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Per- and Polyfluoroalkyl Substances and Risk of Myocardial Infarction and Stroke: A Nested Case–Control Study in Sweden

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Background: Per- and polyfluoroalkyl substances (PFAS) are widespread and persistent pollutants that have been associated with elevated cholesterol levels. However, data on incident cardiovascular disease (CVD) is lacking.

Objectives: We investigated the association of exposure to PFAS with risk of myocardial infarction and stroke and, subsidiary, with baseline blood lipids.

Methods: This population-based nested case–control study included first incident myocardial infarction and stroke cases with matched controls from two Swedish cohorts: the Swedish Mammography Cohort-Clinical (SMC-C) and the Cohort of 60-year-olds (60YO). Baseline blood sampling occurred during 2003–2009 and 1997–1999 with follow-up through 2017 and 2014 for the SMC-C and the 60YO, respectively. Eight plasma PFAS concentrations were measured using targeted liquid chromatography–triple quadrupole mass spectrometry. Five of these were quantifiable in both cohorts; individual values and their standardized sum were categorized into tertiles based on the controls. First incident myocardial infarction ($n = 345$) and ischemic stroke ($n = 354$) cases were ascertained via linkage to the National Inpatient Register and the Cause of Death Register. Controls were randomly selected from each cohort after matching for age, sex, and sample date. Baseline blood lipids were measured in plasma or serum after overnight fasting.

Results: Among the 1,528 case–control subjects, the mean (standard deviation) age was 66 (7.7) y and 67% of them were women. In multivariable-adjusted analyses, the third tertile of the standardized sum of five PFAS associated with higher cholesterol and lower triglyceride levels among controls at baseline ($n = 631$). The corresponding results were odds ratios = 0.70 [95% confidence interval (CI): 0.53, 0.93] for CVD, 0.60 (95% CI: 0.39, 0.92) for myocardial infarction, and 0.83 (95% CI: 0.46, 1.50) for stroke.

Discussion: This study indicated that exposure to PFAS, although associated with increased cholesterol levels, did not associate with an increased risk of myocardial infarction, stroke, or their composite end point. The findings improve our knowledge on potential health effects of environmental contaminants in the CVD context. https://doi.org/10.1289/EHP9791

Introduction

Cardiovascular health may be damaged by certain classes of environmental pollutants (GBD 2015 Risk Factors Collaborators 2016; O'Toole et al. 2008), one such group of interest is the fluorinated synthetic chemicals widely used for their water-, oil-, and stain-repelling properties. Per- and polyfluoroalkyl substances (PFAS) accumulate globally in the environment and, subsequently, also in humans (Lau et al. 2007) via contaminated food, food-contact materials, drinking water, dust, and contact with PFAS-containing products (Sunderland et al. 2019). There is consistent evidence for an association between PFAS and elevated total cholesterol in humans (EFSA CONTAM et al. 2018; Steenland et al. 2020; Sunderland et al. 2019). Underlying mechanisms may involve the disruption of fatty acid metabolism and lipid synthesis in the liver given that PFAS activate transcription factors for genes involved in lipid metabolism, including peroxisome proliferator-activated receptor α (PPARα) (U.S. EPA 2016; Bijland et al. 2011).

PFAS have been shown to be related to atherosclerosis development (Lind et al. 2017, 2018; Osorio-Yáñez et al. 2021) and could impact cardiovascular disease (CVD) risk via elevated cholesterol (Prospective Studies Collaboration et al. 2007), as well as via endocrine disruption (Kahn et al. 2020), oxidative stress (Liu et al. 2007), reduced immune response (DeWitt et al. 2019), and endothelial dysfunction (Lin et al. 2016). However, studies to date on PFAS and CVD are scarce, inconsistent, and with considerable methodological limitations (lack of temporality criterion, small sample sizes, or self-reported end points), as reviewed by the European Food Safety Agency (EFSA CONTAM et al. 2018). Therefore, high-quality epidemiological studies on PFAS and CVD are needed to provide a stronger basis for regulatory decisions. Thus, the present study investigated whether the observed association between PFAS and cholesterol translated into increased risk of CVD, that is, myocardial infarction and stroke. We assessed associations of seven different PFAS with different chain lengths with CVD risk using a nested case–control design, using bio-banked plasma and data from two population-based cohorts. We also assessed baseline associations with blood lipid fractions among the controls.

