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Association of Perfluorooctanoic Acid with HDL Cholesterol and Circulating miR-26b and miR-199−3p in Workers of a Fluorochemical Plant and Nearby Residents

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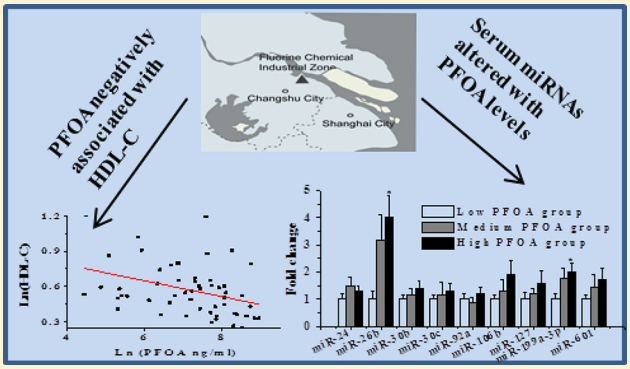
Supporting Information

ABSTRACT: Perfluoroalkyl chemicals (PFCs) are stable man-made compounds with many industrial and commercial uses. Concern has been raised that they may exert deleterious effects, especially on lipid regulation. We aimed to assess exposure to perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and seven other PFCs in occupational workers from a fluorochemical plant and nearby community residents, and to investigate the association between PFOA and serum biomarkers. Serum biomarkers included not only biochemical parameters, such as lipids and enzymes, but also circulating microRNAs (miRNAs). Samples were analyzed by high-pressure liquid chromatography/tandem mass spectrometry (HPLC-MS/MS). Circulating miRNA levels were detected by quantitative polymerase chain reaction (PCR). Analyses were conducted by correlation and linear regression. We detected PFOS, PFOA, perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) in all samples. The median levels of serum PFOA and PFOS were 284.34 ng/mL and 34.16 ng/mL in residents and 1635.96 ng/mL and 33.46 ng/mL in occupational participants, respectively. To our knowledge, we found for the first time that PFOA was negatively associated with high-density lipoprotein cholesterol (HDL-C) in workers using linear regression after adjusting for potential confounders. Circulating miR-26b and miR-199a-3p were elevated with serum concentration of PFOA. Although the limitations of small sample size and the cross-sectional nature of the current study constrained causal inferences, the observed associations between PFOA and these serum biomarkers warrant further study.



INTRODUCTION

Perfluoroalkyl chemicals (PFCs) are a class of highly stable man-made compounds, composed of a fluorinated carbon backbone of varying length terminated by a carboxylate or sulfonate functional group. Perfluoroalkyl chemicals are widely used in industrial and consumer products, from stain- and water-resistant coatings for carpets and fabrics to fast-food contact materials, fire-resistant foams, paints, and hydraulic fluids.[1]

The carbon-fluoride bonds that characterize PFCs make them highly stable and persistent. Many reports have indicated that PFCs are widespread in occupationally exposed populations,[2,3] in the sera of diverse general human populations,[4,5] as well as in the environment and in wildlife, even from remote regions.[6−8] Unlike most persistent organic pollutants, which are lipophilic and accumulate in fatty tissues, PFCs are both lipo- and hydro-phobic, and after absorption will bind to proteins in serum and liver rather than accumulate in lipids.[9,10] Two of the most widely detected and studied compounds are eight-carbon-chain perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Other PFCs of concern include perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), and perfluorotetradecanoic acid (PFTA). In occupationally exposed workers, serum PFOS concentrations appear to vary depending on job type. The highest levels of PFOA were reported by Olsen et al.[11] in ammonium perfluorooctanoate production workers, with serum PFOA concentrations of 114 100 ng/mL. For general populations, the National Health and Nutrition Examination Survey (NHANES) in the United States detected perfluoroalkyl acids in almost all samples, with US-population geometric mean concentrations for PFOA of 5.2 ng/mL and 3.9 ng/mL and for PFOS of 30.4 ng/mL and 20.7 ng/mL in the

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NHANES 1999−2000 and NHANES 2003−2004, respectively.[1] Other PFCs, such as PFNA and PFHxS, have also been quantified in the general population at concentrations approximately an order of magnitude lower than that of PFOS.[12]

