Association of per- and polyfluoroalkyl substance exposure with fatty liver disease risk in US adults

Xinyuan Zhang, Longgang Zhao, Alan Ducatman, Chuanjie Deng, Katherine Ellen von Stackelberg, Christopher J. Danford, Xuehong Zhang
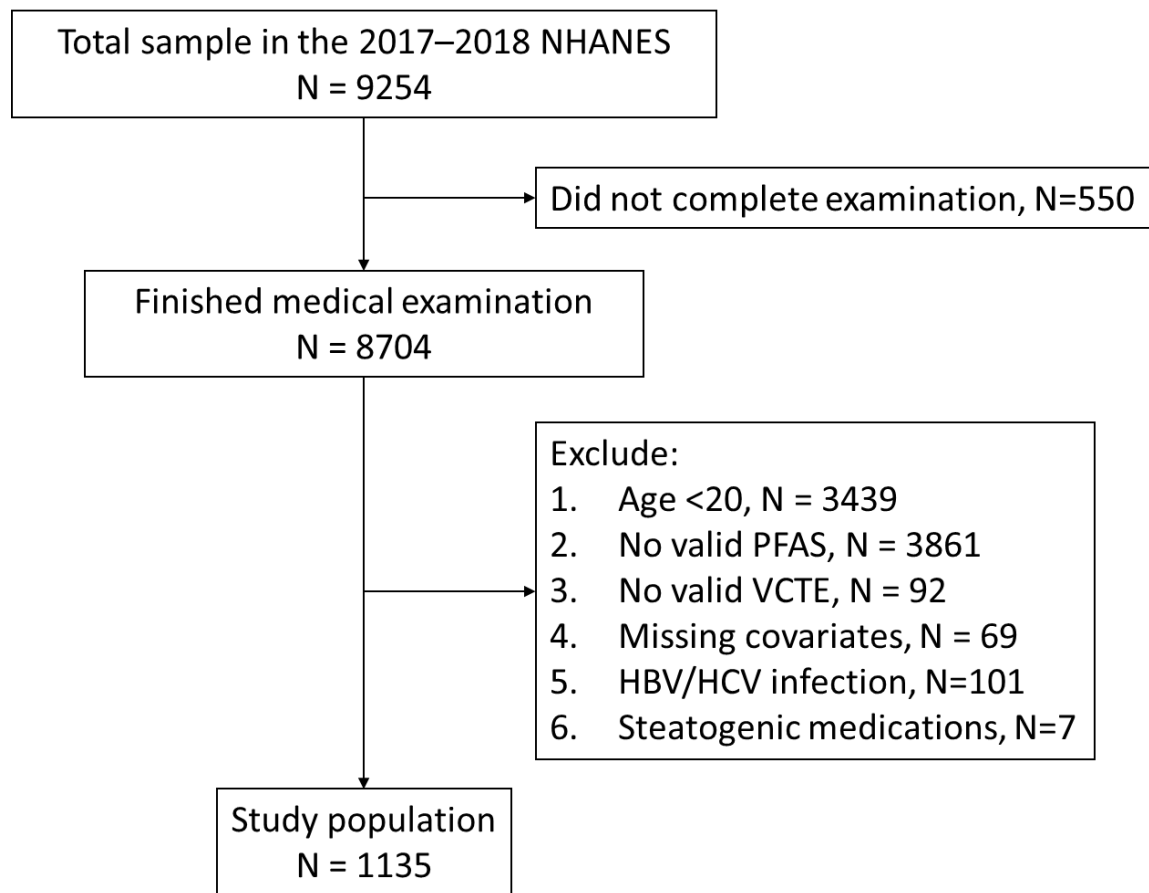
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Supplementary methods

Assessment of PFAS

Substances measured were 2-(N-methyl-perfluorooctane sulfonamido) acetate, perfluorohexane sulfonate (PFHxS), n-PFOS, branched PFOS isomers, n-PFOA, branched PFOA isomers, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), and perfluorododecanoate (PFDoA). Due to changes in the manufacture of PFAS in the US, several additional PFAS were measured in an appendix to the previous list, including perfluoro-1-heptanesulfonate (PFHpS). Total PFOS was calculated as the sum of n-PFOS and branched PFOS isomers. Total PFOA was calculated as the sum of n-PFOA and branched PFOA isomers.

