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Per- and polyfluoroalkyl substances, apolipoproteins and the risk of coronary heart disease in US men and women

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Abstract

Background Existing evidence for associations of per- and polyfluoroalkyl substances (PFASs) with blood lipids, lipoproteins and apolipoproteins (apo), and coronary heart disease (CHD) risk is limited and inconsistent. This study aims to explore associations between plasma PFASs, blood lipoprotein subspecies defined by apolipoproteins, and CHD risk.

Methods A case–control study of CHD was conducted in the Health Professionals Follow-Up Study (HPFS) and Nurses' Health Study (NHS). Among participants initially free of cardiovascular disease at blood collection in 1994 (HPFS) or 1990 (NHS), 101 participants who developed non-fatal myocardial infarction or fatal CHD were identified and confirmed. A healthy control was matched to each case for age, smoking status, and date of blood draw. Plasma levels of perfluorohexane sulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), total perfluorooctane sulfonic acid (PFOS), branched PFOS (brPFOS), linear PFOS (nPFOS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) were measured. Conditional logistic regression and cubic spline regression models were used to examine associations between baseline PFASs and CHD risk. Linear regression models were applied to study PFAS associations with lipids and their subfractions.

Results After multivariate adjustments, total PFOS, brPFOS and nPFOS were significantly associated with increased risk of developing CHD, and HRs (95% CIs) per log(ng/mL) increment of PFASs were 3.66 (1.36–9.89), 3.68 (1.55–8.76), and 3.01 (1.16–7.86), respectively. Significant positive dose–response relationships were identified for these PFASs ($P_{linearity}$ = 0.01, 0.002, 0.02, respectively). Other PFASs were not associated with CHD risk. PFNA and PFDA were positively associated with total apoE levels among HDL particles with or without apoC-III. No associations were observed for other PFASs with blood lipid subspecies. Blood lipid subfractions did not explain the association between PFOS and CHD risk.

Conclusions Plasma PFOS and its isomers were positively associated with CHD risk. These findings suggest that PFOS exposure causes public health risks that are greater than hitherto believed.

Keywords Perfluoroalkyl substance, Cholesterol, Lipid subfractions, Coronary heart disease

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Introduction

Per- and polyfluoroalkyl substances (PFASs) are persistent anthropogenic chemicals that are extensively used in numerous consumer products and widely exist in the environment [1]. Most animal studies have reported that PFASs with structures similar to fatty acids interfere with lipid metabolism [2], possibly through activating peroxisome proliferator-activated receptors (PPAR) [3–5]. However, findings from human epidemiological studies that examined potential associations between plasma PFASs and blood lipids including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TG) are inconsistent [6–13]. Our recent meta-analysis showed overall positive associations of PFOA and PFOS with blood lipid levels in adults, especially LDL-C and HDL-C [14]. In addition, prior studies demonstrated the metabolic and functional heterogeneity of lipoprotein subspecies defined by apolipoproteins (apo) [15, 16]. LDL-C and HDL-C that contain ApoC-III may be closely linked with atherosclerosis and CHD [17–19]. Despite this, evidence regarding potential associations between PFASs and CHD risk is mixed, and less investigated is the links between PFASs and lipoprotein subspecies [20–26]. Thus far, few studies have been conducted to explore the inter-relationships between plasma concentrations of PFASs, blood lipoproteins subspecies, and the subsequent incident CHD risk in free-living individuals.

We therefore aim to fill these important knowledge gaps by conducting a small case–control study to explore the interplay of plasma PFASs, blood lipids and subspecies, and the risk of CHD using the data from the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS). Given the known relationships of PFASs and blood lipids with body weight [18], in a secondary analysis, we also explored the role of BMI in modulating the PFASs-lipids associations.

Materials and methods

Study population

The HPFS was initiated in 1986 and recruited 51,529 male professionals aged 40–75 years at baseline. The NHS was started in 1976 and included 121,700 female registered nurses aged 30–55 years at baseline [27, 28]. Blood samples were collected from subgroups of the cohorts between 1989 and 1990 in the NHS ($n=32,862$) or between 1993 and 1995 in the HPFS ($n=18,019$). In the NHS, blood collection was self-administered into sodium heparin-treated tubes, while in the HPFS, blood samples were collected using EDTA-treated tubes. These samples were placed on ice packs, shipped in Styrofoam containers by overnight courier, then centrifuged, divided into aliquots, and the serum was then stored in liquid-nitrogen freezers at temperatures of − 130 °C or colder until analysis. The cumulative follow-up rate was > 95% in both cohorts.