The ubiquitous presence and persistence of PFCs in the environment and within the human body have led to efforts to understand the toxicological hazards that may be associated with exposure. In particular, the structural resemblance of PFCs to natural fatty acids has raised concern that PFCs may disrupt lipid regulation. Several studies in humans, predominantly medical surveillance studies of male chemical workers, suggest that exposure to PFOA, and possibly to PFOS, may be associated with increased total cholesterol (CHO), low-density lipoprotein cholesterol (LDL-C), and/or triglycerides (TG) in humans.[2,3,11,13−16] Animal studies suggest that the liver is an important target organ for perfluoroalkyl acid physiological activity. Reported hepatic toxicological effects include hepatomegaly, hepatocellular adenomas, and peroxisome proliferation in rodents and nonhuman primates.[17,18] The animal findings on liver toxicity have prompted a number of studies on serum enzymes, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in people exposed to PFCs, with several studies on occupational workers showing that PFOA is associated with increased liver enzymes.[3,14,16]

MicroRNAs (miRNAs) are endogenous, small noncoding RNAs. They play an important role in regulating gene expression by base-pairing to complementary sites on target mRNAs to block translation or trigger the degradation of target mRNAs.[19] Recent studies have shown that human serum contains a large amount of miRNAs and the pattern of serum miRNA is altered in various cancers and could potentially be used as novel noninvasive biomarkers for diagnosis and therapy.[20] A few studies have also researched the alteration in serum miRNAs under chemical injury and found that concentrations of miR-122 increased with alcohol- and chemical-induced liver diseases.[21] We hypothesized that the levels of specific circulating miRNAs may also be used as biomarkers to monitor potential pathological injuries by serum PFCs. In the present study, our study objectives are to (a) assess the level of serum concentrations of 9 PFCs in occupational workers and residents nearly, and (b) analyze the association between PFOA and serum biomarkers for lipids, enzymes, and miRNAs levels.

MATERIALS AND METHODS

Study Location and Participants. Jiangsu High-Tech Fluorine Chemical Industrial Zone is located near the Yangtze River in a suburban area of Changshu City, Jiangsu Province (Figure S1 of the Supporting Information). Twenty-seven fluorochemical plants are located in the fluorochemical industrial zone. Chemical workers from a Changshu fluorochemical plant, as well as residents of the nearby community, were sampled from May 2010 to October 2011. Participants from the plant must have worked there for at least two years, and residents must have lived in the district for at least five years to be considered eligible for the study. The volunteer participants were asked to donate a 5 mL blood sample and respond to a questionnaire that inquired about demographic characteristics and health-related variates. The Biomedical Research Ethics Committee, Institute of Zoology (IOZ), Chinese Academy of Sciences (CAS), approved the research and all participants gave written informed consent. Fifty-five blood samples of male occupational workers were collected. No female occupational individuals were sampled due to the relatively small proportion of female workers. A total of 132 blood samples were obtained from near-by residents.

Blood Collection, Serum Lipids, and Enzymes Detection. Blood samples were obtained in the morning before breakfast. For serum isolation, samples were allowed to sit at room temperature for 30 min between collection and separation to allow the blood to clot. Separation of clots was accomplished by centrifugation at 1000−1300 g at 4 °C for 15−20 min. The serum was removed and dispensed in aliquots of 1 mL. After aliquoting, the serum were frozen and transferred on dry ice and subsequently stored at −80 °C. All serum samples collected were used for biochemistry parameters analysis and PFCs analysis, and part of each sample was used for RNA isolation and miRNA analysis. Standard spectrophotometric methods for the HITAC7170A automatic analyzer were used to measure serum lipid and enzyme parameters, including TG, CHO, LDL-C, high-density lipoprotein cholesterol (HDL-C), ALT, and AST.

Perfluoroalkyl Compounds Analysis. Thawed serum samples were analyzed for nine different PFCs, including PFBS, PFHxS, PFHpA, PFOA, PFOS, PFNA, PFDA, PFDoA, and PFTA in 1 mL of serum using a high-performance liquid chromatography-tandem mass spectrometer (HPLC-MS/MS). One milliliter serum samples were spiked with an internal standard mixture ([^{13}C4]-PFOA, [^{13}C5]-PFNA, [^{13}C2]-PFDA, and [^{13}C4]-PFOS) to correct for matrix effects as well as for losses from sample extraction, concentration, and analysis before extraction. Extraction of PFCs from serum samples was achieved using acetonitrile, and the extract was subjected to further purification using the SPE-Oasis-WAX-method (details given in the Supporting Information). The instrumental chromatographic setup consisted of a P680 binary gradient pump, UltiMate 3000 autosampler, and *Chromeleon 6.70* chromatography workstation (Dionex, Sunnyvale, CA, USA). Mass spectra were collected using an API 3200 triple quadrupole tandem mass spectrometer, fitted with an electrospray ionization source, and operated in the negative ionization mode. Quantification was conducted using *Analyst 1.4.1* software. Chromatographic separations were carried out on an Acclaim 120 C18 column (4.6 × 150 mm, 3 μm) (Dionex, Sunnyvale, CA, USA), with a binary gradient. Methanol (A) and 50 mmol ammonium acetate (B) were employed as mobile phases.