Methods

Study Population

The study used data from the Swedish Mammography Cohort-Clinical (SMC-C) (SIMPLER; https://www.simpler4health.se/) and the Cohort of 60-year-olds (60YO) (Karolinska Institutet; https://ki.se/en/imm/the-cohort-of-60-year-olds). The SMC, established between 1987 and 1990, included women born during 1914–1948 residing in Central Sweden (74% response rate, $n = 61,433$) (Harris 2013). Between 2003 and 2009, all SMC-women <85 years of age living in Uppsala town and surrounding

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areas were invited for health examination (baseline in this study); 5,022 responders (61%) constituted the SMC-C. The 60YO cohort, established to assess CVD etiology, identified residents in Stockholm County turning 60 y old between July 1997 and June 1998 and randomly invited every third man and woman for a health examination between August 1997 and March 1999 (78% response rate, $n = 4{,}232$). Both cohorts donated blood samples and completed a questionnaire (Wändell et al. 2007). Written or oral informed consent was obtained from all participants and the studies were approved by the regional ethical review board in Stockholm.

Ascertainment of Myocardial Infarction and Ischemic Stroke

From baseline blood sampling through 2017 for the SMC-C and 2014 for the 60YO, a total of 135 and 214 first incident cases of primary myocardial infarction and 173 and 183 first incident cases of ischemic stroke, respectively, were ascertained via linkage of the cohort to the National Inpatient Register [*International Classification of Diseases (ICD), 10th Revision* (WHO 2016): I21 and I63], among participants free of prevalent coronary heart disease or cerebrovascular disease. Outside hospital deaths from myocardial infarction from the Cause of Death Register were verified by autopsy reports. Register validation revealed that diagnosis was correct in 98% for myocardial infarction (validation study of men and women 45–70 years of age between 1992 and 1994), 98.6% for stroke (validation study of men, private communication) and 68.5% for nonfatal stroke (men and women 25–74 years of age between 1985 and 1989) as reviewed by Ludvigsson et al. (2011).

Nested Case–Control Study

For each case, controls were randomly matched if alive and free from the case diagnosis at the time the case experienced the event (risk-set sampling). In the SMC-C, controls were matched (1:2 for myocardial infarction and 1:1 for stroke) based on age (± 1 y) and sample date (± 90 d). In the 60YO, controls were matched (1:1) based on sex and sample date (± 90 d). Plasma samples were missing for some subjects, leading to a final study population of 134 cases–264 controls (4 cases were matched 1:1) in the SMC-C and 211 case–control pairs in the 60YO for myocardial infarction and 172 pairs in the SMC-C and 182 pairs in the 60YO for ischemic stroke. Thus, 699 cases and 829 controls were available for total CVD assessment (Figure 1).

Cross-Sectional Assessment of Blood Lipid Levels

For the baseline cross-sectional evaluation of PFAS and blood lipids, we included data from all available controls, plus three controls with a missing case, and removed the duplicated controls (matched for both myocardial infarction and stroke; $n = 70$). Those reporting high baseline cholesterol (either self-reported or ascertained via the prescribed drug register in the SMC-C and as self-reported in the 60YO; $n = 131$) and those with missing lipid measurements ($n = 1$) were excluded, leaving 631 controls (326 from the SMC-C and 305 from the 60YO; Figure 1). Blood lipids