Ascertainments of CHD

In this analysis, the primary disease outcome was total CHD, including both fatal CHD and nonfatal myocardial infarction (MI). In both cohorts, nonfatal MI cases were self-reported on the biennially follow-up questionnaires. Study physicians blinded to the exposure status reviewed participants' medical records to confirm MI cases, using World Health Organization criteria of typical symptoms, along with either elevated enzymes or diagnostic changes in electrocardiography [29]. Telephone interviews were performed when medical records were not available. Deaths were identified using reports from next of kin, postal authorities, or the National Death Index, with over 98% of deaths identified through these methods [30]. Fatal CHD was confirmed by a review of hospital records or autopsy reports if CHD was listed as the primary cause of death and previous CHD was documented in medical records. If the death certificate listed CHD as the cause but no medical records were available and there were no prior CHD reports, the case was considered probable fatal CHD. Sudden deaths without cardiac causes were not classified as fatal CHD in this analysis. Given that similar results were observed in the analysis of definite cases only, both definite and probable cases were included in the final analysis.

Nested case–control study design

We conducted two nested case–control studies of CHD in the NHS and HPFS among participants who provided blood samples. Men and women who were free of cardiovascular disease and cancer at blood draw were eligible for the case–control studies. Eligible, confirmed CHD cases were matched with a control who was randomly selected from participants who were free of CHD when the index case occurred [31]. Cases and controls were matched with a 1:1 ratio by age, smoking, and time and date of blood sampling [19]. In the current study, we included 101 pairs of CHD cases and controls in the NHS ($n=51$) and HPFS ($n=50$). Of the 202 participants, 124 had existing data on all or some lipoprotein subspecies (N range between 106 and 124).

The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required.

Laboratory measurements of PFASs

To avoid systematic bias, we handled samples of matched cases and controls identically, and assayed the paired

samples in the same batch in random order at the University of Southern Denmark. Lab-blind QC samples were inserted in each batch to monitor coefficients of variation (CVs). Plasma concentrations of PFHxS, PFOA, total PFOS, brPFOS, nPFOS, PFNA and PFDA were measured using a highly sensitive and reliable technique involving online solid phase extraction and liquid chromatography paired with a triple quadrupole mass spectrometer [32]. The concentrations of the PFASs were all above the estimated limits of detection (LOD: 0.002–0.05 ng PFASs/ml serum), with the inter- and intra-assay coefficients of variation < 6.3% and < 6.1%, respectively.

Laboratory measurements of lipoprotein and apolipoprotein levels

To quantify the lipoprotein subspecies, the thawed samples were filtered, and incubated overnight at 4 °C in columns with anti-apoC-III immuno-affinity to bind lipoproteins containing apoC-III. Lipoproteins not containing apoC-III were eluted using phosphate-buffered saline and collected via gravity flow, while those bound to apoC-III were eluted with 3 M sodium thiocyanate. The separation efficiency of the apoC-III immunoaffinity method was 99%. The apoC-III-bound and unbound fractions were then ultracentrifuged to isolate very-low density lipoprotein (VLDL-C) (4 °C and 25,000 rpm for 16 h, d < 1.006 g/mL), LDL-C (4 °C and 25,000 rpm for 24 h, 1.006 < d < 1.063 g/mL), and the remaining solution containing HDL-C and other plasma components. Concentrations of apoB, apoC-III, and apoE were measured using sandwich ELISAs (Academy Biomedical, Houston, TX), while TC and TG levels were measured using enzymatic assays (Thermo Electron Corp, Waltham, MA). Any sample with an intra-assay CV greater than 15% was reanalyzed. Further details on the quantification methods were described in a previous study [33].

Assessments of other covariates

The information of age, sex, self-reported anthropometric measurements, alcohol consumption, family history of MI, aspirin use, lipid-lowering medication use, history of hypertension, diabetes, and hypercholesterolemia was collected at baseline and updated using biennial follow-up questionnaires. Body mass index (BMI) was calculated by dividing body weight in kilograms by height in meters squared. Physical activity levels were assessed using the Baecke physical activity questionnaire and quantified in metabolic equivalent (MET) hours per week (METs-hr/wk) [34]. In a food frequency questionnaires (FFQ) administered as baseline and every 4 years, participants were asked to report their intake of individual food items at a pre-specified portion size with frequencies ranging from never to ≥ 6 times per day. The reproducibility and validity of the FFQ were documented previously [35]. Nutrient intake from FFQ assessments was calculated based on the Harvard University Food Composition Database. Diet quality was assessed based on the cumulative average of alternative healthy eating index (AHEI) since baseline. For this analysis, we included these covariates measured in 1990/1994 questionnaire in the NHS and HPFS, respectively.