Data Quality Assurance and Quality Control. Data quality assurance and quality control included instrumental blanks, procedural (method) blanks, matrix spikes, and duplicated analysis. Procedural blanks (Milli-Q water) and recoveries (Milli-Q water spiked with native standards) were assessed following the same procedure as described above with each group of extractions. The method detection limits (LOD) and method quantification limits (LOQ) were calculated for substances found in real samples at a signal-to-noise of 3 and 10, respectively. The LOQs were 0.01 ng/mL for the PFCs detected. The blanks were all below LOQs. All native standards were spiked into samples and analyzed. Mean procedural recoveries ranged between 79% (PFBS) and 115% (PFHpA). More detailed information for the values of LOQ, instrumental blanks, cartridge blanks, procedural blanks, procedural recoveries, and matrix spike recoveries, as well as the parent and daughter ions for detection are given in Table S1 of the Supporting Information. A calibration curve was prepared from

Table 1. Descriptive Statistics for PFCs[a]

group		PFCs (ng/mL)	*n*	mean	std. deviation	median	minimum	maximum	range	geometric mean
nearby residents	all	PFOA	132	378.30	347.11	284.34	10.20	2436.91	2426.71	249.93
		PFNA	132	6.29	6.15	4.84	0.43	50.89	50.46	4.42
		PFDA	132	11.71	10.55	8.07	1.11	67.76	66.65	8.36
		PFDoA	132	0.90	0.90	0.62	0.04	4.71	4.67	0.56
		PFTA	97	1.08	3.33	0.08	0.01	22.64	22.63	0.11
		PFBS	120	0.15	0.14	0.10	0.01	0.62	0.61	0.10
		PFHxS	132	1.12	0.92	0.89	0.04	4.96	4.92	0.74
		PFOS	132	39.42	26.69	34.16	3.00	157.27	154.27	30.92
		PFHpA	116	0.39	0.45	0.25	0.01	2.52	2.51	0.21
	female	PFOA	57	390.57	408.50	298.31	27.80	2436.91	2409.11	232.65
		PFNA	57	6.44	7.33	4.65	0.56	50.89	50.33	4.12
		PFDA	57	11.85	10.89	8.31	1.29	67.76	66.47	8.18
		PFDoA	57	0.83	0.73	0.59	0.08	2.82	2.74	0.55
		PFTA	41	0.08	0.06	0.06	0.01	0.26	0.25	0.05
		PFBS	53	0.17	0.15	0.11	0.01	0.62	0.61	0.11
		PFHxS	57	0.95	1.01	0.56	0.09	4.96	4.87	0.55
		PFOS	57	36.40	25.23	33.86	5.50	119.83	114.33	28.09
		PFHpA	56	0.41	0.41	0.27	0.01	1.88	1.87	0.22
	male	PFOA	75	368.97	294.63	270.16	10.20	1542.95	1532.75	263.92
		PFNA	75	6.18	5.12	4.91	0.43	33.55	33.12	4.65
		PFDA	75	11.61	10.35	7.97	1.11	54.31	53.20	8.49
		PFDoA	75	0.95	1.01	0.63	0.04	4.71	4.67	0.56
		PFTA	56	1.82	4.25	0.13	0.01	22.64	22.63	0.20
		PFBS	67	0.14	0.13	0.10	0.01	0.58	0.57	0.09
		PFHxS	75	1.26	0.83	1.11	0.04	4.03	3.99	0.93
		PFOS	75	41.71	27.69	36.21	3.00	157.27	154.27	33.25
		PFHpA	60	0.38	0.48	0.21	0.01	2.52	2.51	0.20
	age ≤45 years	PFOA	66	358.20	246.63	295.08	27.80	876.38	848.58	259.08
		PFNA	66	6.21	5.12	5.48	0.56	33.55	32.99	4.56
		PFDA	66	11.61	9.68	7.98	1.29	54.31	53.02	8.60
		PFDoA	66	0.95	0.91	0.66	0.04	3.93	3.89	0.61
		PFTA	49	2.07	4.49	0.12	0.01	22.64	22.63	0.23
		PFBS	58	0.16	0.15	0.10	0.01	0.62	0.61	0.10
		PFHxS	66	1.15	0.85	1.03	0.09	4.03	3.94	0.79
		PFOS	66	41.57	27.54	35.63	5.50	157.27	151.77	33.06
		PFHpA	55	0.41	0.47	0.30	0.01	2.52	2.51	0.21
	age >45 years	PFOA	66	398.41	425.65	268.93	10.20	2436.91	2426.71	241.11
		PFNA	66	6.37	7.07	4.51	0.43	50.89	50.46	4.28
		PFDA	66	11.81	11.42	8.19	1.11	67.76	66.65	8.12
		PFDoA	66	0.85	0.89	0.57	0.04	4.71	4.67	0.51
		PFTA	48	0.08	0.07	0.06	0.01	0.29	0.28	0.05
		PFBS	62	0.15	0.13	0.11	0.01	0.44	0.43	0.10
		PFHxS	66	1.10	1.00	0.82	0.04	4.96	4.92	0.70
		PFOS	66	37.26	25.84	30.20	3.00	119.83	116.83	28.91
		PFHpA	61	0.38	0.43	0.23	0.01	1.88	1.87	0.21
occupational workers	all	PFOA	55	2157.74	1942.29	1635.96	84.98	7737.13	7652.15	1272.31
		PFNA	55	7.37	5.51	5.69	0.82	24.45	23.63	5.72
		PFDA	55	9.57	9.05	5.39	0.71	42.27	41.56	6.59
		PFDoA	54	0.82	1.06	0.53	0.04	6.68	6.64	0.49
		PFTA	54	1.80	2.19	1.04	0.01	7.92	7.91	0.71
		PFBS	52	0.14	0.32	0.05	0.01	2.22	2.21	0.05
		PFHxS	55	1.19	1.03	0.98	0.10	6.07	5.97	0.84
		PFOS	55	42.63	31.52	33.46	5.23	165.69	160.46	33.33
		PFHpA	44	0.66	1.25	0.29	0.01	7.57	7.56	0.27