Fig. S1. Study flowchart.

Abbreviations: NHANES, National Health and Nutrition Examination Survey; PFAS, per- and polyfluoroalkyl substances; VCTE, vibration controlled transient elastography; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table S1. Percentage of values above the limit of detection for all per- and polyfluoroalkyl substances assessed in the NHANES 2017–2018.

Codebook variable name	Sample N	Percentage above LOD (%)	Analyzed name
LODPFDE	1929	89	PFDA
LODPFHS	1929	99	PFHxS
LODMPAH	1929	59	Not included
LODPFNA	1929	92	PFNA
LODPFUA	1929	66	Not included
LODNFOA	1929	99	PFOA
LODBFOA	1929	10	
LODNFOS	1929	99	PFOS
LODMFOS	1929	99	
LDDONA	1929	13	Not included
LDGENX	1929	13	Not included
LDCLPF	1929	24	Not included
LDFHPS	1929	81	PFHpS
LDPFHA	1929	14	Not included

Abbreviations: LOD, limit of detection; PFDA, perfluorodecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFHpS, perfluoroheptane sulfonic acid.

Table S2. Detailed stratified results for associations between serum PFAS and fatty liver disease risk according to alcohol intake.

	Never and light drinker					Heavy drinker				
	Tertile 1	Tertile 2	Tertile 3	P_{linear}	Per SD	Tertile 1	Tertile 2	Tertile 3	P_{linear}	Per SD
PFOS										
Median (ng/mL)	2.1	4.9	10.2			2.1	4.7	10.6		
Case/non-case N	113/182	121/176	131/200			29/55	34/46	18/30		
Crude model	1 (ref)	0.85 (0.47-1.54)	1.09 (0.60-1.97)	0.85	1.02 (0.85-1.22)	1 (ref)	1.97 (0.80-4.84)	1.62 (0.44-6.02)	0.67	1.07 (0.75-1.54)
Adjusted model	1 (ref)	0.77 (0.41-1.47)	0.85 (0.42-1.74)	0.62	0.95 (0.77-1.17)	1 (ref)	4.17 (1.08-16.2)	4.72 (0.73-30.6)	0.17	1.47 (0.84-2.57)
PFOA										
Median (ng/mL)	0.8	1.5	2.7			0.8	1.5	2.9		
Case/non-case N	127/196	130/185	108/177			21/53	39/41	21/37		
Crude model	1 (ref)	1.12 (0.63-1.98)	0.88 (0.52-1.49)	0.78	0.97 (0.75-1.25)	1 (ref)	1.95 (1.00-3.82)	1.00 (0.39-2.59)	0.49	1.13 (0.78-1.65)
Adjusted model	1 (ref)	1.10 (0.66-1.82)	0.94 (0.56-1.57)	0.53	0.93 (0.75-1.16)	1 (ref)	2.37 (0.62-9.03)	2.07 (0.50-8.58)	0.03	1.79 (1.07-2.99)
PFHxS										
Median (ng/mL)	0.5	1.2	2.4			0.5	1.1	2.6		
Case/non-case N	100/181	157/200	108/177			21/49	34/48	26/34		
Crude model	1 (ref)	1.37 (0.74-2.56)	1.18 (0.73-1.88)	0.61	1.03 (0.91-1.16)	1 (ref)	1.67 (0.62-4.51)	1.61 (0.46-5.69)	0.22	1.21 (0.87-1.70)
Adjusted model	1 (ref)	1.28 (0.62-2.66)	1.07 (0.63-1.80)	0.76	1.02 (0.90-1.15)	1 (ref)	2.41 (0.97-5.96)	6.12 (0.76-49.1)	0.01	2.06 (1.17-3.65)
PFNA										
Median (ng/mL)	0.2	0.5	0.9			0.2	0.4	0.9		
Case/non-case N	120/187	130/192	115/179			35/57	22/43	24/31		
Crude model	1 (ref)	0.92 (0.62-1.36)	0.92 (0.57-1.48)	0.7	1.03 (0.87-1.23)	1 (ref)	1.22 (0.63-2.36)	1.05 (0.31-3.56)	0.68	1.07 (0.76-1.51)
Adjusted model	1 (ref)	0.75 (0.44-1.28)	0.75 (0.42-1.33)	0.96	1.00 (0.84-1.21)	1 (ref)	1.74 (0.56-5.43)	1.89 (0.49-7.27)	0.40	1.21 (0.78-1.90)
PFHpS										
Median (ng/mL)	0.1	0.2	0.5			0.1	0.2	0.6		
Case/non-case N	93/176	132/192	140/190			24/55	35/56	22/20		
Crude model	1 (ref)	1.13 (0.75-1.71)	1.58 (1.03-2.41)	0.14	1.09 (0.96-1.24)	1 (ref)	1.53 (0.65-3.59)	1.83 (0.55-6.08)	0.22	1.17 (0.89-1.55)
Adjusted model	1 (ref)	1.13 (0.77-1.65)	1.03 (0.64-1.67)	0.94	1.00 (0.87-1.14)	1 (ref)	3.18 (0.67-15.0)	3.90 (0.92-16.5)	0.05	1.44 (1.00-2.07)
PFDA										
Median (ng/mL)	0.2	0.3	0.5			0.2	0.3	0.5		
Case/non-case N	254/357	66/91	45/110			59/88	13/21	9/22		
Crude model	1 (ref)	1.02 (0.59-1.76)	0.57 (0.29-1.12)	0.12	0.79 (0.57-1.09)	1 (ref)	1.05 (0.32-3.40)	0.61 (0.15-2.52)	0.58	0.89 (0.58-1.38)
Adjusted model	1 (ref)	1.17 (0.64-2.16)	0.86 (0.44-1.69)	0.15	0.85 (0.68-1.06)	1 (ref)	1.26 (0.25-6.40)	0.89 (0.27-2.90)	0.39	1.23 (0.77-1.97)
Total PFAS										
Median (ng/mL)	4.5	9.2	17.3			4.5	8.8	16.6		
Case/non-case N	115/178	124/184	126/196			27/56	37/41	17/34		

