A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP11033.

Drinking Water–Associated PFAS and Fluoroethers and Lipid Outcomes in the GenX Exposure Study

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Background: Residents of Wilmington, North, Carolina, were exposed to drinking water contaminated by fluoroethers and legacy per- and polyfluoroalkyl substances (PFAS), such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), with fluoroether exposure occurring from 1980 to 2017. PFOA and PFOS have previously been associated with metabolic dysfunction; however, few prior studies have examined associations between other PFAS and lipid levels.

Objectives: We measured the association between serum fluoroether and legacy PFAS levels and various cholesterol outcomes.

Methods: Participants in the GenX Exposure Study contributed nonfasting blood samples in November 2017 and May 2018 that were analyzed for 20 PFAS (10 legacy, 10 fluoroethers) and serum lipids [total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides] and calculated non-HDL cholesterol. We estimated covariate-adjusted associations between quartiles of exposure to each of the PFAS measures (as well as the summed concentrations of legacy PFAS, fluoroethers, and all 10 targeted PFAS) and lipid outcomes by fitting inverse probability of treatment weighted linear regressions.

Results: In this cross-sectional study of 326 participants (age range 6–86 y), eight PFAS were detected in >50% of the population. For PFOS and perfluorononanoic acid (PFNA), non-HDL cholesterol was approximately 5 mg/dL higher per exposure quartile increase: [PFOS: 4.89; 95% confidence interval (CI): 0.10, 9.68 and PFNA: 5.25 (95% CI: 0.39, 10.1)], whereas total cholesterol was approximately 6 mg/dL higher per quartile [PFOS: 5.71 (95% CI: 0.38, 11.0), PFNA: 5.92 (95% CI: 0.19, 11.7)]. In age-stratified analyses, associations were strongest among the oldest participants. Two fluoroethers were associated with higher HDL, whereas other fluoroether compounds were not associated with serum lipid levels.

Discussion: PFNA and PFOS were associated with higher levels of total and non-HDL cholesterol, with associations larger in magnitude among older adults. In the presence of these legacy PFAS, fluoroethers appeared to be associated with HDL but not non-HDL lipid measures. https://doi.org/10.1289/EHP11033

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals frequently added to consumer products because of their heat-, water-, grease-, and oil-resistant properties.[1] Once in the environment, many PFAS are persistent, mobile, and bioaccumulative.[2] Outside of occupational settings, humans are primarily exposed through consumer products and consumption of contaminated drinking water.[3] Of the thousands of PFAS, relatively few have been measured and analyzed in humans. Of those, the most frequently detected in human blood serum are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), both of which have biological half-lives on the order of years.[4]

In humans, exposure to PFAS has been associated with numerous adverse outcomes, including metabolic dysfunction.[5] The European Food Safety Authority concluded in 2018 that epidemiological evidence provided sufficient support to conclude that there are causal associations between exposure to PFOA and PFOS and increased cholesterol.[6] The strongest evidence exists for the association between PFOA and total cholesterol or low-density lipoprotein (LDL), with fewer studies finding associations with high-density lipoprotein (HDL) or triglycerides.[7] However, the metabolic effects of exposure to other PFAS are largely unstudied, including the metabolic effects of exposure to novel PFAS, such as fluoroethers, that are used or formed as byproducts in industrial processes.[8,9]

Wilmington, North Carolina, a city of approximately 120,000 residents, receives its water from the Cape Fear Public Utility Authority (CFPUA), which sources water from the Cape Fear River and local groundwater. Located about 90 miles upstream of the raw water intake for CFPUA is Fayetteville Works, a fluorochemical manufacturing facility. From 1980 until 2017, Fayetteville Works discharged process wastewater containing PFAS to the Cape Fear River.[10] Since 2013, fluoroethers have been detected in the Cape Fear River downstream of Fayetteville Works, though they have likely discharged into the river since 1980.[9,11,12] These fluoroethers are byproducts of the production of fluoropolymers and their building blocks. Fluoroethers are structurally similar to legacy PFAS, but the fluorinated alkyl chain is interspersed with ether oxygen atoms.[9,11,13] The human health effects of fluoroethers are poorly understood because studies in human population with exposure to fluoroethers are lacking. Hexafluoropropylene oxide dimer acid (HFPO-DA or GenX) is one of the fluoroethers of concern in Wilmington due to the high levels measured in water samples collected downstream of Fayetteville Works and in the finished water for the city of Wilmington.[12] Additional fluoroethers of concern are Nafion by-product 2, perfluoro (3,5,7,9-tetraoxadecanoic) acid (PFO4DA), and perfluoro-3,5,7,9,11-pentaoxadodecanoic acid

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Supplemental Material is available online (https://doi.org/10.1289/EHP11033).

Authors have no conflicts of interest to declare.

Received 31 January 2022; Revised 6 July 2022; Accepted 17 August 2022; Published 7 September 2022.

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(PFO5DoA), which were frequently detected in blood serum samples of people living in Wilmington.[14]

The GenX Exposure Study was initiated in November 2017 in response to community concerns in Wilmington about drinking water exposure to PFAS, including GenX. The study enrolled residents ages 6 y and older and collected biological samples to evaluate PFAS and clinical outcomes. PFAS and fluoroethers were measured in participants' sera and were examined for an association with serum cholesterol levels.

Methods

Population

The GenX Exposure is a community-based study of PFAS exposure.[14] Study participants were enrolled 10–12 November 2017 and 5 May 2018 in Wilmington. Individuals ages 6 y and older who received water from the CFPUA and were current residents of New Hanover County for at least 12 months prior to November 2017 were eligible. Pregnant women, HIV positive, and Hepatitis C positive individuals were ineligible. Individuals were screened prior to enrollment using an anonymous questionnaire, and all exclusion information was based on self-report. At enrollment, individuals provided informed assent and/or consent, had a blood sample collected by trained phlebotomists, had height and weight measured, and completed a questionnaire regarding demographic, health, and drinking water use characteristics. Questionnaires were administered by study staff at the time of blood collection. Study staff included trained phlebotomists, North Carolina State University (NCSU) students, faculty, and staff, as well as trained community volunteers. All received human subjects training prior to data collection. All materials were available in English and Spanish. All procedures were approved by the NCSU institutional review board, IRB number 12229. Participants did not receive monetary compensation for participation but did receive their PFAS results.