[a]For each PFC, cases with concentration lower than LOQ were not included for statistical description. Abbreviations: PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFBS, perfluorobutane sulfonate; PFHpA, perfluoroheptanoic acid; PFDoA, perfluorododecanoic acid; PFTA, perfluorotetradecanoic acid.

a series of PFCs concentrations (0, 10, 50, 100, 500, 1000, 5000, 20 000, and 50 000 pg/mL), and standard deviations were less than 20%. The concentrations of PFCs in the experimental samples were not corrected for their correspond-

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ing recoveries. In addition, reliability of this method was verified through participation in the International Laboratory Calibration Study coordinated by US-EPA.

TaqMan miRNA Microarray. To study the effect of higher serum PFOA content on circulating miRNAs, which can serve as biomarkers for many disease conditions, serum samples from ten occupational workers and ten residents obtained in May 2011 were used for miRNA microarray analysis. Total RNA was extracted from serum using a mirVana miRNA Isolation Kit according to the manufacturer's instructions. The MiRNA profiling was completed using TaqMan Array microRNA Assays (Life Technologies-Applied Biosystems, CA, USA). Total RNA isolated from serum was reverse-transcribed using Human MegaPlex RT Primer Pools, and cDNA was amplified with Human MegaPlex PreAmp Primer Pools and PreAmp Master Mix. Amplified samples were loaded into TaqMan Array Human (v3.0) MicroRNA Cards, which enabled quantification of 754 (human) miRNAs, and were then analyzed using an Applied BioSystems 7900HT fast thermocycler with the manufacturer's recommended cycling conditions. Data were analyzed with *SDS Relative Quantification Software* (v2.2.2), with the automatic Ct setting for assigning baseline and threshold for Ct determination. Those MiRNAs cycle threshold values higher than 40 in any of the 20 samples were filtered out. Statistical analyses of differences in the miRNAs detected in occupational workers and residents were obtained using the $2^{-\text{Delta}-\text{Delta-Ct}}$ method.[22] The U6 RNA was selected as the endogenous control because its Ct value was not changed significantly between the arrays.

TaqMan miRNA PCR Assay. The MiRNA microarray procedure yielded a list of nine miRNAs: miR-127, miR-601, miR-106b, miR-24, miR-199a-3p, miR-92a, miR-26b, miR-30c, and miR-30b, which had differential contents between the two groups. The levels of the nine miRNAs were further detected using 31 serum samples from occupational workers obtained in October 2011. Total RNA extraction, reverse transcription, preamplification, and analysis were finished with the same methods as the microarray assay except that the expression of the above miRNAs was carried out using TaqMan MicroRNA Assay (Life Technologies-Applied Biosystems, Foster City, CA, USA).

