Per- and Polyfluoroalkyl Substances (PFAS) and Hormone Levels during the Menopausal Transition

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Supplemental Materials

Supplemental Table 1. Distributions of PFAS concentrations (ng/mL) in serum collected in 1999-2000 in the Study of Women's Health Across the Nation.

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Supplemental Table 1. Distributions of PFAS concentrations (ng/mL) in serum collected in 1999-2000 in the Study of Women's Health Across the Nation.

Analyte	Detection (%)	GM (GSD)	Percentiles					
	LOD=0.1		5th	25th	50th	75th	95th	Maximum
Perfluoroalkyl carboxylic acids (PFCAs)								
PFOA (total)		4.18 (1.80)	1.6	2.9	4.3	6.0	10.8	56.5
Linear	99.9	4.05 (1.82)	1.5	2.9	4.1	5.8	10.3	56.5
Branched	17.9	0.11 (2.44)	<LOD	<LOD	<LOD	<LOD	1	5.8
PFNA	97.2	0.55 (1.82)	0.2	0.4	0.6	0.8	1.3	5.0
PFDA	41.3	0.13 (2.21)	<LOD	<LOD	<LOD	0.3	0.5	2.7
PFUnDA	32.3	0.12 (2.27)	<LOD	<LOD	<LOD	0.2	0.6	3.8
PFDoDA	3.8	0.08 (1.42)	<LOD	<LOD	<LOD	<LOD	<LOD	9.8
Perfluoroalkane sulfonic acids (PFSAs)								
PFHxS	99.6	1.58 (2.23)	0.5	1.0	1.5	2.4	6.9	46.5
PFOS (total)		25.41 (1.81)	10.3	17.6	24.9	35.8	69.9	376.0
Linear	100	17.87 (1.80)	7.2	12.5	17.5	24.9	48.5	250.0
Branched	99.8	7.15 (2.03)	2.5	4.7	7.3	11.0	22.1	126.0
Perfluoroalkane sulfonamide substances								
MeFOSAA	99.6	1.45 (2.04)	0.5	0.9	1.5	2.3	4.5	14.4
EtFOSAA	99.0	1.24 (2.54)	0.3	0.7	1.2	2.2	6.2	112.5

LOD, limit of detection; GM, geometric mean; GSD, geometric standard deviation; PFOA, perfluorooctanoate; PFNA, perfluorononanoate; PFDA, perfluorodecanoate; PFUnDA, perfluoroundecanoate; PFDoDA, perfluorododecanoate; PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; and EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate.
To compute GMs for the compounds with values below LOD, those values were replaced with LOD/$\sqrt{2}$.

Supplemental Table 2. Percent changes (95% CIs) in serum concentrations of sex hormones for a doubling increase in PFAS concentrations by BMI.