Statistical analysis

The comparisons between cases and controls were tested by paired student's t-test for continuous variables, or by the McNemar chi-square test for categorical variables. The concentrations of plasma PFASs, lipids and apolipoprotein subspecies were natural log-transformed and modeled as continuous variables in all analyses in this study. We analyzed the association between plasma PFAS concentrations and the risk of CHD using conditional logistic regression, which accounts for the matching design. Two models were fit: model one was a basic model, conditioning on matching factors only; model two was additionally adjusted for fasting status (yes or no), BMI (kg/m^2), physical activity (MET-hr/wk), alcohol consumption (never, 0–4.9 g/d, 5.0–14.9 g/d, and > 15 g/d), AHEI (continuous), aspirin use (yes or no), regular lipid-lowering medication use (yes or no), family history of MI (yes or no), history of hypertension (yes or no), diabetes (yes or no) and hypercholesterolemia (yes or no). Since we used risk-set sampling in this case–control study, the odds ratios (ORs) generated using the conditional logistic regression are unbiased estimates of the hazard ratios. Cubic spline regression using the *%LGTPHCURV9* macro with 4 knots was applied to evaluate potential dose–response relationship between PFASs and the risk of CHD [36].

Given that BMI may be an outcome of elevated PFAS exposure [37], we conducted a sensitivity analysis by removing BMI from the multivariable-adjusted model 2. Additionally, we examined the associations of natural log-transformed plasma PFASs concentrations with natural log-transformed lipids, lipoprotein, apolipoprotein subspecies and C-reactive protein (CRP) using generalized linear regression models (GLM) in a subset of participants (N range between 106 and 124), adjusting for matching factors and the same covariates in the abovementioned model 2. In another sensitivity analysis, we also used GLM to examine the interaction between plasma PFASs and BMI (≥ 25 kg/m^2, < 25 kg/

m^2) on the concentrations of lipoprotein and lipoprotein subspecies. To evaluate potential mediation by the apolipoproteins on associations between PFOSs and CHD risk, we used *%MEDIATE* macro to quantify the mediation proportion of the effect of PFOSs on CHD risk that was explained by lipoprotein subspecies [38].

These statistical analyses were performed using SAS version 9.4 and R v.4.3.0. A two-sided *P* value < 0.05 was used to define statistical significance.

Results

Table 1 presents baseline characteristics of CHD cases and controls. Compared with controls, CHD cases were more likely to use aspirin and cholesterol lowering drugs and to have family history of MI or history of hypertension or diabetes at baseline. Concentrations of TC, TG and LDL-C were significantly higher in cases, whereas HDL-C were lower in cases than in controls. Table S1 shows the mean concentrations of apolipoprotein

Table 1 Baseline characteristics of CHD cases and controls in the NHS and HPFS studies[1]

	case ($n=101$)	control ($n=101$)	*P*-values[2]
Age (yr)[a]	56.7 ± 7.9	56.8 ± 7.8	-
Female (%)[a]	50.5	50.5	-
Smoking, %[a]			
Past	32.7	33.6	-
Current	13.8	12.9	-
BMI (kg/m^2)	26.2 ± 4.7	25.7 ± 3.8	0.48
Physical activity (MET/wk)	26.6 ± 37.8	25.6 ± 23.9	0.83
Alcohol intake (g/d)	2.4 ± 1.0	2.4 ± 1.0	0.83
AHEI	46.8 ± 9.4	48.3 ± 10.5	0.27
Fasting, %	62.3	58.6	0.04
Using of cholesterol lowering medication, %	8.9	3.0	< 0.001
Aspirin use, %	39.6	36.6	0.02
Family history of MI, %	44.6	18.8	0.001
History of hypercholesterolemia, %	56.6	35.7	0.47
History of hypertension, %	47.6	24.9	0.01
History of diabetes, %	14.0	2.0	< 0.001
PFAS (ng/mL)			
Total PFOS	36.8 ± 19.8	34.0 ± 15.0	0.26
brPFOS	9.5 ± 4.8	8.6 ± 4.0	0.13
nPFOS	27.2 ± 15.5	25.4 ± 11.5	0.33
PFHxS	4.7 ± 5.1	6.2 ± 13.9	0.32
PFOA	5.6 ± 4.7	5.7 ± 5.8	0.96
PFNA	0.8 ± 0.5	0.9 ± 0.6	0.74
PFDA	0.2 ± 0.2	0.3 ± 0.2	0.89
Blood lipids (mg/dL)			
TC	223.0 ± 40.7	207.6 ± 34.6	0.005
TG	172.4 ± 120.3	129.5 ± 76.3	0.004
HDL-C	47.1 ± 16.2	52.1 ± 13.5	0.02
LDL-C	137.4 ± 37.4	124.5 ± 31.2	0.01
CRP (mg/L)	0.4 ± 0.5	0.3 ± 0.5	0.18