Statistical Description and Comparison. Shapiro-Wilk tests were used to determine whether data sets showed normal distribution. For PFCs, in those instances where a sample was measured below the LOQ, the sample was not used for statistical description or further analysis. When comparing two groups, either unpaired Student's *t*-tests or Mann−Whitney nonparametric tests (two-tailed) were used as determined by normality of data. In the TaqMan miRNA PCR assay experiment, 31 samples were divided into three groups according to PFOA concentrations (low, medium, and high), and the alternation in the expression of serum miRNAs were compared between the three groups by One-Way ANOVA.

Correlation and Regression. We used Spearman nonparametric correlation coefficients and related *P* values to describe correlations between levels of different PFCs. We singled out PFOA for further analysis due to its relatively higher concentration and detectable ratio in serum. Analyses were conducted by linear regression in which the outcomes were TG, CHO, LDL-C, HDL-C, the ratio of HDL-C to LDL-C, ALT, and AST. Potentially confounding model variates were age and body mass index (BMI, weight in kilograms divided by the square of measured height in meters) as continuous variates, and smoking and drinking status (Y/N) as categorical variates. Model covariates were chosen because they were generally significant predictors of the outcomes. Age, weight, height, and smoking and alcohol status were collected through questionnaires. Natural log-transformed values for all continuous variates tended to normalize residuals and led to a better goodness of fit as measured by the *R*-square. For simplicity and consistency, we retained the same set of covariates and log transformed all continuous variates in the model. We also performed a secondary analysis after dividing the residents into two subgroups, with the younger subgroup having a similar age range with occupational workers. We then combined the younger resident subgroup with occupational workers, and linear regression was conducted again with PFOA as the independent variate, HDL-C as the outcome and adjusted for the same covariates as referred to above. All statistical analyses were performed using *SPSS* statistical software, version 16.0 (SPSS Inc., Chicago, IL, USA). For all tests, a $P < 0.05$ was considered significant.

RESULTS

Statistic Description of Covariates and PFCs. Study participants included 132 residents (43.18% females and 56.82% males) and 55 occupational workers (all males). All individuals were in good health based on self-report. Nearly 38% of residents and 40% of occupational workers reported that they smoked, and 26% of residents and 44% of occupational workers reported that they consumed alcohol. Detailed statistical description on parameters such as age, BMI, lipids, and enzymes of the groups is shown in Table S2 of the Supporting Information.

The levels of nine PFCs (PFOA, PFOS, PFBS, PFHxS, PFHpA, PFNA, PFDA, PFDoA, and PFTA) were detected in our study. Table 1 shows the population summary statistics for the nine PFCs in the 55 occupational and 132 resident serum samples. The most detected among the nine PFCs was PFOA, with median values of 284.34 ng/mL and 1635.96 ng/mL in residents and occupational workers, respectively. The second highest was PFOS, with medians of 34.16 ng/mL and 33.46 ng/mL respectively, which were an order of magnitude higher than PFDA and PFNA, the third and fourth highest PFCs. The characteristics of PFCs in resident demographic subgroups by gender and age (two strata, ≤ 45 and >45 years) are also given in Table 1. Using the Mann−Whitney U-test, we assessed whether concentrations of fluorochemicals differed in residents by sex or age. Except for PFTA and PFHxS, no differences were observed in the PFCs between the two genders in residents (Table S3 of the Supporting Information). For PFTA and PFHxS, there was a relatively higher detected concentration in male subgroups. No striking concentration differences were noted between the two age subgroups in eight of the nine PFCs detected, except for PFTA in which a higher level was observed in the younger subgroup. Because no significant differences were observed by sex or age in most of the PFCs in residents, we combined and assessed whether concentrations of fluorochemicals differed between all the residents and the occupational workers. Serum samples from occupational workers had higher concentrations of PFOA and PFTA but lower levels of PFBS compared with samples from residents. No significant differences were observed between the two groups for the other six PFCs, including PFOS, the second most dominant fluorochemical detected.