	BMI (kg/m^2)			
	<25	25-29.9	30+	P for trend
FSH				
n-PFOA	1.02% (-2.70%, 4.89%)	2.88% (-1.03%, 6.95%)	4.07% (-0.21%, 8.54%)[a]	0.540
PFNA	0.70% (-2.80%, 4.33%)	-0.08% (-4.03%, 4.04%)	2.17% (-1.69%, 6.17%)	0.604
PFHxS	-1.52% (-4.22%, 1.25%)	0.80% (-2.16%, 3.86%)	1.28% (-1.58%, 4.23%)	0.212
n-PFOS	0.05% (-3.70%, 3.94%)	3.51% (-0.41%, 7.57%)[a]	3.41% (-0.66%, 7.64%)	0.369
Sm-PFOS	-0.16% (-3.28%, 3.05%)	2.10% (-1.20%, 5.51%)	2.29% (-1.15%, 5.85%)	0.542
Total PFOS	-0.04% (-3.78%, 3.84%)	3.50% (-0.41%, 7.57%)[a]	3.34% (-0.69%, 7.55%)	0.391
MeFOSAA	-2.40% (-5.31%, 0.59%)	3.33% (-0.04%, 6.82%)[a]	-1.31% (-4.85%, 2.36%)	0.553
EtFOSAA	-1.19% (-3.47%, 1.15%)	4.09% (1.51%, 6.74%)[b]	1.40% (-1.24%, 4.12%)	0.164
E_2				
n-PFOA	-2.55% (-6.24%, 1.28%)	-3.27% (-7.31%, 0.94%)	-1.01% (-5.22%, 3.38%)	0.382
PFNA	-4.47% (-7.83%, -0.99%)[b]	-1.81% (-6.24%, 2.83%)	-0.04% (-3.87%, 3.94%)	0.094
PFHxS	0.83% (-1.99%, 3.72%)	-1.74% (-4.98%, 1.62%)	-2.22% (-5.04%, 0.68%)	0.190
n-PFOS	-1.07% (-4.92%, 2.94%)	-3.34% (-7.39%, 0.88%)	-0.68% (-4.75%, 3.55%)	0.633
Sm-PFOS	-0.02% (-3.19%, 3.26%)	-2.45% (-5.93%, 1.16%)	-0.87% (-4.39%, 2.78%)	0.981
Total PFOS	-0.72% (-4.57%, 3.28%)	-3.27% (-7.31%, 0.95%)	-0.67% (-4.70%, 3.53%)	0.692
MeFOSAA	1.75% (-1.32%, 4.92%)	-0.14% (-3.75%, 3.60%)	1.91% (-1.89%, 5.86%)	0.795
EtFOSAA	2.33% (-0.08%, 4.79%)[a]	-1.02% (-3.75%, 1.79%)	-0.42% (-3.15%, 2.38%)	0.214
Testosterone				
n-PFOA	-2.20% (-3.94%, 3.64%)	0.91% (-2.88%, 4.85%)	1.81% (-2.54%, 6.36%)	0.362
PFNA	4.35% (0.66%, 8.17%)[b]	0.68% (-3.15%, 4.66%)	0.52% (-3.40%, 4.60%)	0.095
PFHxS	1.51% (-1.35%, 4.46%)	0.66% (-2.26%, 3.67%)	0.23% (-2.72%, 3.28%)	0.527
n-PFOS	2.47% (-1.39%, 6.48%)	0.90% (-2.85%, 4.79%)	1.97% (-2.18%, 6.29%)	0.840
Sm-PFOS	0.94%	0.79%	1.82%	0.666

	(-2.24%, 4.23%)	(-2.41%, 4.10%)	(-1.80%, 5.57%)	
Total PFOS	2.04% (-1.79%, 6.03%)	1.02% (-2.72%, 4.91%)	2.18% (-1.94%, 6.48%)	0.945
MeFOSAA	-0.73% (-3.77%, 2.40%)	-0.86% (-4.09%, 2.48%)	-1.98% (-5.62%, 1.79%)	0.646
EtFOSAA	-1.77% (-4.09%, 0.62%)	-0.74% (-3.16%, 1.75%)	-0.32% (-2.99%, 2.41%)	0.339
SHBG				
n-PFOA	-1.49% (-5.11%, 2.27%)	1.03% (-2.64%, 4.83%)	0.65% (-3.44%, 4.91%)	0.327
PFNA	-0.47% (-3.94%, 3.12%)	-0.56% (-4.18%, 3.21%)	1.57% (-2.24%, 5.54%)	0.437
PFHxS	1.22% (-1.55%, 4.06%)	1.23% (-1.55%, 4.10%)	-0.62% (-3.42%, 2.27%)	0.319
n-PFOS	-1.96% (-5.56%, 1.79%)	0.39% (-3.19%, 4.11%)	1.39% (-2.53%, 5.47%)	0.170
Sm-PFOS	-1.89% (-4.93%, 1.24%)	-1.31% (-4.32%, 1.80%)	-1.09% (-4.35%, 2.28%)	0.746
Total PFOS	-2.10% (-5.70%, 1.63%)	0.08% (-3.49%, 3.78%)	0.60% (-3.27%, 4.63%)	0.267
MeFOSAA	0.99% (-2.02%, 4.09%)	-0.52% (-3.59%, 2.65%)	1.60% (-1.96%, 5.28%)	0.956
EtFOSAA	-0.71% (-3.00%, 1.64%)	0.20% (-2.14%, 2.59%)	0.69% (-1.87%, 3.31%)	0.390

