjustment for age, race/ethnicity, study site, educational attainment, body mass index, parity, physical activity score, smoking status, and prior hormone use, participants had a relative survival of 0.83 (95% CI: 0.78, 0.88), which means a 17% earlier time to natural menopause per doubling of serum FSH concentrations. Higher estradiol concentrations at visit 3 were related to later natural menopause, although the association did not reach statistical significance (relative survival = 1.02 (95% CI: 0.97, 1.07) per doubling of estradiol concentrations).

Table 3 shows the results of causal mediation analysis decomposing total effects into NDE and NIE. For the total effects, the adjusted relative survival for natural menopause was 0.82 (95% CI: 0.69, 0.96) for n-PFOS, 0.84 (95% CI: 0.69, 1.00) for Sm-PFOS, 0.79 (95% CI: 0.66, 0.93) for n-PFOA, 0.84 (95% CI: 0.71, 0.97) for PFNA, and 0.90 (95% CI: 0.76, 1.05) for PFHxS, comparing the highest tertile of concentrations with the lowest. When FSH was included as a mediator, 26.9% (95% CI: 15.6, 38.4) of the total effect of n-PFOA on natural menopause was attributable to indirect effects operating through FSH. For PFNA and Sm-PFOS, 21.7% (95% CI: 6.8, 37.0) and 13.2% (95% CI: 0.0, 24.5) of the total effects of PFNA and Sm-PFOS were attributable to indirect effects, respectively. No causal mediation effects were observed for n-PFOS (percent mediated = 8.5%, 95% CI: −11.7, 24.0) or PFHxS (percent mediated = 18.3%, 95% CI: −13.0, 40.8).

Table 4 presents causal mediation analysis results for the association between PFAS and time to natural menopause mediated by visit 3 estradiol level. No significant mediation was observed for PFAS compounds. The percent mediated by estradiol was −0.06% (95% CI: −4.8, 3.9) for n-PFOS, −1.6% (95% CI: −10.6, 2.8) for Sm-PFOS, 1.2% (95% CI: −3.8, 6.7) for n-PFOA, 0.4% (95% CI: −4.4, 5.9) for PFNA, and −0.3% (95% CI: −13.0, 10.7) for PFHxS.

Sensitivity analyses

Details of the assumption testing results are provided in Web Tables 2–5. Assuming that an unmeasured confounder decreases the risk of earlier natural menopause and is more prevalent among participants with lower n-PFOA or Sm-PFOS concentrations, the observed NDE seems to be overestimated, and the NIE appears to be underestimated. Our results remained robust after considering an unmeasured confounder with relative survivals of 0.6, 0.8, 1.2, and 1.5. For example, if there is an unmeasured confounder with a relative survival of 1.2 on natural menopause and a higher prevalence of the unmeasured confounder in participants with higher n-PFOA concentrations (10% vs. 25% in the highest and lowest tertiles of n-PFOA concentrations), the estimated NDE would have a relative survival of 0.96, and the estimated NIE would have a relative survival of 0.94 (Web Tables 2 and 3). Therefore, the estimated percentage of mediation would be 59%, larger than the observed 26.9%. The sensitivity analyses also confirmed no interaction between PFAS exposure and FSH (Web Table 6). The mediating role of FSH in the association between n-PFOA and natural menopause remained when using FSH at SWAN visit 4 instead of FSH at visit 3 as the mediator (percent mediated = 36.4%, 95% CI: 1.0, 54.8), as shown in Web Table 7. If the unknown confounder (e.g., other endocrine-disrupting chemicals) increased the risk of earlier natural

Table 1. Characteristics of Multi-Pollutant Study Participants at Baseline (Visit 3) Among 1,120 Premenopausal Women From the Study of Women's Health Across the Nation, 1999–2000

Participant Characteristic	Median (IQR)	No.	%
Age at MPS baseline, years	48.9 (47.0–50.8)		
Physical activity score[a]	7.9 (6.6–9.0)		
Body mass index[b]	26.1 (22.7–31.5)		
FSH level, mIU/mL	21.5 (12.7–41.6)		
Estradiol level, pg/mL	40.2 (24.5–87.1)		
Race/ethnicity			
White		577	51.5
Black		235	21.0
Chinese		142	12.7
Japanese		166	14.8
Study site			
Southeastern Michigan		202	18.0
Boston, Massachusetts		182	16.3
Oakland, California		242	21.6
Los Angeles, California		299	26.7
Pittsburgh, Pennsylvania		195	23.4
Educational attainment			
High school or less		197	17.7
Some college		350	31.4
College degree		271	24.3
Postgraduate study		296	26.6
Parity			
Nulliparous		215	19.2
Parous		905	80.8
Prior hormone use		248	22.1
Smoking status			
Never smoker		720	64.4
Former smoker		291	26.0
Current smoker		107	9.6

Abbreviations: FSH, follicle-stimulating hormone; IQR, interquartile range; MPS, Multi-Pollutant Study.