Abbreviations: *CHD* Coronary heart disease, *BMI* Body mass index, *METs-hr/wk* Metabolic equivalent-hours per week, *AHEI* Alternative healthy eating index, *MI* Myocardial infarction, *TC* Total cholesterol, *HDL-C* High-density lipoprotein-cholesterol, *LDL-C* Low-density lipoprotein-cholesterol, *TG* Triglycerides, *PFOS* Perfluorooctane sulfonate, *brPFOS* Branched PFOS, *nPFOS* Linear PFOS, *PFOA* Perfluorooctanoate, *PFHxS* Perfluorohexanesulfonate, *PFNA* Perfluorononanoic acid, *PFDA* Perfluorodecanoic acid, *CRP* C-reactive protein

Data are mean ± SD or percentage (%)

[1] Data on women are from the Nurses' Health Study, and data on men are from the Health Professionals Follow-up Study

[2] *P*-values for the difference between cases and controls were determined by paired student's t-test for variables expressed as means ± SD, and by the McNemar chi-square test for variables expressed as percentages

[a] Matching factors

subspecies by CHD cases and controls. CHD cases had significantly higher concentrations of TC, apoB, apoA-II, apoC-II, and apoC-III in LDL particles that carry apoC-III and other species than controls.

Conditioning on matching factors only, none of the 7 PFASs were associated with the risk of CHD (Table 2). However, after additional adjustments in model 2, participants with higher concentrations of total PFOS, brPFOS and nPFOS had a significantly increased risk of developing CHD. The HRs and 95% confidence intervals (CIs) for CHD per log(ng/mL) increment of PFOS, brPFOS and nPFOS were 3.66 (1.36, 9.89), 3.68 (1.55, 8.76) and 3.01 (1.16, 7.86), respectively. Multivariate adjustment, particularly for family history of MI, as well as history of hypertension, diabetes, and hypercholesterolemia, led to strengthened associations. Similar trends were observed in PFOA, PFHxS and PFNA, but the associations did not achieve statistical significance. Non-significant inverse associations were observed for PFDA in relation to risk of CHD. When removing BMI from model 2, the results remained robust. The HRs and 95% CIs for CHD per log(ng/mL) increment of PFOS, brPFOS, and nPFOS were slightly attenuated to 3.58 (1.33, 9.60), 3.55 (1.51, 8.33), and 2.97 (1.14, 7.69), respectively.

Figure 1 shows the dose–response relationship between concentrations of PFOS and the risk of CHD. Significant positive linear dose–response relationships were found for the association of total PFOS, brPFOS and nPFOS with the risk of CHD (*P* for linearity = 0.01, 0.002, or 0.02, respectively).

Figure 2 shows correlations between plasma PFASs concentrations and apolipoprotein subspecies among the 124 participants (N range between 106 and 124). After multivariate adjustments, positive associations were primarily observed between plasma PFAS concentrations and apoE levels in HDL particles. Significant, positive associations were found between PFHxS, PFNA, PFDA and apoE in HDL without apoC-III (0.32 mg/dL increment of ln-apoE per ln-PFNA increase, $p = 0.02$; 0.36 mg/dL increment of ln-apoE per ln-PFDA increase, $p = 0.004$; 0.20 mg/dL increment of ln-apoE per ln-PFHxS increase, $p = 0.04$), and in HDL with apoC-III (0.26 mg/dL increment of ln-apoE per ln-PFNA increase, $p = 0.04$; 0.24 mg/dL increment of ln-apoE per ln-PFDA increase, $p = 0.03$) (Fig. 2). Additionally, significant positive associations were observed between PFNA, PFDA and apoA-II, apoC-I, and apoC-II levels in VLDL with or without apoC-III. For example, we observed 0.46 mg/dL increment of ln-apoC-I per ln-PFNA increase ($p = 0.02$) (Fig. 2). No significant association was found between PFASs and TC or TG in VLDL, LDL, or HDL that do or do not carry apoC-III (Figure S1). For the associations between PFASs and total blood lipids and CRP, we also found no significant associations, except for a positive association between PFHxS and HDL-C ($p = 0.04$) (Figure S3).

In the sensitivity analysis, potential effect modification of BMI was observed on the associations of brPFOS with apoC-III levels in LDL. Specifically, brPFOS was associated with lower apoC-III levels in LDL fractions among lean participants (BMI < 25 kg/m^2) but higher apoC-III levels among overweight and obese individuals (BMI ≥ 25 kg/m^2; $P_{interaction} = 0.04$; Table S2). Total PFOS and brPFOS were also inversely associated with apoC-III levels in VLDL and LDL among lean individuals, although the test for interaction was not significant. Lastly, among overweight and obese individuals, but

Table 2 Association of per unit increment of log-transformed PFASs levels (ng/mL) with the risk of CHD in the NHS and HPFS[a]