PFAS, per- and polyfluoroalkyl substances; n-PFOA, linear perfluorooctanoate; PFNA, perfluorononanoate; PFHxS, perfluorohexane sulfonate; n-PFOS, linear perfluorooctane sulfonate; Sm-PFOS, sum of perfluoromethylheptane sulfonate isomers; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; and EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; FSH, follicle-stimulating hormone; E_2, estradiol; SHBG, sex hormone-binding globulin.
All models were adjusted for age, race/ethnicity, site, smoking status, menopausal status, and parity.
[a]*P<0.10,* [b]*P*<0.05.

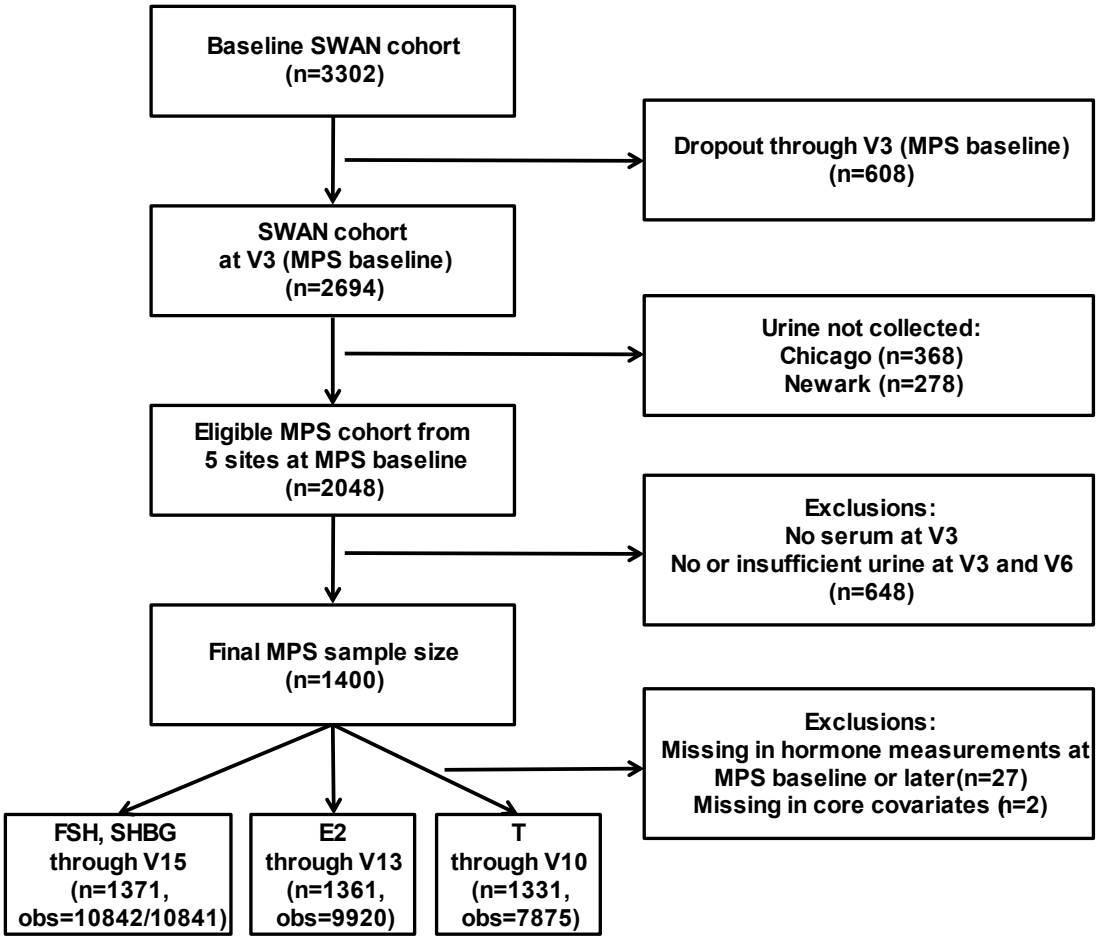
Supplemental Table 3. Sensitivity analysis results with adjustment for education, physical activity and secondhand smoking.