[a] Physical activity was assessed using an adaptation of the Kaiser Physical Activity Survey (57). The total physical activity score ranged from 3 to 15, with 15 indicating the highest activity level.

[b] Weight (kg)/height (m)^2.

menopause and was more prevalent among participants with higher n-PFOA or Sm-PFOS concentrations, the NDE would be overestimated, and then the NIE would be underestimated. The degree of mediation of exposure to n-PFOA and Sm-PFOS by FSH would be underestimated without adjustment in these scenarios. Otherwise, the observed NDE underestimates the true NDE, whereas the observed NIE overestimates the true NIE. The addition of terms for interaction between PFAS and estradiol to the mediation analysis did not change the study results (Web Table 8). Using estradiol at visit 4, however, we still did not observe the mediating effect of estradiol on the associations between PFAS and time to natural menopause (Web Table 9).

DISCUSSION

In this population-based cohort study, we found that the shortening of time to natural menopause by n-PFOA, Sm-PFOS, and PFNA exposure is partially explained by increasing serum FSH concentrations. Although PFAS exposure has been linked to earlier menopause and change in hormone concentrations, the potentially mediating role of FSH and estradiol in the association between PFAS and menopause has never been examined formally. This study is the first, to our knowledge, that supports a potential mechanistic pathway for the associations between PFAS exposure and reproductive outcomes in human populations using a causal mediation approach.

Table 2. Serum PFAS Concentrations at Multi-Pollutant Study Baseline (Visit 3) Among 1,120 Premenopausal Women From the Study of Women's Health Across the Nation, 1999–2000

Type of PFAS	% > LOD	Median (IQR)
n-PFOS	100	17.1 (12.2–24.5)
Sm-PFOS	99.9	7.2 (4.6–10.8)
PFHxS	99.6	1.5 (0.9–2.3)
PFNA	97.0	0.6 (0.4–0.8)
n-PFOA	99.9	4.0 (2.8–5.7)
Sb-PFOA	18.2	<LOD
PFUnDA	30.0	<LOD (<LOD–0.2)
PFDoDA	4.0	<LOD
PFDA	39.3	<LOD (<LOD–0.3)

Abbreviations: IQR, interquartile range; LOD, limit of detection; n-PFOA, linear-chain perfluorooctanoate; n-PFOS, linear-chain perfluorooctane sulfonate; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFDoDA, perfluorododecanoate; PFUnDA, perfluoroundecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; Sb-PFOA, sum of branched-chain perfluorooctanoate; Sm-PFOS, sum of branched-chain perfluorooctane sulfonate.

FSH and estradiol are highly variable during the menopausal transition and have not been standardized to ascertain cutoff levels. The STRAW+10 (Stages of Reproductive Aging Workshop) study incorporated the results of longitudinal studies of women across the menopausal transition and suggested an FSH level greater than 25 IU/L as an indication of being in the late menopausal transition (39). Among women without hormone use, typical ranges of circulating hormone concentrations after menopause are less than 10–40 pg/mL for estradiol and 30–240 mIU/mL for FSH (62). Serum FSH and estradiol concentrations in SWAN are comparable to those observed in middle-aged women during the menopausal transition in the general population (36, 63).

As proposed by Baron and Kenny (64) in 1986, mediation requires strong relationships 1) between the exposure and the mediating variable and 2) between the mediating variable and the outcome of interest. Previous studies have documented associations between PFAS exposure and circulating levels of FSH in the human body (65). Exposure to PFOS and PFHxS has been associated with increased serum concentrations of FSH in patients with premature ovarian insufficiency (65). In a longitudinal study using SWAN data, Harlow et al. (40) also detected positive relationships between n-PFOA exposure and increases in FSH concentrations among midlife women. Experimental studies have confirmed the endocrine-disrupting role of PFAS, possibly through activation of peroxisome proliferator-activated receptors (33).

The second criterion, an association between FSH and natural menopause, is well established. FSH is a glycoprotein hormone that is critical for ovarian folliculogenesis. FSH acts through FSH receptors located on the membrane of granulosa cells to facilitate antral follicle development and, in combination with luteinizing hormone, stimulate preovulatory follicle growth and form a corpus luteum after ovulation (66, 67). FSH serum concentrations begin increasing about 7 years before the final menstrual period; the rates of change accelerate approximately 2 years before the final menstrual period; and FSH concentrations stabilize approximately 2 years after the final menstrual period (36). FSH is also a candidate biomarker for estimation of ovarian reserve as a reflection of decreased negative feedback from a diminishing cohort of follicles, with elevated FSH levels commonly used to confirm the onset of menopause (68).