Blood Collection and Sample Processing

Location for the November sampling was the New Hanover County Health Department. All samples were collected inside and then processed in the health department clinical laboratory. Location for the May sampling was the MLK Community Center in Wilmington. All samples were collected inside and then transported to the New Hanover County Health Department for processing.

Four tubes of blood (2 red top borosilicate glass tube for serum, 2 lavender top K_2EDTA tubes) were collected from individuals ages 11 y and older; children 6–10 y provided two red top tubes for serum. All blood samples were processed on site to separate the serum for clinical analyses and PFAS measurement. Serum tubes held at room temperature for 30–60 min, then spun at $1{,}300 \times g$ for 10 min in a Sorvall RT 600D centrifuge at room temperature. Room temperature was approximately 24°C (75°F). Serum was aliquoted into transfer tubes, immediately frozen on dry ice, transported to East Carolina University (Greenville, North Carolina), and stored at −80°C (−112°F) prior to analysis. All blood samples were nonfasting. PFAS and cholesterol were from the serum tubes that were collected first. If possible, both were aliquots from the same tube. Otherwise, the cholesterol sample was from the second tube. Additional detail on collection procedures have been previously described.[14]

PFAS Measurement

The measurement of PFAS in serum has been detailed in Kotlarz et al.[14] Briefly, 50 μL of serum was combined with 100 μL 0.1 M formic acid containing mass-labeled standards (6.25 ng/mL) to denature serum proteins. The sample was vortex mixed again and centrifuged at $12{,}500 \times g$ for 5 min at room temperature. Finally, a 100-μL aliquot of the acetonitrile supernatant was placed with 100 μL 0.4 mM ammonium formate buffer (1:1 mixture).

Measurements for PFAS (10 fluoroethers, 10 legacy PFAS) in serum were conducted using a Thermo Vanquish ultra-performance liquid chromatograph coupled to a Thermo Orbitrap Fusion mass spectrometer (LC-HRMS). A full list of the measured compounds is displayed in Table S1. Using a 25-μL injection volume, PFAS were separated on an Accucore Vanquish C18+LC column (100×2.1 mm, 1.5 μL particle diameter). Mass-labeled analytical standards were not available for all analytes. Native standards were commercially available (Wellington Labs) for 11 of the 20 PFAS (1 fluoroether, 10 legacy PFAS); mass-labeled standards were available for 8 of these 11. Analytical standards for the remaining fluoroethers, including Nafion by-product 2, PFO4DA, PFO5DoA, and perfluoro-3,5,7-trioxaoctanoic acid (PFO3OA) were acquired as aqueous solutions (1,000 ng/μL) from the Chemours Company because there were no commercial sources at the time. The identity of each standard was confirmed by high-resolution mass spectrometry. A mixed PFAS standard stock solution was prepared in methanol at 0.1 ng/μL. Calibration standards were prepared in newborn calf serum (ThermoFisher Scientific) by spiking PFAS standard stock solution into the serum; calibration standards were processed using the protocol for human serum samples described above. Compounds were quantified using a relative response ratio of the native standard and either the available matching or a closely eluting isotopically labeled internal standard. Integration of PFAS isomers was consistent with U.S. Environmental Protection Agency (U.S. EPA) Method 537.1 (2018); that is, for compounds with branched and linear isomers (PFOA, PFOS, PFHxS), peaks for the branched and linear isomers were integrated together to report total concentrations. The method reporting limit (MRL) varied over the eight batches (Table S2). For the PFAS that were detected in blood serum, MRLs were 0.1 ng/mL for Nafion by-product 2, PFO4DA, and PFO5DoA; 0.1–0.5 ng/mL for PFOS; 0.1–0.5 ng/mL for PFOA; 0.1–1.8 ng/mL for PFHxS; 0.1–0.9 ng/mL for PFNA, and 0.1–0.3 ng/mL for PFHpA. Batch-specific MRLs were considered when imputing values below the MRL.

Outcome Measurements

Clinical analyses for lipids were conducted at Vidant Medical Center, Greenville, approximately 2–4 wk following sample collection. Serum lipid levels were determined using enzymatic methods employing an Abbott ARCHITECHT automated clinical analyzer platform. The lipid panel included total cholesterol, HDL cholesterol, triglycerides, and LDL cholesterol. Low-density lipoprotein cholesterol (LDL-c) concentrations were determined using the Friedewald Formula (FF). Because the FF is not valid for triglyceride (TG) levels of 400 mg/dL or greater, LDL-c values were not calculated/reported for subjects with triglycerides levels ≥400 mg/dL; these participants were excluded from the analysis ($n = 14$).[15]

Participants were not specifically instructed to fast prior to blood collection; therefore, non-HDL levels, which have been shown to be robust to fasting status,[16–18] were an outcome of particular interest. We calculated non-HDL cholesterol as total cholesterol minus HDL cholesterol.

Statistical Analyses

Univariate and bivariate analyses of the outcome variables and exposures were conducted to summarize distributions of these variables and their associations with covariates. Linear regression

models were used to quantify the association between measurements of PFAS in serum and the outcome variables (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, and non-HDL cholesterol).