Crude model	1 (ref)	0.72 (0.41-1.27)	1.01 (0.55-1.83)	0.74	1.03 (0.83-1.28)	1 (ref)	3.26 (1.33-7.95)	0.83 (0.26-2.69)	0.3	1.22 (0.81-1.83)
Adjusted model	1 (ref)	0.66 (0.39-1.10)	0.78 (0.39-1.55)	0.87	0.98 (0.79-1.22)	1 (ref)	7.51 (1.81-31.2)	3.30 (0.84-13.1)	0.05	2.12 (0.99-4.54)

Values presented from the models are odds ratio (95% confidence interval). Model was adjusted for age group (20–<40, 40–<60, ≥60 years), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), ever smoker (only in analyses for never/light drinkers; yes, no), ever drink alcohol (yes, no), physical activity (min/day), body mass index (kg/m2), history of diabetes (yes, no), cancer (yes, no), hypertension (yes, no), aspirin use (yes, no), and high-fat diet (yes, no).

p-trend values were calculated using log-transformed continuous exposure variables.

Abbreviations: PFAS, per-and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFDA, perfluorodecanoate; SD, standard deviation.

Table S3. Associations between serum PFAS and advanced liver fibrosis according to alcohol intake.

	Total	Never and light drinker	Heavy drinker
PFOS	0.97 (0.82-1.15)	0.92 (0.76-1.11)	1.21 (0.72-2.04)
PFOA	1.00 (0.84-1.20)	0.89 (0.72-1.10)	1.75 (1.10-2.79)
PFHxS	1.08 (0.95-1.22)	0.99 (0.87-1.14)	1.61 (1.10-2.35)
PFNA	1.00 (0.87-1.14)	0.96 (0.81-1.13)	1.23 (0.81-1.85)
PFHpS	1.05 (0.93-1.17)	1.01 (0.90-1.14)	1.28 (0.92-1.78)
PFDA	0.85 (0.71-1.02)	0.80 (0.64-0.99)	1.25 (0.78-2.00)
Total PFAS	1.03 (0.86-1.24)	0.94 (0.76-1.16)	1.74 (0.98-3.11)

Values presented from the models are odds ratio (95% confidence interval) per long-transformed standard deviation from ordinal logistic regression. Adjusted model included age group (20–<40, 40–<60, ≥60 years), sex, race/ethnicity (non-Hispanic whites, non-Hispanic black, Hispanic, other), ever smoker (yes, no), ever drink alcohol (yes, no), physical activity (minutes/day), body mass index (kg/m^2), history of diabetes (yes, no), cancer (yes, no), hypertension (yes, no), aspirin use (yes, no), and high-fat diet (yes, no). Ever drink alcohol was not adjusted among heavy drinkers.