PFASs	Model 1			Model 2		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Total PFOS	1.50	(0.76, 2.95)	0.24	3.66	(1.36, 9.89)	0.01
brPFOS	1.43	(0.80, 2.55)	0.23	3.68	(1.55, 8.76)	0.003
nPFOS	1.40	(0.72, 2.70)	0.32	3.01	(1.16, 7.86)	0.02
PFOA	0.99	(0.59, 1.68)	0.98	1.63	(0.82, 3.25)	0.17
PFHxS	0.89	(0.63, 1.27)	0.53	1.20	(0.75, 1.94)	0.45
PFNA	1.11	(0.68, 1.82)	0.68	1.05	(0.54, 2.04)	0.89
PFDA	0.98	(0.64, 1.49)	0.92	0.88	(0.49, 1.59)	0.68

Abbreviations: *PFOS* Perfluorooctane sulfonate, *brPFOS* branched PFOS, *nPFOS* linear PFOS, *PFOA* Perfluorooctanoate, *PFHxS* Perfluorohexanesulfonate, *PFNA* Perfluorononanoic acid, *PFDA* Perfluorodecanoic acid

Model 1 adjusted only for matching factors, which are conditioned by conditional logistic regression, including age, gender, smoking status and date of blood sampling

Model 2 was based on Model 1 and further adjusted for fasting status (yes or no), BMI (kg/m^2), physical activity (MET-hr/wk), alcohol consumption (never, 0–4.9 g/d, 5.0–14.9 g/d, and > 15 g/d), AHEI (continuous), aspirin use (yes or no), regular lipid-lowering medication use (yes or no), family history of MI (yes or no), and history of hypertension (yes or no), diabetes (yes or no) and hypercholesterolemia (yes or no)

[a] Data are selected from HPFS and NHS. HRs are interpreted as hazard ratios per log(ng/mL) increment of PFAS

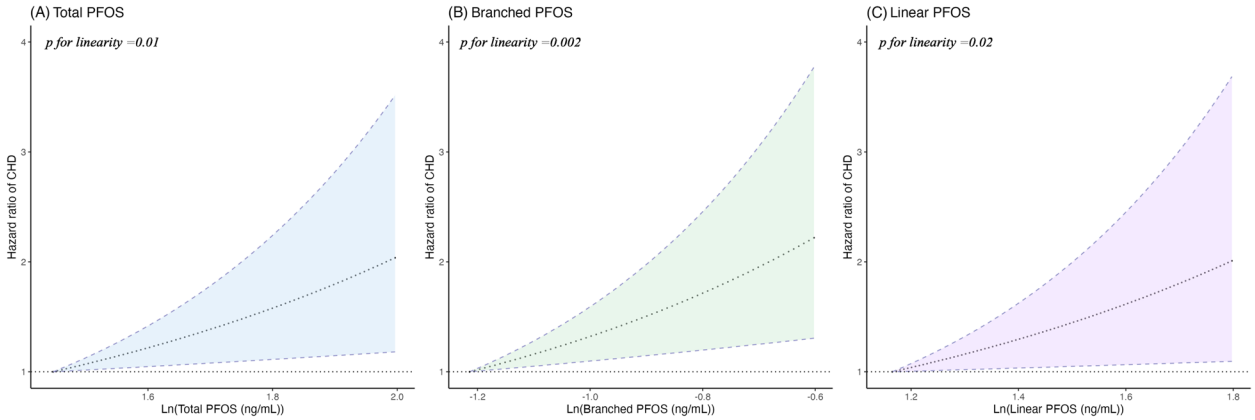

Fig. 1 Dose–response associations of plasma total PFOS, branched PFOS and linear PFOS with the risk of CHD. Note: This analysis was run in the case–control cohort based on conditional logistic regression. The models were adjusted for matching factors (including age, gender, smoking status and date of blood sampling), fasting status (yes or no), BMI (kg/m^2), physical activity (MET-hr/wk), alcohol consumption (never, 0–4.9 g/d, 5.0–14.9 g/d, and > 15 g/d), AHEI (continuous), aspirin use (yes or no), regular lipid-lowering medication use (yes or no), family history of MI (yes or no), and history of hypertension (yes or no), diabetes (yes or no) and hypercholesterolemia (yes or no). The x-axis is natural log-transformed total PFOS, brPFOS and nPFOS, respectively; the y-axis represents the hazard ratio of CHD. The lines in the plot show the trend for the association of interest and *p*-values are showed on the top-left; the shade denotes 95% confidence interval. Abbreviations: PFOS, Perfluorooctane sulfonate; brPFOS, branched PFOS; nPFOS, linear PFOS

not lean counterparts, significant positive associations between PFNA, PFDA and PFOA and apoE in HDL without apoC-III were observed (Table S2).