In the present study, exposure to PFAS was related to shorter time to natural menopause in midlife women. In experiments conducted in vitro and in in-vivo animal models, inverse associations were reported for PFAS exposure with diminished ovarian reserve (i.e., the number of ovarian follicles and oocytes) (69–76) and reduced steroidogenic enzyme activities (30, 77, 78). We found that FSH was a statistically significant mediator of the associations of n-PFOA and Sm-PFOS exposure with natural menopause incidence, with the proportion mediated by FSH being 26.8% and 23.8%, respectively. Other possible mechanisms for the effect of PFAS on menopause timing include interruption of gap junction intercellular communication between oocytes and granulosa cells (74), oxidative stress (79), and distribution of thyroid hormone homeostasis (80–82).

Mediation by estradiol at MPS baseline or SWAN visit 4 was almost 0. Linear-chain PFOA and PFNA have been associated with a lower estradiol level in midlife women (40). Estradiol concentrations begin to decrease around 2.0 years before the final menstrual period and achieve the maximal rate of change at the final menstrual period, unlike FSH, which starts to increase approximately 6.1 years before the final menstrual period (36). It is possible that PFAS influence age at menopause through their impact on hormonal changes that occur during the menopausal transition. However, we were unable to examine longitudinal changes in hormone concentrations due to a lack of statistical methods for dealing with repeatedly measured mediators in the survival setting. Additionally, there is a lack of statistical methods to account for multiple pollutants or chemical mixtures in mediation analysis. Future research is needed to explore the role of environmental mixtures (83, 84).

This study had several strengths. First, the prospective design minimized the possibility of reverse causation. Second, the use of standard annual follow-up visits instead of 1-time questionnaire responses provided reliable estimates of the date of the final menstrual period. Furthermore, the availability of data on serum FSH concentrations at visit 3 (MPS baseline) allowed assessment of the mediating role of FSH in the association between PFAS exposure and incident natural menopause. Additionally, our sensitivity analysis using FSH and estradiol measured at visit 4 confirm the robustness of the study findings.

There are limitations of this study that should be acknowledged. First, the age range for the cohort was restricted to 45–56 years at MPS baseline. Participants who experienced menopause before MPS baseline, especially those with premature menopause (before age 40 years) or early menopause (before age 45 years), were not included in the MPS cohort. It is possible that participants with higher PFAS

Table 3. Results of Causal Mediation Analysis of the PFAS–Natural Menopause Association by Follicle-Stimulating Hormone Level Among 1,120 Premenopausal Women From the Study of Women's Health Across the Nation, 1999–2017[a–c]

Type of PFAS	Natural Direct Effects		Natural Indirect Effect		Total Effect		% Mediated	
	Relative Survival[d]	95% CI	Relative Survival	95% CI	Relative Survival	95% CI	%	95% CI
n-PFOS	0.84	0.71, 0.97	0.98	0.94, 1.02	0.82	0.69, 0.96	8.5	−11.7, 24.0
Sm-PFOS	0.86	0.72, 1.02	0.97	0.94, 1.00	0.84	0.69, 1.00	13.2	0.0, 24.5
n-PFOA	0.85	0.72, 0.98	0.93	0.89, 0.97	0.79	0.66, 0.93	26.9	15.6, 38.4
PFNA	0.88	0.74, 1.01	0.96	0.92, 0.99	0.84	0.71, 0.97	21.7	6.8, 37.0
PFHxS	0.92	0.79, 1.07	0.98	0.94, 1.01	0.90	0.76, 1.05	18.3	−13.0, 40.8

Abbreviations: CI, confidence interval; n-PFOA, linear-chain perfluorooctanoate; MPS, Multi-Pollutant Study; n-PFOS, linear-chain perfluorooctane sulfonate; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; Sm-PFOS, branched-chain perfluorooctane sulfonate.

[a] Causal mediation analysis was based on 1) accelerated failure time for the outcome model with time to natural menopause as the dependent variable and both the exposure (PFAS) and the mediator (follicle-stimulating hormone) as independent variables, with adjustment for confounders; and 2) linear regression for the mediator model with follicle-stimulating hormone as the dependent variable and PFAS as an independent variable, with adjustment for confounders. PFAS and follicle-stimulating hormone levels were measured at MPS baseline (Study of Women's Health Across the Nation visit 3, 1999–2000).