Abbreviations: PFAS, per-and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFDA, perfluorodecanoate.

Table S4. Stratified results for associations between serum PFAS and non-alcoholic fatty liver disease risk.

Strata factor	Subgroups	PFOS	PFOA	PFNA	PFHpS	PFDA	Total PFAS
Sex							
	Male	1.08 (0.75-1.55)	0.87 (0.60-1.25)	0.99 (0.72-1.35)	1.00 (0.83-1.21)	0.82 (0.64-1.05)	1.11 (0.71-1.74)
	Female	0.96 (0.70-1.34)	1.18 (0.81-1.71)	1.04 (0.79-1.38)	1.09 (0.85-1.39)	0.95 (0.69-1.29)	1.05 (0.72-1.55)
	P for interaction	0.75	0.24	0.54	0.34	0.86	0.44
Race/ethnicity							
	Whites	1.00 (0.72-1.39)	1.00 (0.71-1.40)	0.99 (0.78-1.26)	1.02 (0.83-1.25)	0.95 (0.73-1.23)	1.03 (0.73-1.46)
	Others	1.00 (0.86-1.16)	1.14 (0.90-1.44)	1.08 (0.87-1.33)	1.14 (0.96-1.35)	0.86 (0.68-1.09)	1.12 (0.90-1.40)
	P for interaction	0.9	0.44	0.80	0.33	0.28	0.46
Smoking							
	Non-smoker	1.17 (0.95-1.44)	1.04 (0.77-1.40)	1.11 (0.91-1.37)	1.03 (0.89-1.20)	1.00 (0.75-1.35)	1.23 (0.93-1.63)
	Smoker	0.89 (0.69-1.14)	1.08 (0.78-1.50)	0.91 (0.71-1.15)	1.02 (0.81-1.29)	0.74 (0.52-1.05)	0.95 (0.69-1.30)
	P for interaction	0.51	0.65	0.72	0.79	0.14	0.82
Obesity							
	Non-obese	0.86 (0.67-1.10)	0.81 (0.54-1.22)	0.91 (0.71-1.16)	1.03 (0.90-1.17)	0.69 (0.54-0.88)	0.87 (0.63-1.21)
	Obese	1.03 (0.83-1.28)	1.27 (0.98-1.66)	1.17 (0.97-1.41)	1.06 (0.88-1.27)	1.06 (0.80-1.41)	1.17 (0.96-1.43)
	P for interaction	0.78	0.12	0.13	0.66	0.80	0.18
Waist circumference							
	WC<102(M)/88 (F) cm	0.91 (0.65-1.27)	0.71 (0.48-1.06)	0.78 (0.60-1.00)	1.12 (0.91-1.38)	0.63 (0.45-0.87)	0.93 (0.62-1.39)
	WC≥102(M)/88 (F) cm	0.92 (0.78-1.10)	1.07 (0.83-1.38)	1.09 (0.92-1.31)	1.04 (0.87-1.24)	0.97 (0.75-1.25)	1.00 (0.81-1.24)
	P for interaction	0.75	0.47	0.43	0.88	0.89	0.58
High-fat diet							
	No	0.93 (0.73-1.20)	1.11 (0.80-1.54)	1.01 (0.80-1.27)	1.01 (0.86-1.18)	0.90 (0.69-1.18)	1.02 (0.74-1.40)
	Yes	1.07 (0.86-1.35)	1.01 (0.75-1.36)	1.03 (0.79-1.35)	1.06 (0.86-1.30)	0.85 (0.63-1.16)	1.15 (0.87-1.50)
	P for interaction	0.23	0.57	0.54	0.40	0.37	0.10