The blood lipids and apolipoproteins subspecies did not significantly explain the association between PFASs and the risk of CHD. For example, total apoE level non-significantly mediated the association between CHD and total PFOS (54.5%, $p=0.42$) and brPFOS (1.7%, $p=0.49$). nPFOS and CHD associations were non-significantly mediated by apoC-III levels in VLDL (9.8%, $p=0.47$) and LDL (27.3%, $p=0.43$) (data not shown).

Discussion

In this study, we found that concentrations of the PFOS were significantly associated with CHD after adjustment for established CHD risk factors. The associations might be potentially dose dependent. Furthermore, PFAS concentrations were not consistently associated with lipoprotein subspecies defined by the presence or absence of apoC-III or apoE, except that PFNA, PFDA, and PFHxS were associated with apoE levels among HDL particles. The apolipoprotein subspecies did not significantly explain the associations between PFOS and CHD risk. To our knowledge, the current study is among the first prospective studies that elucidated the inter-relationships along the PFAS—lipoprotein and apolipoprotein species—CHD risk axis among U.S. men and women.

The epidemiological evidence concerning exposure to PFASs and cardiovascular events is limited and inconsistent [20]. In cross-sectional studies, positive associations between total or individual PFASs and cardiovascular disease were reported [21, 39, 40], whereas other studies found null associations [11, 41, 42] or even inverse associations [22]. Two studies using NHANES data reported positive relationships between PFOA and cardiovascular disease [39], and between PFNA and CHD [21]. A higher prevalence of MI has also been noted among those exposed to PFOA in contaminated drinking water [40]. However, the studies using data from C8 Health Project showed inverse associations of PFHxS and PFOS with stroke [41], and of PFOA, PFHxS and PFOS with CHD among diabetes patients [22]. One study using data from both C8 Health Project and an occupational cohort found no association between PFOA and CHD [11]. A recent study based on the U.S. national NHANES survey showed that increased exposure to PFOS was associated with a higher risk of total, cardiovascular, and cancer mortality [43]. Cardiovascular risk factors associated with elevated levels of PFASs also include hypertension and artery calcification [44–49]. Among occupational health studies, current PFOA and PFOS concentrations in Italian production workers were positively associated with systolic blood pressure [50]. These workers also showed increased all-cause mortality [51].

Evidence from prospective studies remains sparse and controversial and is exclusively from Swedish and Italian populations. In one Swedish study, PFASs were not significantly associated with risk of CHD [24]. Another Swedish cohort observed that higher perfluoroheptanoic acid (PFHpA) was significantly associated with a higher CHD risk [23]. Intriguingly, a further study in a different Swedish population demonstrated that increased levels