Table 2. Results for Linear Multivariate Regression of Lipids and Hepatic Enzymes on PFOA[a]

		Ln PFOA						
outcome	group	coef.	std. Err.	standardized coef	t	P	95% CI	
Ln CHO	residents	−0.00	0.02	−0.02	−0.18	0.85	−0.04	0.03
	workers	0.02	0.02	0.13	0.93	0.36	−0.03	0.07
Ln HDL-C	residents	0.02	0.02	0.07	0.86	0.39	−0.02	0.05
	workers	−0.07	0.03	−0.33	−2.59	0.01[b]	−0.12	−0.01
Ln LDL-C	residents	−0.00	0.03	−0.00	−0.04	0.97	−0.05	0.05
	workers	0.03	0.03	0.11	0.79	0.43	−0.04	0.09
Ln HDL-C/LDL-C	residents	0.02	0.03	0.05	0.57	0.57	−0.04	0.08
	workers	−0.09	0.04	−0.29	−2.52	0.01[b]	−0.16	−0.02
Ln TG	residents	0.02	0.05	0.03	0.34	0.73	−0.08	0.11
	workers	−0.05	0.06	−0.12	−0.91	0.37	−0.17	0.06
Ln ALT	residents	−0.10	0.05	−0.17	−1.96	0.05	−0.19	0.00
	workers	0.04	0.05	0.08	0.88	0.38	−0.06	0.15
Ln AST	residents	−0.04	0.03	−0.10	−1.22	0.22	−0.10	0.02
	workers	−0.12	0.05	−0.30	−2.33	0.02[b]	−0.22	−0.02

[a]Abbreviations: CHO, total cholesterol (mmol/L); HDL-C, high-density lipoprotein cholesterol (mmol/L); LDL-C, low-density lipoprotein cholesterol (mmol/L); TG, Triglycerides (mmol/L); ALT, alanine aminotransferase (IU/L); AST, aspartate aminotransferase (IU/L). Adjusted for BMI and age. [b]Statistically significant ($P < 0.05$).

Correlation among PFCs. Unadjusted, nonparametric Spearman's rho rank-order correlation analysis was conducted for the nine PFCs in residents and occupational workers (Table S4 of the Supporting Information). In general, the PFCs were significantly correlated to each other in residents, though to different degrees (correlation coefficients ranged from 0.33 to 0.93), except for between PFTA and PFBS (correlation coefficient 0.16, $P = 0.15$). Except for PFTA, all PFCs showed similar correlation patterns, though to different degrees in occupational workers compared with residents. The lowest and highest correlations were observed between PFOS and PFHpA (correlation coefficient 0.37) and between PFOS and PFNA (correlation coefficient 0.88) in occupational workers, respectively. However, only a relatively moderate correlation (correlation coefficient 0.37) was observed between PFTA and PFOS, and no significant correlations were observed between PFTA and the other eight PFCs detected in occupational workers. For PFOA and PFOS, stronger correlations were observed in residents (correlation coefficient 0.73) compared with occupational workers (correlation coefficient 0.46).

Results for Linear Regression Analyses. Because of its highest values in serum, PFOA was selected for regression analysis. The frequency distribution of PFOA and most outcome graphs suggested log-normal distributions, which were further confirmed by the Shapiro-Wilk test. Using PFOA as a continuous predictor, a model using log transformed variates fit the data better than a model using untransformed ones for most outcomes. Smoking and drinking status were not significantly associated with any outcome. Therefore, we did not include smoking and drinking in the final analyses. Age and BMI were not associated with levels of PFOA in either group and as expected, CHO, TG, AST, and ALT outcomes varied with BMI and age in predictable ways. For example, BMIs showed significant positive association with CHO, LDL-C, TG, ALT, and AST, and negative association with HDL (or HDL/LDL cholesterol) in at least one of the two groups. Detailed information about the associations between covariates with outcomes in our model is shown in Table S5 of the Supporting Information. Multivariate regression model results of CHO, LDL-C, HDL-C and HDL-C/LDL-C, TG, ALT, and AST on PFOA concentrations adjusted for age and BMI, as described above, are given in Table 2. In the multivariate models in residents, PFOA was not associated with the outcomes significantly. In contrast, a statistically significant negative association was observed between PFOA and HDL-C (standardized coefficient −0.33, $P = 0.01$) and between PFOA and HDL-C/LDL-C (standardized coefficient −0.29, $P = 0.01$) in occupational workers. To increase sample size, we also ran an analysis between HDL-C and PFOA including all subjects ≤45 years ($n = 121$) regardless of whether they were residents or occupational workers, and results were substantively similar as those presented above (data not shown). Because of the reported potential association between PFOS and lipids,[13,15] we also analyzed their relationship, although no significant associations were obtained (data not shown). For hepatic enzymes, a statistically negative association was also observed between PFOA and AST (standardized coefficient −0.30, $P = 0.02$) in occupational workers and increased levels of PFOA had a marginal significance with decreased ALT (standardized coefficient −0.17, $P = 0.05$).