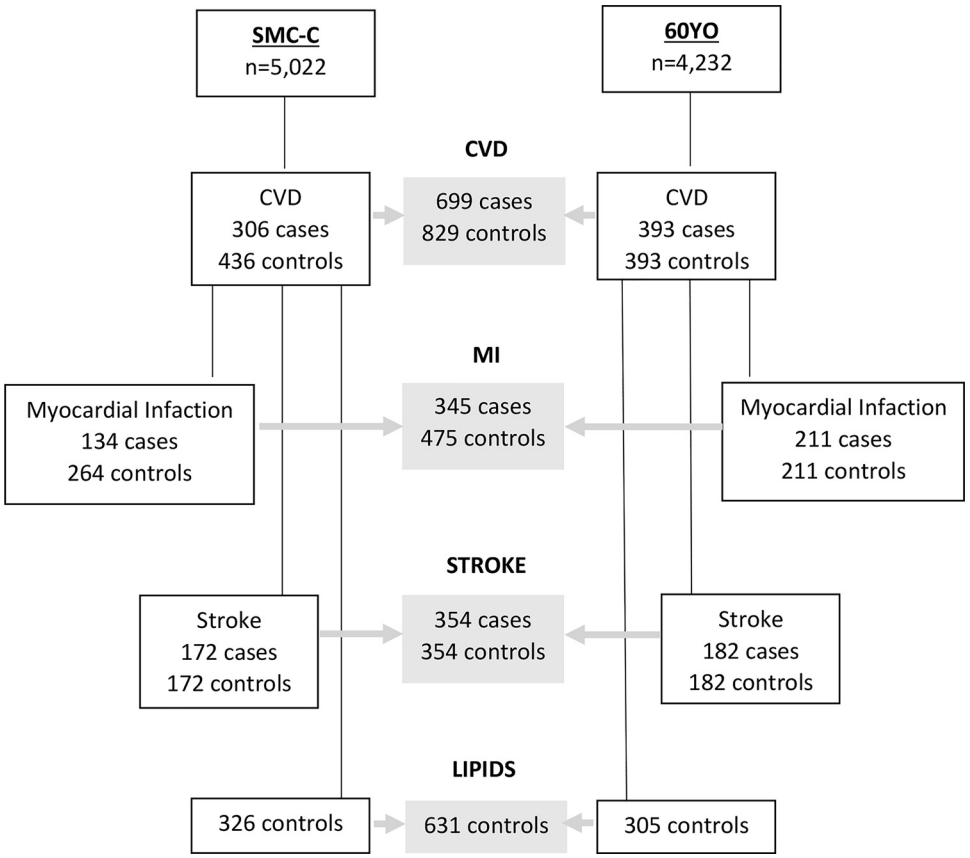

Figure 1. Flow chart of the prospective nested case–control design and the cross-sectional assessment of lipids using two pooled Swedish cohorts, SMC-C and 60YO. For MI in the SMC-C, there is a 1:1 match in four cases, due to missing missing/broken samples. For lipid analyses, controls without a matched case (due to missing/broken samples) that were excluded from the CVD/MI/stroke analyses were included in the lipid analyses, whereas controls used in both MI and stroke data sets were used only once. Controls on lipid-lowering medication at baseline and missing lipid concentrations were excluded ($n = 5$ additional for LDL analyses). Note: 60YO, Cohort of 60-year-olds; CVD, cardiovascular disease; LDL, low-density lipoprotein; MI, myocardial infarction; SMC-C, Swedish Mammography Cohort-Clinical cohort.

[i.e., total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides] and apolipoproteins (apoB and apoA1, in 60YO) were measured in blood plasma (SMC-C) or serum (60YO) after overnight fasting using routine hospital laboratories in the SMC-C and automated measurement systems in the 60YO (Halldin et al. 2007).

Baseline PFAS Measurements

Serum PFAS were measured at the Division of Occupational and Environmental Medicine at Lund University, applying a modified method previously described (Norén et al. 2021). In short, the proteins were precipitated using acetonitrile by vigorous shaking for 30 min of thawed samples. After centrifugation, an aliquot of the supernatant was analyzed using liquid chromatography–triple quadrupole linear ion trap mass spectrometry (QTRAP 5500, AB Sciex), using selected reaction monitoring in negative ion mode. For quality control (QC), five QC reference samples, four chemical blanks (water), and calibration standards were analyzed for each sample batch. The limit of detection (LOD) was three times the standard deviation of responses in chemical blanks (Table S1). QC samples results were used to calculate the between-run precision as the coefficient of variation (2-14%; Tables S1–S2).

All samples were analyzed within 5 wk. The laboratory participates in a quality control program from the University of Erlangen-Nuremberg, Germany, for perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and in Interlaboratory Comparison Investigations/External Quality Assurance Schemes exercises for the analysis of perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA) and is approved by quality controls performed by the European Human Biomonitoring Initiative (HBM4EU) project (see "Appendix S1. Certificates quality control PFAS measurements (University of Erlangen-Nuremberg, Germany)" and "Appendix S2. Certificate quality control PFAS measurements (HBM4EU)." in the Supplemental Material).