hs-CRP							
	≤3.0 mg/L	1.01 (0.76-1.34)	0.83 (0.58-1.18)	0.98 (0.80-1.21)	0.99 (0.83-1.19)	0.86 (0.67-1.10)	0.99 (0.71-1.39)
	>3.0 mg/L	1.03 (0.80-1.31)	1.29 (0.98-1.70)	1.05 (0.80-1.39)	1.13 (0.97-1.30)	0.92 (0.72-1.18)	1.21 (0.93-1.56)
	P for interaction	0.77	0.03	0.66	0.22	0.67	0.16
ALT							
	≤33 (M)/25 (F)	1.02 (0.84-1.23)	1.04 (0.82-1.32)	1.02 (0.86-1.21)	1.05 (0.91-1.22)	0.88 (0.73-1.07)	1.07 (0.86-1.33)
	>3 3(M)/25 (F)	1.23 (0.95-1.60)	1.13 (0.58-2.19)	1.11 (0.72-1.71)	0.94 (0.78-1.13)	0.88 (0.62-1.24)	1.38 (0.99-1.94)
	P for interaction	0.68	0.43	0.83	0.56	0.22	0.80

Values are odds ratio (95% confidence interval) per one standard deviation increase of log-transformed PFAS. Model was adjusted for age group (20–<40, 40–<60, ≥60 years), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), ever smoker (yes, no), ever drink alcohol (yes, no), physical activity (minutes/day), body mass index (kg/m^2), history of diabetes (yes, no), cancer (yes, no), hypertension (yes, no), aspirin use (yes, no), and high-fat diet (yes, no), while excluding the corresponding strata factor in each analysis.

Abbreviations: PFAS, per-and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFDA, perfluorodecanoate; M, male; F, female; hs-CRP, high-sensitivity C-reactive protein; ALT, alanine aminotransferase.

Table S5. Sensitivity analyses on PFAS and fatty liver disease risk.

	Tertile 1 (lowest)	Tertile 2	Tertile 3 (highest)	*p-trend*	Per log SD
PFOS					
Exclude N=98 history of cancers	1 (reference)	1.29 (0.83-2.00)	1.33 (0.72-2.45)	0.34	1.10 (0.90-1.35)
Exclude N=164 albuminuria	1 (reference)	1.14 (0.70-1.84)	1.32 (0.72-2.44)	0.38	1.08 (0.91-1.29)
PFOA					
Exclude N=98 history of cancers	1 (reference)	1.32 (0.84-2.08)	1.15 (0.64-2.06)	0.32	1.10 (0.91-1.32)
Exclude N=164 albuminuria	1 (reference)	1.13 (0.72-1.78)	1.00 (0.59-1.69)	0.56	1.07 (0.85-1.35)
PFHxS					
Exclude N=98 history of cancers	1 (reference)	1.56 (0.84-2.88)	1.49 (0.87-2.54)	0.01	1.20 (1.04-1.37)
Exclude N=164 albuminuria	1 (reference)	1.58 (0.91-2.77)	1.71 (0.99-2.93)	0.009	1.25 (1.06-1.47)
PFNA					
Exclude N=98 history of cancers	1 (reference)	0.96 (0.58-1.59)	0.97 (0.59-1.59)	0.46	1.06 (0.91-1.23)
Exclude N=164 albuminuria	1 (reference)	0.85 (0.53-1.37)	0.86 (0.50-1.48)	0.96	1.00 (0.84-1.21)
PFHpS					
Exclude N=98 history of cancers	1 (reference)	1.53 (0.88-2.66)	1.30 (0.69-2.44)	0.41	1.07 (0.91-1.25)
Exclude N=164 albuminuria	1 (reference)	1.53 (0.90-2.61)	1.55 (0.97-2.50)	0.16	1.10 (0.96-1.26)
PFDA					
Exclude N=98 history of cancers	1 (reference)	1.12 (0.61-2.06)	0.81 (0.49-1.32)	0.14	0.88 (0.75-1.04)
Exclude N=164 albuminuria	1 (reference)	1.27 (0.65-2.48)	0.93 (0.56-1.55)	0.49	0.94 (0.78-1.12)
Total PFAS					
Exclude N=98 history of cancers	1 (reference)	1.32 (0.77-2.26)	1.22 (0.66-2.27)	0.12	1.19 (0.96-1.48)
Exclude N=164 albuminuria	1 (reference)	1.06 (0.60-1.89)	1.05 (0.58-1.92)	0.14	1.16 (0.95-1.43)

Values presented from the models are odds ratio (95% confidence interval). Model was adjusted for age group (20–<40, 40–<60, ≥60 years), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), ever smoker (yes, no), ever drink alcohol (yes, no), physical activity (minutes/day), body mass index (kg/m^2), history of diabetes (yes, no), cancer (yes, no), hypertension (yes, no), aspirin use (yes, no), and high-fat diet (yes, no).

p-trend values were calculated using log-transformed continuous exposure variables.