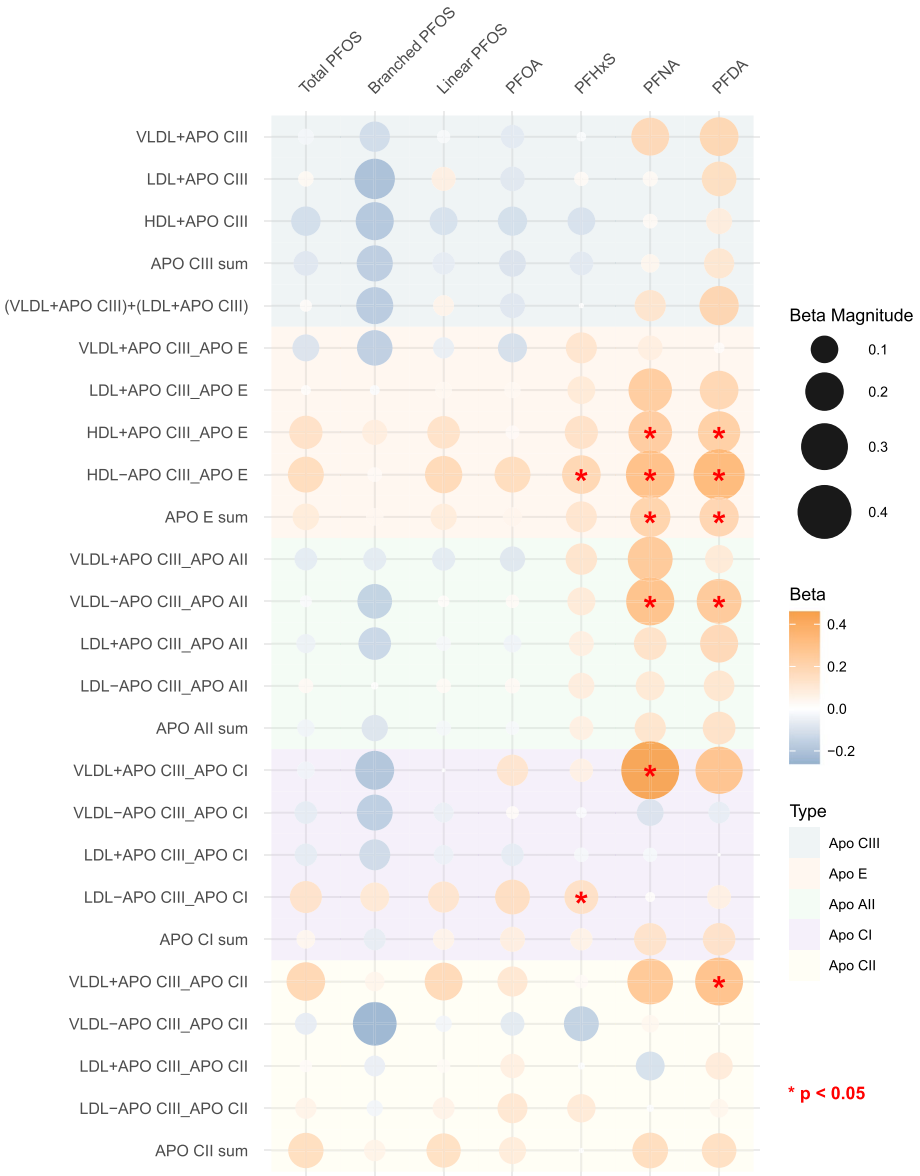

Fig. 2 Heatmap of the associations between PFASs (ng/mL) and lipoprotein and lipoprotein subspecies (mg/dL) in the NHS and HPFS. Note: PFASs and lipoprotein subspecies are log transformed. Generalized linear regressions were used for the association of interest in the 124 study participants with available lipoprotein subspecies data (N range between 106 and 124) and adjusted for age (continuous), gender (female, male), smoking status (never, former, and current smoker), date of blood sampling (continuous), fasting status (yes or no), BMI (kg/m^2), physical activity (MET-hr/wk), alcohol consumption (never, 0–4.9 g/d, 5.0–14.9 g/d, and > 15 g/d), AHEI (continuous), aspirin use (yes or no), regular lipid-lowering medication use (yes or no), family history of MI (yes or no), and history of hypertension (yes or no), diabetes (yes or no) and hypercholesterolemia (yes or no). The x-axis are the concentrations of PFASs, including PFOS, brPFOS, nPFOS, PFOA, PFHxS, PFNA and PFDA; the y-axis are the levels of 5 types of lipoprotein subspecies, including Apo CIII, Apo E, Apo AII, Apo CI and Apo CII. A nomenclature scheme was used to define each lipoprotein subspecies in this figure. For example, in the "HDL + APO CIII_APO E", " + APO CIII" (or "-APO CIII") means that the HDL particles do (or do not) carry apo CIII, and "_APO E" means the total apo E concentration. Thus, "HDL-APO CIII_APO E" means the apo E concentration among HDL particles without apo CIII. Colors and sizes of circles indicate association coefficients, and asterisks denote association significance (* $p < 0.05$). Abbreviations: HDL, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; VLDL, very low-density lipoprotein-cholesterol; APO, apolipoprotein; PFOS, Perfluorooctane sulfonate; PFOA, Perfluorooctanoate; PFHxS, Perfluorohexanesulfonate; PFNA, Perfluorononanoic acid; PFDA, Perfluorodecanoic acid

of multiple PFASs, including PFOA, PFOS, and PFHpA, over 10 years were significantly associated with contemporaneous increased carotid artery intima-media thickness [25]. The current prospective case–control study, considering both men and women, showed a significant positive association between total PFOS, brPFOS, nPFOS and CHD. We also observed non-significant positive associations between PFOA and PFHxS and CHD. The potential reasons for the differing observations between the current study and the Swedish studies are not fully understood. One possible explanation could be the assessment of variations in PFASs exposures between the studies in the U.S. and Sweden. For example, compared with the Swedish population, samples collected in 1999–2000 in the U.S. showed higher levels of PFOS (U.S. median: 30.2 ng/ml) but lower levels of PFNA (U.S. median: 0.6 ng/ml) and PFDA (below LOD) [52]. Clearly, more large-scale prospective studies are needed to substantiate or refute these associations.