The LOD ranged from 0.01 ng/mL for PFHpA to 0.09 ng/mL for PFOA. Of the eight PFAS that had measurable levels, concentrations of PFHxS, PFOS, PFOA, PFNA, and PFDA were >LOD for all participants. Concentrations of PFHpA and PFUnDA were >LOD for all participants in the SMC-C, but <LOD for 2.2% and 0.25% of participants in the 60YO, respectively, which were substituted with the LOD divided by the square root of 2. However, concentrations of PFDoDA were <LOD in 50.1% and 60.7% in the SMC-C and the 60YO cohorts, respectively, and because tertiles could not be accurately assessed, they were therefore excluded from the analysis. Furthermore, concentrations of PFOA and PFHpA were remarkably high in the SMC-C (50% of participants had values between 80 and 400 ng/mL and 0.3 and 4 ng/mL for PFOA and PFHpA, respectively), likely owing to contamination of the samples during sampling/storage, and they were therefore not considered in that cohort.

Thus, eventually, five long-chain PFAS—three carboxylated (PFNA, PFDA, and PFUnDA) and two sulfonated (PFHxS and PFOS)—were available in both the SMC-C and the 60YO cohorts, whereas two carboxylated PFAS—one short-chain (PFHpA) and one long-chain (PFOA)—were additionally available in the 60YO cohort.

Baseline Assessment of Covariates

Questionnaire information included age, sex, attained education, body mass index (BMI), comorbidities (i.e., diabetes, hypertension, and high cholesterol), family history of CVD (i.e., heart attack in a relative before 60 years of age in the SMC-C cohort and in any siblings, father, or mother in the 60YO cohort), smoking habits and physical activity (i.e., active when reported walking/biking was ≥40 min/d and exercise ≥1 h/wk for the SMC-C and when reported activity was moderate or heavy for the 60YO). Covariates were selected based on *a priori* knowledge of CVD risk factors and lifestyle factors that could impact PFAS levels (Lindbohm et al. 2021; Rosengren et al. 2019).

We obtained information on food consumption (from a semi-quantitative 124-item food frequency questionnaire in the SMC-C and a questionnaire with 17 food-related questions in the 60YO). For the SMC-C, we created a healthy diet score based on the eight-point scoring system (low to high adherence) of the modified Mediterranean diet score, reflecting consumption of fruits and vegetables, fermented dairy foods, whole grain/fiber-rich foods, legumes and nuts, fish, olive/rapeseed oil, and alcohol (in moderation) as positive components and with red and processed meat (as a negative component) (Tektonidis et al. 2015), which was collapsed into three categories. For the 60YO, the healthy diet score was constructed from a six-point scoring system based on the intake of fruits, vegetables, fish, alcohol (in moderation) as positive components and with meat and snacks as negative components, and also collapsed into three categories. Missing information on covariates (<5%, with the exception of 16% for physical activity in the SMC-C) were replaced by a missing indicator category.

Statistical Analyses

Spearman's rank correlation was used to assess pairwise relationships between different PFAS. Individual PFAS plasma concentrations were natural log-transformed and assessed as a continuous variable per 1 standard deviation (SD) increment. To create a sum of the PFAS, individual PFAS were standardized (rescaled with mean = 0 and SD = 1) and summed (ΣPFAS). Individual PFAS and ΣPFAS were also categorized into tertiles according to the cohort-specific distribution among the controls.

The baseline cross-sectional associations among the controls between PFAS and blood lipids were assessed using multivariable-adjusted linear regression analysis. Pooled results from both cohorts, using linear mixed effects models, are presented as β-coefficients with corresponding 95% confidence intervals (CIs).

The prospective associations between baseline PFAS and risk of CVD, myocardial infarction, and stroke were assessed using conditional logistic regression. Pooled results from the two cohorts are presented as odds ratios (ORs) with corresponding 95% CIs. To maximize statistical power, simple pooling was used in assessing total CVD because of low between-cohort heterogeneity (I^2 statistic range: 0–34%, lowest $p = 0.22$). Random-effects meta-analysis was used for assessing separate risk of myocardial infarction and stroke because the heterogeneity between the cohorts was larger (I^2 statistic range: 0–86%, $p < 0.05$) for separate outcomes.