[b] Model results were adjusted for age at MPS baseline, race/ethnicity, study site, education, body mass index (weight (kg)/height $(m)^2$) at MPS baseline, parity, physical activity, smoking status, and prior hormone use at MPS baseline.

[c] The effects of PFAS exposure were interpreted in terms of relative survival and its related 95% CI, comparing the highest tertile of PFAS concentrations with the lowest.

[d] A relative survival less than 1 means that PFAS exposure is associated with earlier onset of natural menopause.

concentrations could have had a premature or early natural menopause. Thus, the observed results may underestimate the true effects of PFAS on natural menopause through alterations in hormone levels. Second, more than 40% of the cohort was censored at the initiation of hormone therapy before the participant was classified as postmenopausal. The right-censoring may have resulted in underestimation of age at the final menstrual period, because participants who

Table 4. Results of Causal Mediation Analysis of the PFAS–Natural Menopause Association by Estradiol Level Among 1,120 Premenopausal Women From the Study of Women's Health Across the Nation, 1999–2017[a–c]

Type of PFAS	Natural Direct Effects		Natural Indirect Effect		Total Effect		% Mediated	
	Relative Survival[d]	95% CI	Relative Survival	95% CI	Relative Survival	95% CI	%	95% CI
n-PFOS	0.83	0.70, 0.98	1.00	0.99, 1.01	0.83	0.70, 0.98	−0.1	−4.8, 3.9
Sm-PFOS	0.85	0.72, 0.99	1.00	0.99, 1.02	0.85	0.72, 0.99	−1.6	−10.6, 2.8
n-PFOA	0.82	0.68, 0.98	1.00	0.98, 1.01	0.82	0.68, 0.97	1.2	−3.8, 6.7
PFNA	0.86	0.74, 1.01	1.00	0.99, 1.01	0.86	0.74, 1.01	0.4	−4.4, 5.9
PFHxS	0.93	0.80, 1.10	1.00	0.99, 1.01	0.93	0.80, 1.09	−0.3	−13.0, 10.7

Abbreviations: CI, confidence interval; MPS, Multi-Pollutant Study; n-PFOA, linear-chain perfluorooctanoate; n-PFOS, linear-chain perfluorooctane sulfonate; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; Sm-PFOS, branched-chain perfluorooctane sulfonate.

[a] Causal mediation analysis was based on 1) accelerated failure time for the outcome model with time to natural menopause as the dependent variable and both the exposure (PFAS) and the mediator (estradiol) as independent variables, with adjustment for confounders; and 2) linear regression for the mediator model with estradiol as the dependent variable and PFAS as an independent variable, with adjustment for confounders. PFAS and estradiol levels were measured at MPS baseline (Study of Women's Health Across the Nation visit 3, 1999–2000).

[b] Model results were adjusted for age at MPS baseline, race/ethnicity, study site, education, body mass index (weight (kg)/height $(m)^2$) at MPS baseline, parity, physical activity, smoking status, and prior hormone use at MPS baseline.

[c] The effects of PFAS exposure were interpreted in terms of relative survival and its related 95% CI, comparing the highest tertile of PFAS concentrations with the lowest.

[d] A relative survival less than 1 means that PFAS exposure is associated with earlier onset of natural menopause.

used hormone therapy had a higher educational level, which has been associated with later age at natural menopause. However, our previous analysis imputed age at the final menstrual period for participants with hormone therapy use, and the bias was likely to be small (13). Moreover, although we considered many confounding variables in the analyses, residual or unmeasured confounding might still have biased our effect estimates. Given the expected direction of residual confounding, our sensitivity analyses suggest that the observed indirect effects may have underestimated the true mediated effect while the observed direct effects were overestimated. Furthermore, age at the final menstrual period was measured prospectively, since all participants were premenopausal at baseline. Bleeding-related questions on the (approximately annual or biannual) visit questionnaire yielded prospective classification of menopausal status and date of the final menstrual period. It is unlikely that self-reporting of this outcome biased effect estimates. Finally, FSH concentrations assessed at MPS baseline cannot account for longitudinal variations in FSH over time. Thus, the observed mediation effects may be underestimated (85).

In conclusion, to our knowledge, this study was the first to investigate and quantify the degree to which FSH is an explanatory factor for the shortened time to natural menopause in midlife women with higher exposure to PFAS. Although mediation analysis still does not concretely establish a causal pathway, our findings provide evidence that PFAS exposure may accelerate ovarian aging through endocrinological mechanisms associated with changing serum concentrations of FSH. Any potential mechanism underlying the relationships between PFAS exposure and natural menopause is likely to involve an interplay of hormones, beyond the action of single hormone levels. Future replication of our findings should consider other hormones such as luteinizing hormone in the analysis.

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