Abbreviations: PFAS, per-and polyfluoroalkyl substances; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFDA, perfluorodecanoate; SD, standard deviation.

Table S6. Linear regression coefficients β (denoted by significance) of log-transformed PFAS and liver function biomarkers stratified by obesity.

	AST	ALT	ALP	GGT	Bilirubin	hs-CRP	Albumin
PFOS							
Non-obese	0.023	0.025	0.03	0.03	0.045	-0.027	0.014*
Obese	0.043	0.028	-0.031	-0.003	0.061	-0.204*	0.022*
PFOA							
Non-obese	0.076*	0.078	0.03	0.088	0.087*	-0.09	0.026*
Obese	0.047	0.048	-0.007	0.094	0.094	-0.06	0.026*
PFHxS							
Non-obese	0.056*	0.064	0.027	0.045	0.024	-0.001	0.018*
Obese	0.032	0.026	-0.032	0.014	0.084	-0.143	0.029*
PFNA							
Non-obese	0.063*	0.077	0.041	0.067	0.045	-0.036	0.014*
Obese	0.022	0.026	0.021	0.105	0.011	-0.006	0.013*
PFHpS							
Non-obese	-0.028	-0.026	0.004	-0.033	0.042	0.029	0.007
Obese	0.053	0.089*	-0.019	0.031	-0.006	-0.036	0.01
PFDA							
Non-obese	0.041	0.059	0.018	0.09	0.061	-0.133	0.012
Obese	0.009	0.012	-0.041	0.095	-0.005	-0.25	0.018*
Total PFAS							
Non-obese	0.023	0.025	0.03	0.03	0.045	-0.027	0.014*
Obese	0.043	0.028	-0.031	-0.003	0.061	-0.204*	0.022*

Adjusted model included age group (20–<40, 40–<60, ≥60 years), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), ever smoker (yes, no), ever drink alcohol (yes, no), physical activity (minutes/day), history of diabetes (yes, no), cancer (yes, no), hypertension (yes, no), aspirin use (yes, no), and high-fat diet (yes, no).
* $p < 0.05$.
** $p < 0.001$.

Abbreviations: PFAS, per-and polyfluoroalkyl substances; HDL, high-density lipoproteins; LDL, low-density lipoproteins; LDL, low-density lipoproteins; HbA1c, hemoglobin A1c; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFDA, perfluorodecanoate.

Table S7. Linear regression coefficients of log-transformed PFAS and other serum biomarkers.

	Triglyceride N = 546	LDL N = 535	HDL N = 1134	Cholesterol N = 1134	Glucose N = 546	Insulin N = 546	HbA1c N = 1134
PFOS	0.057	0.019	-0.009	0.035*	0.027	-0.07	0.014*
PFOA	0.02	0.032	0.027	0.042*	-0.002	-0.205*	-0.001
PFHxS	0.068	0.033	-0.014	0.023	0.006	-0.125	< 0.001
PFNA	0.031	0.039	0.021	0.04**	0.02	-0.087	0.013*
PFHpS	0.083	0.019	-0.016	0.02	0.028	0.035	0.011
PFDA	-0.062	0.024	0.068*	0.045*	-0.003	-0.166*	-0.004
Total PFAS	0.056	0.033	-0.003	0.043*	0.023	-0.125	0.011

Adjusted model included age group (20–<40, 40–<60, ≥60 years), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), ever smoker (yes, no), ever drink alcohol (yes, no), physical activity (min/day), body mass index (kg/m^2), history of diabetes (yes, no), cancer (yes, no), hypertension (yes, no), aspirin use (yes, no), and high-fat diet (yes, no).
* p <0.05.
** p <0.001.
Abbreviations: PFAS, per-and polyfluoroalkyl substances; HDL, high-density lipoproteins; LDL, low-density lipoproteins; LDL, low-density lipoproteins; HbA1c, hemoglobin A1c; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFDA, perfluorodecanoate.