Both assessments of PFAS with lipids and with CVD were adjusted in Model 1 for matching factors [i.e., age (in SMC-C), sex (in 60YO) and sample year] and in Model 2 were additionally adjusted for attained education (≤12 y/ > 12 y/missing), BMI (as continuous), diabetes (yes/no), hypertension (yes/no), family history of CVD (yes/no), smoking habits (never/former/current/missing), physical activity (active/inactive/missing), and healthy diet score (4 categories, including "missing" category). To explore potential mediation by lipids on the PFAS and CVD risk association, lipids were included in additional models (Model 3 included LDL, whereas Model 4 included HDL and triglycerides). Furthermore, the potential effect modification by BMI (normal ≤25 kg/m^2 vs. overweight and obese >25 kg/m^2) was investigated using interaction terms for continuous PFAS. Adjusted

Table 1. Baseline (2003–2009 and 1997–1999, respectively) characteristics by cardiovascular disease (CVD) case–control status of 742 women from the Swedish Mammography Cohort-Clinical and of 786 men and women from the Swedish Cohort of 60-year-olds.

	SMC-C cohort		60YO cohort	
Characteristics	CVD cases ($n = 306$)	Controls ($n = 436$)	CVD cases ($n = 393$)	Controls ($n = 393$)
Sex [% (n)]				
Female	100 (306)	100 (436)	36 (141)	36 (141)
Male	0 (0)	0 (0)	64 (252)	64 (252)
Age (y)	72 (7.3)	72 (7.3)	61 (0.1)	61 (0.1)
Sample year	2006 (1.5)	2006 (1.5)	1998 (0.4)	1998 (0.3)
Education (y) [% (n)]				
<12	70 (212)	68 (296)	79 (289)	67 (254)
≥12	30 (93)	32 (138)	21 (79)	33 (125)
Missing (n)	1	2	25	14
BMI (kg/m^2)	27 (4.6)	26 (4.4)	27 (4.3)	27 (4.3)
History of diabetes [% (n)]				
No	95 (292)	97 (424)	91 (356)	94 (370)
Yes	4.6 (14)	2.8 (12)	9.4 (37)	5.9 (23)
History of hypertension [% (n)]				
No	48 (146)	59 (257)	50 (198)	62 (243)
Yes	52 (160)	41 (179)	50 (195)	38 (150)
History of high cholesterol [% (n)]				
No	72 (221)	77 (336)	93 (365)	90 (353)
Yes	28 (85)	23 (100)	7.1 (28)	10 (40)
Family history of CVD [% (n)]				
No	62 (191)	64 (280)	56 (222)	56 (220)
Yes	38 (115)	36 (156)	44 (171)	44 (173)
Smoking status [% (n)]				
Never	52 (152)	59 (249)	29 (108)	46 (172)
Former	32 (92)	32 (133)	37 (135)	38 (144)
Current	16 (47)	8.8 (37)	34 (123)	16 (61)
Missing (n)	15	17	27	16
Physical activity [% (n)]				
Active	31 (77)	33 (120)	28 (100)	33 (127)
Inactive	70 (175)	67 (249)	72 (257)	67 (255)
Missing (n)	54	67	36	11
Healthy diet score [% (n)]				
Unhealthy	21 (62)	13 (56)	40 (146)	33 (127)
Moderately healthy	61 (180)	61 (256)	35 (130)	32 (124)
Healthy	18 (53)	25 (106)	25 (92)	34 (132)
Missing (n)	11	18	25	10
Total cholesterol (mmol/L)	5.8 (1.1)	5.8 (1.0)	6.0 (1.0)	5.9 (1.0)
LDL (mmol/L)	3.5 (1.0)	3.5 (0.9)	4.0 (0.9)	3.9 (0.9)
HDL (mmol/L)	1.5 (0.4)	1.6 (0.4)	1.3 (0.4)	1.4 (0.4)
Triglycerides (mmol/L)	1.5 (0.7)	1.3 (0.6)	1.6 (1.0)	1.4 (0.9)
ApoB (mmol/L)[a]	—	—	1.2 (0.2)	1.0 (0.2)
ApoA1 (mmol/L)[a]	—	—	1.4 (0.3)	1.5 (0.3)
PFHxS (ng/mL)	5.28 (7.81)	5.96 (7.64)	3.21 (4.60)	3.46 (6.45)
Median (IQR)	2.69 (2.07–4.62)	2.73 (2.01–5.59)	2.34 (1.85–2.93)	2.33 (1.84–2.91)
PFHpA (ng/mL)[a]	—	—	0.08 (0.10)	0.08 (0.08)
Median (IQR)	—	—	0.05 (0.03–0.09)	0.06 (0.03–0.1)
PFOS (ng/mL)	18.4 (10.5)	19.2 (17.6)	26.5 (13.2)	27.8 (14.9)
Median (IQR)	16.8 (11.8–22.1)	16.9 (12.5–21.8)	24.8 (19.1–31.2)	25.2 (19.0–34.4)
PFOA (ng/mL)[a]	—	—	5.59 (3.44)	5.91 (3.91)
Median (IQR)	—	—	5.05 (3.85–6.63)	5.31 (3.99–6.93)
PFNA (ng/mL)	1.01 (0.52)	1.02 (0.48)	0.74 (0.39)	0.81 (0.42)
Median (IQR)	0.95 (0.67–1.19)	0.92 (0.72–1.21)	0.69 (0.48–0.89)	0.71 (0.51–0.99)
PFDA (ng/mL)	0.40 (0.21)	0.43 (0.23)	0.28 (0.15)	0.30 (0.16)
Median (IQR)	0.35 (0.27–0.48)	0.38 (0.29–0.52)	0.25 (0.18–0.34)	0.26 (0.19–0.39)
PFUnDA (ng/mL)	0.32 (0.19)	0.36 (0.21)	0.25 (0.14)	0.29 (0.17)
Median (IQR)	0.27 (0.19–0.4)	0.31 (0.21–0.44)	0.22 (0.16–0.31)	0.24 (0.17–0.36)