	Education	Physical Activity	Secondhand Smoking
FSH	N=1363	N=1367	N=1370
n-PFOA	3.32% (0.54%, 6.17%)[b]	3.12% (0.36%, 5.95%)[b]	3.19% (0.43%, 6.02%)[b]
PFNA	1.15% (-1.39%, 3.76%)	1.15% (-1.38%, 3.75%)	1.20% (-1.33%, 3.79%)
PFHxS	0.03% (-1.92%, 2.01%)	0.01% (-1.93%, 1.98%)	-0.01% (-1.95%, 1.95%)
n-PFOS	3.01% (0.30%, 5.78%)[b]	2.84% (0.16%, 5.60%)[b]	2.93% (0.25%, 5.69%)[b]
Sm-PFOS	2.34% (0.09%, 4.64%)[b]	2.23% (-0.004%, 4.52%)[a]	2.32% (0.08%, 4.60%)[b]
Total PFOS	3.15% (0.46%, 5.91%)[b]	2.99% (0.32%, 5.73%)[b]	3.08% (0.42%, 5.82%)[b]
MeFOSAA	0.06% (-2.17%, 2.35%)	0.08% (-2.15%, 2.36%)	0.12% (-2.10%, 2.40%)
EtFOSAA	1.85% (0.15%, 3.58%)[b]	1.69% (-0.005%, 3.41%)[a]	1.71% (0.02%, 3.43%)[b]
E_2	N=1354	N=1358	N=1361
n-PFOA	-2.54% (-5.08%, 0.08%)[a]	-2.42% (-4.96%, 0.19%)[a]	-2.48% (-5.02%, 0.13%)[a]
PFNA	-2.29% (-4.66%, 0.14%)[a]	-2.45% (-4.82%, -0.03%)[b]	-2.49% (-4.85%, -0.08%)[b]
PFHxS	-1.01% (-2.88%, 0.88%)	-0.86% (-2.73%, 1.04%)	-0.87% (-2.73%, 1.02%)
n-PFOS	-1.85% (-4.35%, 0.72%)	-1.81% (-4.32%, 0.76%)	-1.86% (-4.36%, 0.71%)
Sm-PFOS	-1.32% (-3.43%, 0.84%)	-1.26% (-3.37%, 0.90%)	-1.32% (-3.43%, 0.84%)
Total PFOS	-1.76% (-4.25%, 0.80%)	-1.71% (-4.20%, 0.85%)	-1.76% (-4.24%, 0.79%)
MeFOSAA	1.11% (-1.08%, 3.36%)	1.01% (-1.19%, 3.25%)	0.98% (-1.21%, 3.21%)
EtFOSAA	0.27% (-1.37%, 1.93%)	0.38% (-1.25%, 2.05%)	0.36% (-1.27%, 2.03%)
Testosterone	N=1324	N=1328	N=1331
n-PFOA	-0.04% (-3.04%, 3.05%)	0.30% (-2.70%, 3.39%)	0.31% (-2.68%, 3.40%)
PFNA	2.10% (-0.74%, 5.03%)	2.23% (-0.60%, 5.15%)	2.21% (-0.62%, 5.11%)
PFHxS	0.72% (-1.47%, 2.95%)	0.91% (-1.26%, 3.14%)	0.90% (-1.27%, 3.12%)
n-PFOS	1.26% (-1.69%, 4.30%)	1.44% (-1.50%, 4.47%)	1.41% (-1.53%, 4.43%)
Sm-PFOS	0.32% (-2.13%, 2.84%)	0.50% (-1.95%, 3.01%)	0.52% (-1.93%, 3.03%)
Total PFOS	1.05% (-1.87%, 4.06%)	1.24% (-1.68%, 4.24%)	1.22% (-1.69%, 4.22%)
MeFOSAA	-1.55% (-4.00%, 0.96%)	-1.56% (-4.01%, 0.95%)	-1.55% (-3.99%, 0.95%)
EtFOSAA	-1.50% (-3.34%, 0.37%)	-1.53% (-3.36%, 0.34%)	-1.51% (-3.34%, 0.35%)
SHBG	N=1363	N=1367	N=1370
n-PFOA	0.76% (-2.30%, 3.92%)	0.88% (-2.17%, 4.03%)	0.85% (-2.19%, 4.00%)
PFNA	0.42% (-2.44%, 3.36%)	0.17% (-2.67%, 3.08%)	0.20% (-2.63%, 3.11%)
PFHxS	0.39% (-1.82%, 2.65%)	0.43% (-1.77%, 2.69%)	0.38% (-1.82%, 2.62%)
n-PFOS	1.15% (-1.85%, 4.24%)	0.92% (-2.05%, 3.98%)	0.99% (-1.98%, 4.04%)
Sm-PFOS	0.25% (-2.24%, 2.81%)	0.16% (-2.31%, 2.69%)	0.18% (-2.30%, 2.71%)
Total PFOS	1.07% (-1.91%, 4.13%)	0.85% (-2.09%, 3.89%)	0.91% (-2.03%, 3.95%)
MeFOSAA	1.83% (-0.73%, 4.47%)	1.69% (-0.86%, 4.31%)	1.77% (-0.77%, 4.39%)
EtFOSAA	1.09% (-0.82%, 3.03%)	1.15% (-0.75%, 3.08%)	1.15% (-0.75%, 3.08%)