Evidence for the association between plasma PFASs and blood lipids is also highly mixed. In our recent meta-analysis, PFOA and PFOS were positively related to TC and LDL-C, and inversely associated with HDL-C and TG [14]. PFHxS was positively associated with blood HDL-C levels, which was also reported in previous studies and in line with the current study [53, 54]. There were no significant findings for PFNA, PFDA and cholesterol outcomes. Few studies have been conducted to understand PFASs in relation to blood lipid subspecies. It is well-elucidated that blood lipid particles comprise a broad category of lipoproteins and apolipoproteins subspecies bearing diverse functions [17–19, 55, 56], but most previous human studies focused on lipoproteins or individual apolipoproteins in total plasma [6–13, 57–59]. Recently, we found that LDL-C and HDL-C that contain apoC-III were positively associated with the risk of CHD, whereas HDL-C without apoC-III was negatively associated with CHD [17, 19]. We also reported positive associations of plasma PFOA with apoC-III and subspecies in HDL and LDL with apoC-III in individuals with overweight and obesity participating in the POUNDS-Lost study while null associations were observed for TC and TG [18]. In the present study, we did not observe positive associations between PFASs and apoC-III levels. Instead, we observed significant correlations of PFDA and PFNA with apoE levels among HDL particles with or without apoC-III, which seems to be consistent with the non-significant inverse associations with CHD risk for PFDA. In light of the established relationship between BMI and blood lipids and the fact that the positive associations between PFOA and apoC-III were observed in individuals with overweight and obesity [18], we explored whether the associations between PFASs and lipoprotein subspecies may depend on participants' body weight. In this exploratory analysis, we observed inverse associations between brPFOS and apoC-III levels among lean individuals. We also observed significant positive associations between PFNA/PFDA and PFOA and apoE in HDL without apoC-III in individuals with overweight and obesity. The discrepancy between the current analysis and our previous analysis in the POUNDS-Lost study is unknown, although the small sample size and chance findings may potentially account for some of the inconsistency. Nonetheless, it remains unknown regarding how the apolipoproteins or lipoprotein subspecies may play a role in the associations of PFASs with CHD.

At present, the mechanisms underlying associations between PFASs and lipoprotein or CHD risk are still poorly understood, but experimental evidence is accruing. Based on current experimental evidence and the structural similarity with fatty acids, plasma PFASs may modulate the expression of certain lipid metabolism regulating proteins through interfering with peroxisome proliferator-activated receptor alpha (PPARα) [3, 60, 61]. PFASs may also influence blood lipid metabolism through interfering with bile acid metabolism [62]. Salihović and colleagues showed concomitant enterohepatic reabsorption of both bile acids and PFASs in the intestine, and decreased reabsorption of bile acids led to reduced blood cholesterol levels [63]. Other factors that affect enterohepatic reabsorption of both PFASs and bile acids could also introduce a correlation between PFAS and lipids. As another possibility, PFASs may disrupt the expression of genes involved in the cholesterol clearance pathway by reducing expression in genes involved in cholesterol clearance (NR1H2, NR1H3, ABCG1, and NPC1) and an up-regulated expression in genes coding for proteins responsible for efflux of free cholesterol (NCEH1) [2]. In addition, in vitro studies suggested that PFASs may also change the functionality of platelets, bind to phospholipid membranes, alter cell permeability and ion channel structure [64]. More experimental and multi-omics studies are warranted to explore potential pathways and substantiate the findings in mediation analysis.

This study has several limitations. First, the main results for PFASs and apolipoprotein subspecies were based on a cross-sectional analysis, which might raise concerns about reverse causality, while the persistent nature of PFASs may mitigate these concerns. Second, the small sample size limits our statistical power to examine associations between PFASs and apolipoprotein subspecies, or to explore interactions by other factors for the associations of interest. The mediation analysis was therefore exploratory, limited by small sample size and potential selection bias, requiring cautious interpretation of the findings on the pathways linking PFAS, apolipoproteins,

and CHD risk. Third, the observed associations may be due to chance as they did not hold after adjustments for multiple comparisons. Fourth, our analysis was based on a single baseline assessment of PFASs and lipoprotein subspecies, so we could not derive changes during the follow-up. Fifth, unmeasured confounding may contribute to our findings, while it is less likely to significantly impact our findings because of our ability to adjust for many known and potential risk factors for CHD. Finally, the homogeneous socioeconomic status of our study participants limits the generalizability of our results to populations with different characteristics, even though the underlying biological mechanisms involved are likely consistent across groups.

Conclusion

Plasma PFOS and its isomers concentrations were significantly associated with a higher risk of CHD. However, PFOS and other PFASs were not consistently associated with lipoprotein subspecies that are considered relevant to CHD risk. The positive association of PFOS and CHD risk appears to be independent of lipoprotein subspecies. Further evidence from large-scale prospective studies is needed to elucidate the complex inter-relationships between PFASs, blood lipids and CHD risk.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12940-024-01147-2.

Supplementary Material 1: Supplementary Tables and Figures.

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Author's contributions

QS supervised the research project; LZ and BL conducted the research and drafted the manuscript. All authors contributed to the manuscript and read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

Dr. Grandjean has provided paid expert assistance in legal cases involving PFAS-exposed populations. All other authors declare no competing interests associated with the manuscript.

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