Circulating miRNA. To explore the effect of PFOA on circulating miRNA, serum samples from 10 occupational workers and 10 residents obtained in May 2011 were used for miRNA microarray analysis. The median PFOA concentrations for the 10 residents and ten occupational workers were 173.06 ng/mL and 4995.62 ng/mL, respectively. However, ages between the two groups were quite dissimilar, with individuals in the resident group much older (mean = 40 years) than the occupational workers (mean = 27 years). Among the 754 miRNAs checked, 63 miRNAs were detected in all 20 serum samples. Most miRNAs detected were unchanged between the ten residents and ten occupational workers (Student's t-tests, $P > 0.05$), whereas 9 miRNAs were significantly elevated in the serum samples from workers compared with the residents (Table 3). To further assess the 9 miRNAs, another independent sample set consisting of 31 subjects was analyzed using TaqMan MicroRNA Assay. The 31 subjects were occupational workers grouped according to serum PFOA concentration (low, medium, and high), with age matched between the three groups. All 9 circulating miRNAs were elevated with increasing serum PFOA concentrations, with miR-26b and miR-199−3p significantly increased in the high

Table 3. Changing Levels of 9 miRNAs in 10 Residents and 10 Workers (Student'S *t*-Tests)

	Mean Delta Ct		Fold Change	
miRNA	residents (n = 10)	workers (n = 10)	workers/ residents	P value
miR-24	2.84	1.71	2.50	0.02
miR-26b	4.05	3.11	1.90	0.03
miR-30b	5.66	4.51	2.54	0.04
miR-30c	4.64	3.60	1.91	0.04
miR-92a	4.25	3.01	3.16	0.03
miR-106b	6.95	5.51	2.98	0.01
miR-127	15.77	11.93	8.31	0.00
miR-199a-3p	8.98	6.90	3.47	0.02
miR-601	6.81	5.39	2.33	0.01

PFOA group compared with the low level one (One-Way ANOVA, $P < 0.05$) (Figure 1). These results indicated that all

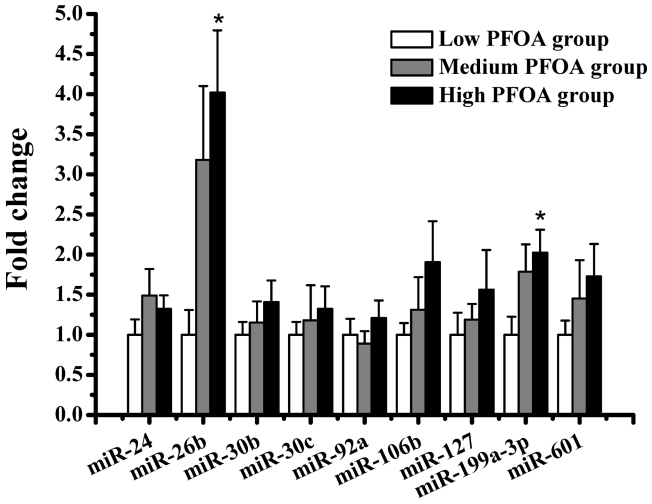

Figure 1. Changes in 9 circulating miRNAs in samples from 31 occupational workers. Samples were divided into 3 groups according to PFOA concentrations. Differences in serum miRNAs concentrations were analyzed by one-way ANOVA. $P < 0.05$ (*) was considered significant.

nine serum miRNAs in this set of individuals were concordant with the first set of 20 individuals. When using simple correlation analysis, we also found significant association between the two serum miRNAs (miR-26b and miR-199−3p) and PFOA levels. The above results implied that high serum PFOA levels may alter the level of circulating miRNAs such as miR-26b and miR-199−3p.

DISCUSSION

PFCs Concentrations and Correlations. We detected PFOA, PFOS, PFHxS, PFNA, and PFDA in all occupational workers and nearby residents, among which PFOA showed the highest detected concentrations. The concentrations in exposed workers were very high, among the highest in any occupational group (median concentrations 1100−1300 μg/L) ever reported.[23] The high value of PFOA in the Changshu region, especially in fluorochemical workers indicated that the fluorochemical industry in Changshu City was the main regional source of serum PFOA. Higher levels of PFOA in Changshu City residents confirmed that PFOA products and usage is more prevalent in China, although producers have committed to reducing manufacturing emissions of PFOA (including its salts and precursors) and global emissions have reportedly decreased.[24]

Significant correlations between concentrations of fluorochemical types have been observed in general populations, especially between PFOA and PFOS, suggesting they had a similar or common source(s) or pathway(s) of exposure.[12,25] Most of the PFCs detected, except for PFTA, exhibited similar concentrations between the two age groups in residents. Similar to our results, previous studies have reported no significant age difference in the concentration of many PFCs.[12,25] No gender difference in residents was observed in seven of the nine PFCs, except for PFTA and PFHxS where higher levels were observed in male subgroups. These sex differences may reflect variability in exposure patterns between genders as a result of differences in factors such as lifestyle and use of products containing these chemicals.