We conducted regression analyses for those PFAS for which at least 50% of the study sample had measured values above the MRL. For the purposes of regression analyses, we categorized each PFAS into quartiles based on its distribution in the overall study sample. Measured values below the MRL were assigned a value equal to $\mathrm{MRL}/\sqrt{2}$.[19] We also fitted models in which the exposure of interest represented summed PFAS concentrations; specifically, we calculated the summed concentration of fluoroethers [Nafion by-product 2, PFO4DA, PFO5DoA, PFO3OA, and 1,1,2,2-Tetrafluoro-2-(1,2,2,2-tetrafluoroethoxy)ethanesulfonic acid (NVHOS)], the summed concentration of legacy PFAS (PFOS, PFOA, PFNA, PFHxS, PFHpA), and the summed concentration of 10 detected PFAS (Σ_{10} PFAS).[14] In these summed variables, values below the MRL were recoded to 0 to avoid artificially inflating overall levels.[14]

A directed acyclic graph (DAG) was used to identify potential confounders of the associations of interest (Figure S1). We identified the following variables as confounders in our minimally sufficient set: gender, self-reported race/ethnicity (White non-Hispanic, Black non-Hispanic, Hispanic regardless of race, other), body mass index (BMI; quartiles), age (quartiles), and ever smoker (yes, no, not asked). Smoking information was not included on the questionnaire completed by those ≤18 y old. Race/ethnicity was included as a confounder because it is known to be associated with both PFAS levels and lipid outcomes.[20,21] Participants indicated their race from the following provided options: Multiracial; American Indian/Alaska Native/Native American/Pacific Islander; Asian; Black; White; Other. Participants selected their ethnicity as Hispanic or non-Hispanic. A combined race/ethnicity variable was created and due to small sample size, participants not identifying as White, Black, or Hispanic were categorized as "other."

Inverse probability of treatment weighting (IPTW) was used to control for potential confounding; this approach balances, in expectation, the distributions of confounders within each quartile of the exposure distribution, with the target population being the total study population.[22] Separate IPT weights were calculated for each PFAS, where each set of weights was derived from a multinomial logistic regression model for the quartiles of that PFAS, with the same set of independent variables included in each model. To avoid imprecision in estimated weights, categories with small samples were collapsed (e.g., one transgender participant collapsed into male; one person with unknown race collapsed into other). Participants with missing values of covariates were dropped from the analyses ($n=4$). The resultant weights were then applied in linear regression analyses of the outcomes to obtain estimates of the associations of interest.

Applying the IPT weights, we examined the weighted average level of non-HDL cholesterol across each quartile of PFAS exposure. We fit IPT-weighted linear regression models to estimate the association between PFAS exposure (expressed in quartiles) and each individual cholesterol outcome. All analyses were conducted in SAS (version 9.4; SAS Institute, Inc.).

To examine potential modification of exposure–outcome associations by attained age, we conducted analyses among those >18 y of age in which we allowed for an interaction between exposure and tertiles of age (19–44, 45–62, 63–86 y) at time of blood draw. We examined the effect estimate within each tertile as well as the *p*-value for the interaction term between age tertile and exposure. In a separate analysis of those ≤18 y of age, we examined age as a dichotomous variable (6–11 vs. ≥12–18). Smoking was not included in the latter model (because smoking information was not elicited from children), and quartiles of PFAS and BMI were recalculated based on the distribution in the children.

Sensitivity Analyses

Several adult participants reported taking medications to lower their cholesterol. This question was not asked of those younger than 18 y or those who did not report high cholesterol. We assumed that individuals without a high cholesterol diagnosis were not using cholesterol medications. To assess the impact of use of these medications on our findings, we conducted a sensitivity analysis in which use of cholesterol-lowering medications (yes vs. no) was included as a weighting variable. To assess residual confounding by age, we restricted to those in the highest tertile of age and reweighted the age quartiles to the distribution of age to those in this stratum.

Results

Our sample consisted of 326 individuals in the GenX study who provided blood samples in Wilmington between 2017 and 2018. The population was primarily female and White non-Hispanic, with an average age of approximately 50 y (Table 1). Total cholesterol tended to be higher among women than among men and to correlate positively with BMI. Those who identified as Hispanic had lower average total, HDL, and non-HDL cholesterol in comparison with people who identified as non-Hispanic. Mean triglyceride levels were higher among those with a BMI of 25–29.99 or 30+, former or current smokers, and among participants with a high school education or less. Total cholesterol and LDL were highly correlated (0.93), total cholesterol and non-HDL were highly correlated (0.95), and non-HDL and LDL were highly correlated (0.94) (Table S3). In this population, 114 participants (35%) had total cholesterol ≥200 mg/dL, which exceeds both the desirable level of <200 mg/dL set by the National Cholesterol Education Program (NCEP)[18] and 150 mg/dL recognized by the American Heart Association.[23] Of these, 55 participants had information on use of cholesterol-lowering medications, and 45% ($n=25$) reported use.

Ten PFAS were detected in this study (Table 2). PFOS, PFOA, and Nafion by-product 2 were detected in >99% of participants. PFOS and PFOA had the highest measured levels in participants, with geometric mean (geometric standard deviation) levels of 8.08 (2.2) ng/mL and 4.13 (2.0) ng/mL, respectively. NVHOS (detected in 15.8% of participants) and PFO3OA (detected in 28.8% of participants) were the least frequently detected PFAS and were not evaluated individually further in statistical analyses. Spearman's correlation coefficients between exposure quartiles ranged from 0.17 (PFOS and PFHpA) to 0.85 (Nafion by-product 2 and PFO5DoA) (Table S4).

After applying IPT weights to account for potential confounders, levels of non-HDL cholesterol were generally lowest in the lowest quartile of the legacy PFAS (relative to the upper three quartiles of their respective PFAS) but did not increase in a monotonic fashion across quartiles (Table 3). For example, study participants in the lowest quartile of PFOS exposure had measured non-HDL of 117 mg/dL, well within the cut point of <130 mg/dL considered optimal by NCEP,[18] whereas the non-HDL levels of those in quartiles 2 through 4 ranged from 132 to 136 mg/dL, levels that fall in the near optimal/above optimal range of 130–159 mg/dL.[18] For both PFOS and PFNA, the lowest quartile of these chemicals had significantly lower non-HDL cholesterol than all other quartiles. For the $\sum_5$ legacy PFAS, participants in quartiles 2 and 3 of exposure had the

Table 1. Characteristics of 326 GenX exposure study participants with data on PFAS and cholesterol (mg/dL), 2017–2018.