Note: Continuous variables are shown as mean (SD) if not otherwise stated. PFAS concentrations are presented as mean (SD) followed by median (IQR). —, not applicable; 60YO, Cohort of 60-year-olds; apo, apolipoprotein; BMI, body mass index; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid; SD, standard deviation; SMC-C, Swedish Mammography Cohort-Clinical cohort.
[a]Available for the 60YO cohort alone.

(Model 2) β coefficients (95% CIs) from linear mixed effects models stratified by BMI were visualized for a 1-SD increase in PFAS.

All statistical analyses were performed using the statistical software STATA (version 15.1; Stata Corp LP) and using the *metan* package for the meta-analysis. *p*-Values were calculated based on two-sided tests, and the level of statistical significance was set at 0.05.

Results

Study population characteristics by case–control status in each cohort are summarized in Table 1 (see Tables S3 and S4 for summaries of myocardial infarction/stroke). The SMC-C compassed an older, female population with a later sampling date than the 60YO, which had mostly male cases and matched controls. The SMC-C cohort showed a lower prevalence of diabetes but had a

higher prevalence of high cholesterol and fewer smokers compared with the 60YO cohort. Furthermore, except for PFOS, PFAS levels were higher in the SMC-C. Overall, the controls were more educated and less often smokers and had a lower prevalence of diabetes and hypertension and lower triglycerides levels than the cases. High correlations were observed between PFNA, PFDA, and PFUnDA ($r > 0.79$), whereas the lowest correlation was between PFHpA and PFUnDA ($r = 0.16$) (Figure S1).

Overall, the vast majority of PFAS showed statistically significant associations, with higher total and LDL cholesterol, whereas associations in a favorable direction were observed with higher HDL cholesterol and apoA1 and lower triglycerides. Results for apoB showed mainly null associations (Figure 2; Table S5). An interaction between PFAS and BMI ($p_{\text{Interaction}} < 0.05$) was observed, with stronger associations of most PFAS with higher LDL and apoB among the overweight and obese, whereas no associations were found among the lean participants (Figure S2). The same effect modification by BMI was observed for total cholesterol, whereas there was no significant interaction for HDL (for most PFAS), triglycerides, or apoA1 ($p_{\text{interaction}} > 0.05$; Table S6).

PFAS levels were overall inversely associated [ΣPFAS: OR = 0.70 (95% CI: 0.53, 0.93)] with risk of CVD after pooling the cohorts (Table 2). Further adjusting for baseline lipid levels (LDL or HDL and triglycerides) had only marginal impact (Models 3 and 4, Table 2). Similar associations were found in individual cohorts (Table S7). Specific assessment of myocardial