PFOA and Serum Biomarkers. Both PFOA and PFOS were positively correlated with serum lipids, specifically with CHO and LDL-C in occupational and community studies.[26] For example, 66 294 adult participants living in six water districts near the DuPont Washington Works facility near Parkersburg, West Virginia (C8 Health Project) were found to have significant positive associations between PFOA and PFOS concentrations and all lipid outcomes, except HDL-C, with a relatively lower serum level of PFOA (median 27 ng/mL) than occupational groups.[13] We investigated PFOA and cholesterol outcomes in both occupational workers and communities near fluorochemical plants. Although no trends between PFOA and serum lipids were observed in residents, PFOA showed significant negative trends in occupational workers with HDL-C, the good cholesterol that transports cholesterol back to the liver for excretion. The ratio of HDL to LDL cholesterol (HDL-C/LDL-C) is considered a predictor of cardiovascular outcomes[27] and showed a significant negative association with PFOA in our study. Additionally, another important predictor for HDL-C in our model was BMI, which exhibited a significant negative association with the outcome. These two variates explained 23% of the variation in HDL-C.

The liver is an important target organ for toxicity of PFCs. The association between serum PFCs and liver enzymes, such as ALT and AST, the two most commonly measured parameters for hepatic injury in clinical and epidemiologic studies has been investigated in numerous studies.[2,3,14,16,28] However, results are inconsistent and the clinical significance is uncertain.[26] In our study, we observed a negative association between PFOA and AST in occupational workers, whereas increased levels of PFOA had a marginal significance with decreased ALT. Taken together, the negative trend between PFCs and AST (or ALT) observed in occupational workers did not support a harmful effect of PFOA on human liver.

The miRNAs are evolutionarily conserved noncoding RNAs involved in the regulation of many physiological responses in organs and tissues. Recently, miRNAs in the blood have become of interest for diagnosis of certain diseases, though their source and biological role is not yet fully understood.[29,30] Our study is the first known assessment of associations between serum chemical pollutants and circulating miRNA. Nine circulating miRNAs were differentially expressed in occupational workers compared with residents, and the PFOA levels in the occupational group were an order of magnitude higher than that in residents. Although preliminary, this result implied that the high concentration of PFOA in occupational workers

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potentially aroused physiological responses and led to an alteration in the level of circulating miRNAs. However, age between the two groups differed, with residents being much older than the occupational workers. Therefore, we further assessed the 9 miRNAs in another independent sample set consisting of 31 occupational workers of similar age, and found that all 9 circulating miRNAs were generally concordant with the first set of 20 individuals, in which the circulating miRNAs increased with serum PFOA concentrations. Most notably, miR-26b and miR-199−3p increased significantly in the high PFOA group compared with the low level group. The elevated levels of miRNAs in high PFOA burdened individuals may exert detrimental effects on these individuals, despite the pathological damage remaining unidentified.

Limitations and Implications. Our research was limited by the relatively small population, which increased the possibility of chance findings and potential selection biases, and the cross-sectional nature of the study. Despite associations between PFOA and HDL-C, as well as several miRNAs, causal inference was limited. In addition, survey data such as weight and height were self-reported, and no information on socioeconomic data such as income, education, and social status were provided. Potential limitations for miRNA analysis included that covariates associated with miRNAs were not known and exact pathological meanings for altered serum miRNAs were unavailable.

■ ASSOCIATED CONTENT

Supporting Information

Location of Changshu High-Tech Fluorine Chemical Industrial Zone in Changshu City, Jiangsu Province; procedural recovery and limits of quantification (LOQ) of the used analytical procedure for each tested compound; descriptive statistics for outcomes and covariates; comparison of the serum concentrations (ng/mL) of fluorochemicals in residents, its subgroups (by sex and age), and occupational workers (Mann−Whitney U test); correlations between PFCs in residents and occupational workers; coefficients from multivariate regression of lipids and hepatic enzymes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS:

PFC: perfluoroalkyl chemical; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonic acid; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid; PFBS: perfluorobutane sulfonate; PFHpA: perfluoroheptanoic acid; PFDoA: perfluorododecanoic acid; PFTA: perfluorotetradecanoic acid; HPLC-MS/MS: high-performance liquid chromatography-tandem mass spectrometer; BMI: body mass index; CHO: total cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; AST: aspartate aminotransferase; ALT: alanine aminotransferase; miRNA: microRNA

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