		Total cholesterol	HDL cholesterol	LDL cholesterol	Non-HDL cholesterol	Triglycerides
	n (%)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (y)						
6–34	82 (25.2)	156.1 ± 29	51.0 ± 10	84.9 ± 24	105.2 ± 29	101.5 ± 63
35–51	85 (26.1)	190.9 ± 39	54.9 ± 14	111.5 ± 34	136.0 ± 38	122.9 ± 67
52–64	78 (23.9)	200.8 ± 42	56.6 ± 15	115.5 ± 35	144.3 ± 36	143.7 ± 70
65+	81 (24.8)	199.2 ± 50	57.8 ± 16	113.6 ± 41	141.4 ± 44	138.6 ± 71
BMI						
<18.5	23 (7.1)	157.3 ± 26	52.2 ± 9	86.8 ± 22	105.1 ± 24	91.6 ± 31
18.5–24.99	114 (35.0)	186.9 ± 46	61.5 ± 14	106.5 ± 38	125.4 ± 41	94.8 ± 48
25–29.99	84 (25.8)	188.6 ± 48	53.2 ± 15	107.3 ± 38	135.4 ± 41	140.6 ± 75
30+	105 (32.2)	191.0 ± 41	50.0 ± 12	109.5 ± 35	140.9 ± 38	157.0 ± 73
Gender						
Male	116 (35.6)	172.1 ± 37	49.9 ± 12	97.0 ± 32	122.2 ± 37	126.3 ± 68
Female	209 (64.1)	194.8 ± 46	57.9 ± 15	111.6 ± 38	136.9 ± 41	126.9 ± 70
Transgender	1 (0.3)	—	—	—	—	—
Adult education						
Not applicable (age <18 y)	52	149.6 ± 22	50.7 ± 10	80.8 ± 19	98.9 ± 20	90.5 ± 55
High school/GED or less	34 (12.5)	194.0 ± 49	51.5 ± 14	111.5 ± 41	142.5 ± 44	154.7 ± 83
Some college	56 (20.6)	195.2 ± 51	54.6 ± 17	111.7 ± 43	140.6 ± 43	144.4 ± 77
College graduate	96 (35.3)	190.8 ± 39	55.0 ± 13	110.0 ± 34	135.8 ± 37	128.7 ± 62
Post college	86 (31.6)	195.8 ± 43	59.4 ± 14	111.8 ± 35	136.4 ± 40	123.3 ± 66
Missing	2	—	—	—	—	—
Race/ethnicity						
White non-Hispanic	254 (77.9)	188.8 ± 44	56.3 ± 14	107.2 ± 36	132.5 ± 40	126.8 ± 68
Black non-Hispanic	31 (9.5)	192.6 ± 51	54.4 ± 16	116.3 ± 40	138.2 ± 44	109.7 ± 58
Hispanic, regardless of race	31 (9.5)	162.0 ± 39	47.4 ± 11	87.7 ± 34	114.6 ± 37	134.3 ± 88
Other	10 (3.1)	187.6 ± 29	48.4 ± 10	110.5 ± 27	139.2 ± 28	143.6 ± 58
Alcohol consumption						
Any	199 (72.6)	194.6 ± 43	56.8 ± 15	110.6 ± 37	137.8 ± 40	135.9 ± 72
None	75 (27.4)	189.8 ± 46	52.9 ± 14	111.7 ± 38	136.9 ± 40	126.2 ± 62
Not asked (age <18 y)	52	—	—	—	—	—
Smoking status						
Never	154 (56.2)	190.3 ± 44	55.3 ± 15	109.7 ± 36	134.9 ± 39	126.3 ± 68
Former	93 (33.9)	198.5 ± 48	57.1 ± 14	113.3 ± 41	141.4 ± 43	140.8 ± 72
Current	27 (9.9)	192.7 ± 30	53.3 ± 15	110.0 ± 25	139.3 ± 29	146.9 ± 72
Not asked (age <18 y)	52	—	—	—	—	—
Total population	326 (100)	186.6 ± 44	55.0 ± 14	106.3 ± 36	131.3 ± 40	126.4 ± 69

Note: —, no data; GED, general education diploma; PFAS, per- and polyfluoroalkyl substances; SD, standard deviation.

highest levels of non-HDL cholesterol (139 and 135 mg/dL, respectively), whereas those in quartile 4 (126 mg/dL) had a somewhat lower mean level of non-HDL cholesterol; quartile 1 (117 mg/dL) was significantly lower than quartiles 2 and 3. The same trend was not true of the fluoroethers. There were no significant differences noted across quartiles of PFOA, PFHpA, Nafion by-product 2, PFO4DA, and total summed PFAS.

Relationships between PFAS quartiles and other lipid measures are presented in Table S5. HDL cholesterol exhibited the least variability across quartiles, whereas total and LDL cholesterol tended

Table 2. Distribution of PFAS chemicals (nanograms per milliliter) among those with cholesterol measures, 326 participants, GenX exposure study, Wilmington, North Carolina, 2017–2018.