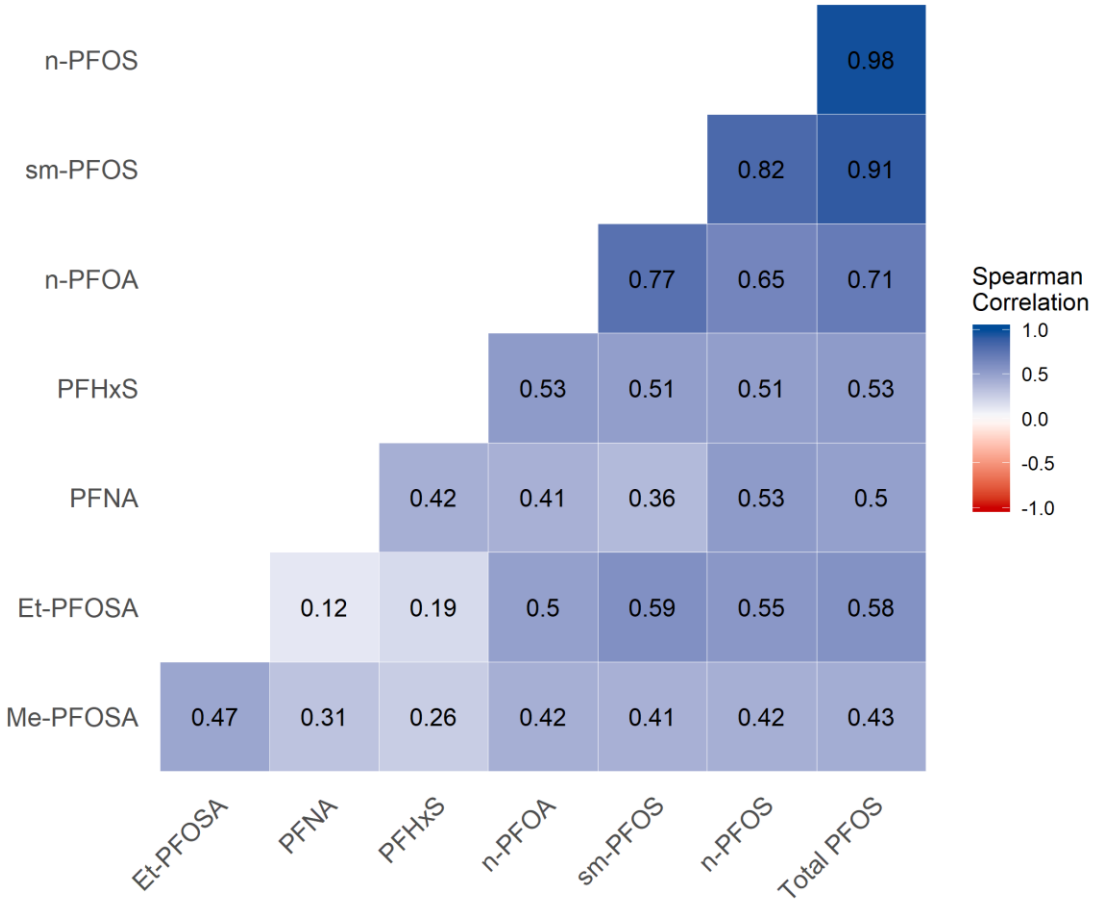
PFAS, per- and polyfluoroalkyl substances; n-PFOA, linear perfluorooctanoate; PFNA, perfluorononanoate; PFHxS, perfluorohexane sulfonate; n-PFOS, linear perfluorooctane sulfonate; Sm-PFOS, sum of perfluoromethylheptane sulfonate isomers; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetate; and EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; FSH, follicle-stimulating hormone; E_2, estradiol; SHBG, sex hormone-binding globulin.