	Percentage >MRL[a]	Geometric mean (GSD)	Median (25th, 75th)	Min–Max
Legacy				
PFOS	99.4	8.08 (2.2)	8.47 (5.00, 13.62)	<MRL–54.27
PFOA	99.7	4.13 (2.0)	4.34 (2.72, 6.93)	<MRL–20.23
PFNA	97.0	1.19 (2.0)	1.19 (0.78, 1.98)	<MRL–7.52
PFHxS	97.6	2.97 (2.1)	3.19 (1.80, 5.19)	<MRL–15.22
PFHpA	62.4	0.31 (2.3)	0.26 (0.18, 0.58)	<MRL–4.46
$\sum_5$ legacy PFAS	—	17.24 (2.0)	18.80 (11.05, 28.69)	0.48–79.08
Fluoroethers				
Nafion by-product 2	99.1	2.50 (2.4)	2.75 (1.51, 4.67)	<MRL–16.87
PFO4DA	98.5	2.13 (3.5)	2.41 (0.83, 5.49)	<MRL–51.17
PFO5DoA	87.3	0.28 (2.3)	0.31 (0.15, 0.51)	<MRL–2.02
PFO3OA[b]	28.8	—	<MRL (<MRL, 0.14)	<MRL–4.20
NVHOS[b]	15.8	—	<MRL (<MRL, <MRL)	<MRL–4.46
$\sum_5$ Fluoroethers	—	5.84 (2.5)	6.17 (3.03, 10.87)	0.43–78.31
$\sum_{10}$ PFAS	—	23.99 (2.0)	25.29 (15.44, 39.85)	1.54–132.87

Note: —, no data; GSD, geometric standard deviation; Mas, maximum; Min, minimum; MRL, method reporting limit; NVHOS, 1,1,2,2-Tetrafluoro-2-(1,2,2,2-tetrafluoroethoxy)ethanesulfonic acid; PFAS, per- and polyfluoroalkyl substances; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFO3OA, perfluoro-3,5,7-trioxaoctanoic acid; PFO4DA, perfluoro-3,5,7,9-tetraoxadecanoic acid; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

[a]MRL for all exposures was typically 0.1 ng/mL. Full list of MRLs displayed in table S1.

[b]Means are not presented for NVHOS and PFO3OA due to their low frequency of detection.

Table 3. Mean level (SD) of non-HDL cholesterol (milligrams per deciliter) by quartile of PFAS exposure, weighted for age, sex, BMI, race/ethnicity, and smoking status.

	PFOS	PFOA	PFNA	PFHxS	PFHpA	$\sum_5$ legacy PFAS	Nafion by-product 2	PFO4DA	PFO5DoA	$\sum_5$ Fluoroethers	$\sum_{10}$ PFAS
Q1	116.5 ± 37	122.3 ± 37	116.1 ± 34	114.7 ± 39	132.7 ± 31	117.4 ± 37	129.2 ± 37	129.9 ± 38	125.0 ± 36	126.2 ± 35	119.9 ± 37
Q2	136.4 ± 34	132.7 ± 38	132.2 ± 36	138.4 ± 38	132.1 ± 57	138.6 ± 37	138.6 ± 37	134.1 ± 40	137.2 ± 42	133.2 ± 43	134.8 ± 41
Q3	135.0 ± 38	134.1 ± 40	138.1 ± 41	128.0 ± 41	127.0 ± 36	134.9 ± 38	134.5 ± 37	125.4 ± 38	136.1 ± 37	133.2 ± 38	131.5 ± 34
Q4	131.7 ± 37	130.9 ± 40	130.8 ± 36	130.6 ± 33	131.7 ± 40	126.4 ± 36	121.8 ± 41	129.0 ± 37	118.5 ± 42	126.0 ± 37	127.3 ± 40

Note: BMI, body mass index; HDL, high-density lipoprotein; PFAS, per- and polyfluoroalkyl substances; PFNA, perfluorononanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFO4DA, perfluoro-3,5,7,9-tetraoxadecanoic acid; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; SD, standard deviation.

to be highest in quartiles 2 and 3 of PFAS exposures. Triglycerides were lowest in the lowest quartile of exposure, but no consistent trends were noted across compounds for quartiles 2 through 4.

In the weighted regression model estimating the change in non-HDL cholesterol per quartile increase of PFAS, we noted the strongest associations among the legacy PFAS, specifically PFOS and PFNA (Figure 1 and Table 4). On average, a 1-quartile increase in PFOS was associated 4.9 ng/dL higher non-HDL cholesterol [95% confidence interval (CI): 0.10, 9.68] (Table 4). For a one-quartile increase in PFNA, non-HDL was higher on average by 5.25 ng/dL (95% CI: 0.39, 10.10). Positive trends of small magnitude were also noted for associations between PFOA and PFHxS and non-HDL cholesterol. We did not observe any associations between exposure to fluoroether PFAS and non-HDL cholesterol, either individually or in a summed measure.

PFOS and PFNA were positively associated with total cholesterol, with effect estimate sizes similar to those observed with non-HDL cholesterol (Table 4). Nafion by-product 2 and PFO5DoDA were positively associated with HDL cholesterol, where HDL cholesterol was elevated by approximately 2 mg/dL for each quartile of exposure. No other associations were noted between any exposure and outcome pair. Including use of cholesterol medications in the weighting model did not meaningfully change estimates (Table S6).

In models with adult participants stratified by tertile of age, we noted statistically significant effect modification, such that the associations were of largest magnitude among the oldest participants (Table 5). Associations between exposure quartile (treated continuously) and outcome (total cholesterol and non-HDL cholesterol) increased with age across multiple compounds. Significant interaction ($p < 0.1$) was noted for PFOS, PFNA (non-HDL only), PFHpA, summed legacy PFAS (non-HDL only), all fluoroethers, and summed total PFAS (non-HDL only), though the estimates themselves were largely not statistically significant.

Effect estimates for all exposures were null among those 19–44 y old. Among those 45–62 y old, associations were noted between legacy PFAS but not for fluoroethers. In this age group, for each quartile increase in summed legacy PFAS measures, total cholesterol was 8.71 mg/dL higher (95% CI: 0.84, 16.58) and non-HDL cholesterol was 7.17 mg/dL higher (95% CI: 0.35, 13.98). Associations were strongest among those 63–86 y old and larger for legacy PFAS than fluoroethers. For each quartile increase of total summed PFAS, total cholesterol was 9.62 mg/dL (95% CI: 1.22, 18.02) higher, whereas non-HDL cholesterol was 8.90 mg/dL (95% CI: 1.38, 16.41) higher on average for those age 63 y and older (Table 5). Associations were not meaningfully different between the reweighted population and the original population for those in the highest tertile of age (Table S7).