Models adjusted for age, race/ethnicity, site, smoking status, menopausal status, and parity.

[a]*P<0.10,* [b]*P*<0.05, [c]*P*<0.01.



Supplemental Figure 1. A schematic diagram of analytic samples.



Supplemental Figure 2. A correlation heatmap. Spearman correlation coefficients are shown. More intense color indicates a stronger correlation. PFOA, perfluorooctanoate; PFNA, perfluorononanoate; PFDA, perfluorodecanoate; PFUA, perfluoroundecanoate; PFDoA, perfluorododecanoate; PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamido) acetic acid; and EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid.

Supplemental Methods

Description of the mixture analysis

To evaluate the associations of hormones with PFAS as mixtures as an exploratory analysis, we conducted a two-stage modeling approach to account for correlations for both dependent and independent variables (1). In stage-1, to account for correlations in repeatedly measured outcomes within each participant, hormone levels were regressed on time-varying covariates (age, menopausal status, BMI) in linear mixed effects models and participant-specific hormone levels (random intercepts) were estimated. In stage-2, to account for correlations among exposure variables, adaptive elastic-net was used (2). Participant-specific hormone estimates from the stage-1 as the outcome and a linear combination of standardized PFAS variables as exposures were fit with adjustment for site/race-ethnicity, smoking status, and parity (time-constant covariates).

Elastic-net is a hybrid approach of LASSO (least absolute shrinkage and selection operator) and ridge regression that was developed to not only perform variable selection by shrinking coefficients of predictors to zero's but deal with complex correlations among predictors (3). Adaptive elastic-net is an adaptive version of elastic-net that allows it to conduct statistical inference and hypothesis testing with the asymptotic normality assumption (2). The adaptive elastic-net models were fitted as follows:

$$Y_i = \beta_0 + \Sigma\beta_j X_{ji} + Z_i + \varepsilon_i$$

where Y_i represents participant-specific hormone estimates from the stage-1, X_{ji} indicates serum concentration of j^{th} PFAS, Z_i represents the vector of time-constant covariates (site/race-ethnicity, smoking status, and parity), and ε_i indicates error terms.

Then the estimates from the adaptive elastic-net method are defined by

$$\widehat{\beta_\iota} = \left(1 + \frac{\lambda_2}{N}\right)\left\{argmin\left(\sum_{i=1}^{N}(Y_i - \mathrm{X}_i\beta)^2 + \lambda_1\sum_{j=1}^{P}\widehat{\omega}_j|\beta_j| + \lambda_2\sum_{j=1}^{P}{\beta_j}^2\right)\right\},$$

where $\widehat{\omega}_j$ represents the weight of j^{th} PFAS, which can be determined by the beta coefficient from elastic net. These weights can allow coefficients of relatively less important variables (components of PFAS) to be shrunk to zero's more efficiently. Optimal tuning parameters (λ_1 and λ_2) were chosen based on 5-fold cross-validations to minimize prediction errors. The R package gcdnet (version 1.0.5) was used to implement adaptive elastic-net.

References

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