When restricting to those study participants who were age 18 y or younger, associations between PFAS and the outcomes of interest were largely null (Table S8). However, similar to findings for the total population, PFNA was positively associated with total cholesterol. We additionally observed a positive association between PFO5DoDA and total cholesterol. Among those >18 y old, associations were larger for PFOA and PFHxS, driving larger associations with summed legacy PFAS and total PFAS (Table S9).

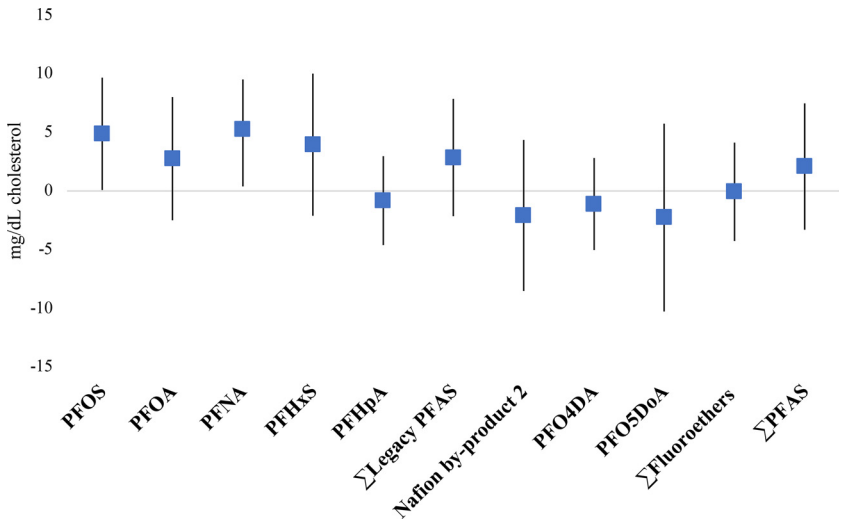

Figure 1. Weighted beta estimates (95% CI) for association between PFAS and non-HDL cholesterol, per quartile increase of PFAS. Corresponding numeric data for this figure is available in Table 4. Estimates are weighted for age, sex, BMI, race/ethnicity, and smoking status. Note: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; PFAS, per- and polyfluoroalkyl substances; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFO4DA, perfluoro-3,5,7,9-tetraoxadecanoic acid; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

Table 4. Betas (milligrams per deciliter) (95% CIs) for a per quartile increase in selected chemical, 326 people from the GenX exposure study, Wilmington, North Carolina, 2017–2018, weighted for age, sex, BMI, race/ethnicity, and smoking status.

	Total cholesterol	HDL	LDL	Non-HDL	Triglycerides
Legacy PFAS					
PFOS	5.71 (0.38, 11.04)	0.82 (−1.19, 2.84)	3.46 (−0.67, 7.58)	4.89 (0.10, 9.68)	7.16 (−1.32, 15.64)
PFOA	2.77 (−2.48, 8.02)	−0.08 (−1.91, 1.74)	2.00 (−2.04, 6.04)	2.86 (−1.82, 7.63)	4.41 (−3.35, 12.17)
PFNA	5.92 (0.19, 11.65)	0.68 (−1.49, 2.84)	3.86 (−0.24, 7.97)	5.25 (0.39, 10.10)	6.94 (−1.69, 15.56)
PFHxS	3.83 (−2.96, 10.62)	−0.13 (−2.51, 2.25)	3.41 (−1.63, 8.45)	3.96 (−2.10, 10.02)	2.87 (−5.72, 11.45)
PFHpA	−0.54 (−4.80, 3.73)	0.30 (−1.14, 1.74)	−1.70 (−5.21, 1.81)	−0.83 (−4.63, 2.97)	4.56 (−2.53, 11.66)
$\sum_5$ legacy PFAS	3.78 (−1.82, 9.38)	0.93 (−1.03, 2.90)	1.84 (−2.38, 6.07)	2.85 (−2.16, 7.85)	5.11 (−3.96, 14.18)
Fluoroethers					
Nafion by-product 2	−0.39 (−6.87, 6.09)	1.69 (0.21, 3.17)	−1.78 (−6.86, 3.30)	−2.08 (−8.52, 4.36)	−1.42 (−11.44, 8.59)
PFO4DA	−1.31 (−5.51, 2.90)	−0.19 (−1.60, 1.23)	−2.08 (−5.47, 1.32)	−1.12 (−5.04, 2.80)	4.95 (−3.16, 13.06)
PFO5DoA	−0.40 (−8.32, 7.52)	1.85 (0.37, 3.33)	−1.29 (−7.42, 4.83)	−2.25 (−10.26, 5.76)	−4.77 (−17.34, 7.81)
$\sum_5$ Fluoroethers	0.12 (−4.72, 5.00)	0.18 (−1.46, 1.83)	−0.73 (−4.49, 3.02)	−0.06 (−4.26, 4.14)	3.55 (−4.19, 11.29)
$\sum_{10}$ PFAS	2.83 (−2.92, 8.60)	0.75 (−1.12, 2.62)	1.42 (−2.92, 5.76)	2.09 (−3.31, 7.48)	3.38 (−5.99, 12.74)

Note: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; PFAS, per- and polyfluoroalkyl substances; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFO4DA, perfluoro-3,5,7,9-tetraoxadecanoic acid; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

Discussion

In this cross-sectional analysis of Wilmington residents, we noted positive associations between quartiles of legacy PFAS measured in serum and non-HDL and total cholesterol measured in serum. Associations were strongest for PFOS and PFNA. Nafion by-product 2 and PFO5DoA were positively associated with HDL, but fluoroethers were not associated with any other outcome. Associations for all exposures increased in magnitude with age, with the strongest associations observed for those in the highest tertile of age (≥63 y). This may be due to the higher cumulative exposure among older participants or to changes in underlying susceptibility. Because of the cross-sectional design, we cannot rule out the possibility of reverse causation.

Levels of legacy PFAS in our population are significantly higher than levels in the general public. The geometric means of PFOA, PFOS, PFNA, and PFHxS from a nationally representative sample during the same time window are all within the lowest quartile of exposure in our population.[24] For PFOA, PFNA,

Table 5. Betas (milligrams per deciliter) (95% CIs) for a unit change in selected chemical, per quartile increase ($n = 273$), stratified on age, weighted for age, sex, BMI, race/ethnicity, and smoking status.

	19–44 y	45–62 y	63–86 y	p for interaction
Legacy PFAS				
PFOS				
Total cholesterol	1.15 (−7.48, 9.78)	5.72 (−1.47, 12.92)	10.30 (2.56, 18.04)	0.04
Non-HDL	−0.33 (−8.42, 7.77)	4.47 (−2.10, 11.04)	9.27 (2.34, 16.20)	0.03
PFOA				
Total cholesterol	4.39 (−4.05, 12.83)	6.83 (0.30, 13.36)	9.27 (2.12, 16.41)	0.27
Non-HDL	2.52 (−4.99, 10.03)	5.26 (−0.63, 11.16)	8.01 (1.59, 14.43)	0.16
PFNA				
Total cholesterol	1.08 (−8.67, 10.82)	5.32 (−1.04, 11.67)	9.56 (3.12, 15.99)	0.15
Non-HDL	−0.11 (−7.62, 7.39)	4.52 (−0.90, 9.94)	9.15 (3.32, 14.99)	0.06
PFHxS				
Total cholesterol	5.91 (−2.71, 14.52)	7.49 (0.49, 14.50)	9.08 (1.21, 16.95)	0.47
Non-HDL	3.32 (−4.15, 10.79)	5.97 (−0.17, 12.11)	8.62 (1.79, 15.44)	0.16
PFHpA				
Total cholesterol	−5.02 (−10.68, 0.64)	−0.69 (−5.56, 4.18)	3.64 (−2.51, 9.78)	0.01
Non-HDL	−5.11 (−10.64, 0.41)	−1.32 (−5.80, 3.16)	2.48 (−2.93, 7.90)	0.02
$\sum_5$ legacy PFAS				
Total cholesterol	5.98 (−4.32, 16.28)	8.71 (0.84, 16.58)	11.43 (3.46, 19.40)	0.26
Non-HDL	3.87 (−4.35, 12.10)	7.17 (0.35, 13.98)	10.46 (3.37, 17.54)	0.08
Fluoroethers				
Nafion by-product 2				
Total cholesterol	−5.86 (−11.82, 0.11)	−1.42 (−5.95, 3.12)	3.02 (−2.43, 8.47)	0.02
Non-HDL	−7.39 (−12.59, −2.18)	−2.67 (−6.80, 1.45)	2.04 (−2.94, 7.02)	0.01
PFO4DA				
Total cholesterol	−2.94 (−8.70, 2.82)	0.74 (−4.12, 5.59)	4.41 (−1.81, 10.63)	0.04
Non-HDL	−2.65 (−8.63, 3.32)	0.32 (−4.26, 4.90)	3.29 (−2.18, 8.76)	0.08
PFO5DoA				
Total cholesterol	−1.91 (−8.36, 4.53)	1.93 (−2.91, 6.77)	5.78 (−0.25, 11.81)	0.06
Non-HDL	−3.78 (−9.42, 1.85)	0.18 (−4.09, 4.45)	4.14 (−1.19, 9.47)	0.03
$\sum_5$ fluoroethers				
Total cholesterol	−1.78 (−8.20, 4.64)	2.44 (−2.97, 7.84)	6.65 (0.26, 13.04)	0.02
Non-HDL	−1.93 (−8.76, 4.90)	1.54 (−3.56, 6.65)	5.01 (−0.57, 10.60)	0.05
$\sum_{10}$ total PFAS				
Total cholesterol	4.70 (−5.44, 14.83)	7.16 (−1.04, 15.36)	9.62 (1.22, 18.02)	0.28
Non-HDL	2.40 (−6.08, 10.88)	5.65 (−1.59, 12.89)	8.90 (1.38, 16.41)	0.08

Note: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; PFAS, per- and polyfluoroalkyl substances; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFO4DA, perfluoro-3,5,7,9-tetraoxadecanoic acid; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

and PFHxS, the geometric means of our population are at or greater than the 90th percentile of the general population.[24] To our knowledge, there are currently no population-based estimates against which to compare the fluoroether levels in our sample.

Non-HDL cholesterol is derived from total and HDL cholesterol values—both measures that are valid in a nonfasted state.[16–18] Furthermore, non-HDL is the sum of LDL and very low-density lipoprotein cholesterol and is therefore highly correlated with total apolipoprotein B (apo B) levels. Apo B is the major apolipoprotein of the atherogenic lipoproteins and is predictive of severity of coronary atherosclerosis and coronary heart disease.[18] Because our population was not specifically asked to fast prior to participation and because of its utility in assessing risk, we chose non-HDL cholesterol as our primary outcome. Prior studies have generally been nonfasted, but not all of them examined non-HDL cholesterol as an outcome.[25–28]

Our positive findings with cholesterol are consistent with a substantial body of literature on this topic.[29,30] Prior studies have primarily examined PFOA, PFOS, PFNA, and PFHxS and like our study have largely been cross-sectional. In populations with background-level exposure of PFAS, consistent positive associations have been noted between PFOA and PFOS with total cholesterol.[25,26,31]

A longitudinal study of 888 prediabetic adults found associations between PFOA, PFOS, and PFNA with both total cholesterol and non-HDL cholesterol.[31] Participants in this study were fasted prior to outcome measurement. In a cross-sectional study of 753 participants age 12–80 y from the 2003–2004 wave of NHANES, Nelson et al. found associations between PFOA, PFOS, and PFNA with both total and non-HDL cholesterol. An inverse association was detected between PFHxS and total/non-HDL cholesterol.[25] Approximately half of the population was fasted prior to outcome measurements. Eriksen et al. detected cross-sectional associations between PFOS and PFOA with total cholesterol in 753 adults age 50-65 y.[26] No other outcomes were examined, and the population was not fasted. These studies were conducted in the early 2000s or earlier, and blood levels of most PFAS have declined since that time.[24] Accordingly, in all three studies, levels of PFOA were comparable to those levels in our population, whereas levels of PFOS were significantly higher than those measured in our population. Levels of PFNA and PFHxS were moderately lower than in our population.

Associations have also been noted in populations with elevated PFAS levels, including positive but nonlinear associations observed with all PFAS measured and total cholesterol, but no association with triglycerides in 1,945 participants ages 20–60 y.[28] In a population with higher levels of PFOA and PFHxS but lower PFOS relative to the Wilmington population, associations were observed between all measured PFAS and both total and non-HDL cholesterol.[27] This population consisted of 15,720 adults ages 20–39. Again, associations were less consistent with triglycerides. Both studies were cross-sectional and nonfasted. Multiple studies found that the largest cholesterol increases per unit PFAS were seen in the lower range of PFAS exposure and that associations were nonlinear.[27,28,32] This is consistent with our findings in which the largest increase in non-HDL cholesterol level was generally observed between the first two quartiles of exposure for legacy PFAS.

Identifying associations with individual compounds may provide clues to mechanism. PFAS are thought to exert potential metabolic effects through perturbations of lipid homeostasis.[29] In human hepatocytes, PFOA and PFOS decrease HNF4A expression, leading to down-regulation of CYP7A1 and higher cholesterol levels.[33] Using rodent models, studies have shown that exposure to PFAS causes activation of the peroxisome proliferator–activated receptor alpha, dysregulation of lipid homeostasis genes, and liver steatosis.[29,34,35] In humans, evidence for an association is consistent whether exposure is measured in serum or estimated through reconstruction models.[25,26,36,37]

We saw no strong evidence of an association with fluoroethers and total cholesterol and non-HDL cholesterol in the overall population, though stronger estimated effects were noted among older participants. However, it should be noted that levels of fluoroethers were significantly lower than those of legacy PFAS, likely due to their much shorter half-lives. Legacy PFAS refer to PFAS that have been historically produced but are now being phased out of production in many developed nations. Because of their long-term use, they are detectable in nearly all individuals.

Participants are simultaneously exposed to multiple PFAS, and it may be difficult to observe associations specifically for fluoroethers. Surprisingly, we saw higher levels of HDL cholesterol in association with fluoroethers, which is consistent with information on legacy PFAS.[25,28,38] Given our small sample size and our complex mixture of PFAS exposures, we are unable to make definitive statements about their impact on lipid outcomes, but given the paucity of available data, our results suggest that these chemicals are more likely to be associated with HDL cholesterol rather than total cholesterol and non-HDL cholesterol in this population with generally acceptable levels of cholesterol.

The GenX Study population represents a highly exposed PFAS population but is a relatively small study in comparison with other PFAS studies. Our study population is representative of the greater U.S. adult population in regard to prevalence of high cholesterol (defined as total cholesterol $\geq$240 mg/dL): 35% in our population vs. 38% in general U.S. adult population.[39] In a sample of U.S. adults from 2007 to 2010, the mean level of non-HDL cholesterol was 146 mg/dL, slightly higher than the average level observed in our population.[40]

The cohort is unique with respect to inclusion of fluoroethers as well as multiple legacy PFAS. Assessment of fluoroether exposure effects is critical, given their increasing use and structural similarity to legacy PFAS, which are known metabolic toxicants. We used IPTW to address confounding which allows for interpretation of a marginal estimate and is robust even in the presence of effect measure modification. Other studies noted significant PFOA associations although we did not, potentially indicating that we were underpowered, especially because associations with PFOA and PFHxS are consistent in direction to the other legacy PFAS. Additionally, the compounds examined in this study are moderately correlated with each other and participants are exposed to multiple compounds simultaneously. We attempted to address this issue by creating summed exposure variables to approximate total exposure; this measure assumes that each PFAS has the same impact on lipid measures, which may be a big assumption. Findings for individual chemicals suggest potentially different potency by chemical. Furthermore, our study has some limitations specifically regarding the assessment of triglycerides. First, we did not have information on whether participants were using triglyceride-lowering medications. Second, our triglyceride measures were nonfasted and thus may be impacted by prior diet. However, research suggests that nonfasted triglyceride measures are still strongly predictive for adverse cardiovascular outcomes.[41,42]

Evaluating the impact of individual PFAS is challenging given that most individuals are exposed to many PFAS at the same time. Because these chemicals have many sources and differing half-lives, the correlation among them in serum can vary widely. For example, the correlation of PFOS with PFO4DA was 0.26, whereas with PFNA it was 0.81, which will make it difficult

to control for PFNA in a model with PFOS. Given that we found the strongest and most consistent results for PFOS and PFNA for both total cholesterol and non-HDL cholesterol, we cannot rule out potential confounding by PFOS in our estimates for PFNA. Studies with a larger sample size may be better able to control for multiple exposures in the statistical models. Due to the cross-sectional nature of this study, caution is warranted with regard to whether these associations are causal.

In conclusion, this study adds to the body of evidence between PFAS exposure and elevated cholesterol levels. To our knowledge, this is the first study to examine fluoroether exposure in association with adverse health effects. We found that fluoroethers were largely not associated with cholesterol outcomes, apart from some positive associations between Nafion by-product 2 and PFO5DoA with HDL cholesterol.

Acknowledgments

This research was funded by the National Institute of Environmental Health Sciences (NIEHS; 1R21ES029353; P42 ES031009), the Center for Human Health and the Environment at NCSU (P30ES025128), and the North Carolina Policy Collaboratory. E.M.R. was funded in part by a training grant from the NIEHS (T32 ES007018).

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