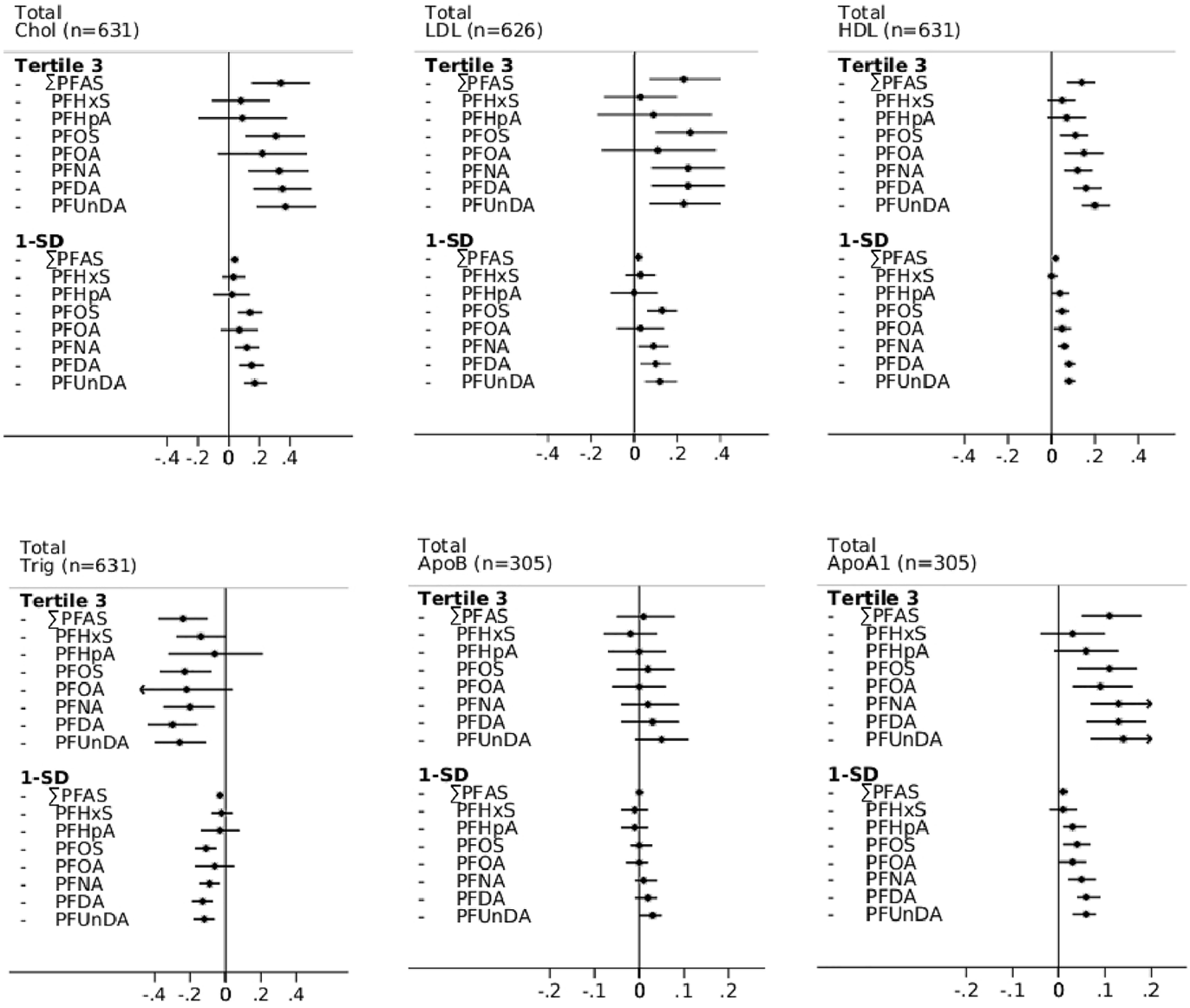

Figure 2. Multivariable-adjusted cross-sectional associations in controls between baseline PFAS plasma concentrations and total cholesterol, LDL, HDL, triglycerides, apoB, and apoA1 of two Swedish pooled cohorts (SMC-C baseline: 2003–2009 and 60YO baseline: 1997–1999), estimated using linear mixed effects models—apoB, apoA1, PFHpA, and PFOA results are from the 60YO cohort alone. Adjusted β-coefficients (95% CIs) are presented according to PFAS tertiles (using Tertile 1 as reference), as well as by 1-SD increment in natural log-transformed plasma PFAS concentrations (ng/mL). Models were adjusted for age, sex, sampling date, education, BMI, diabetes, hypertension, family history of CVD, smoking habits, physical activity, and healthy diet score. Individual PFAS were standardized (rescaled with mean = 0 and SD = 1) and summed (ΣPFAS). Note: 60YO, Cohort of 60-year-olds; BMI, body mass index; apo, apolipoprotein; Chol, cholesterol; CI, confidence interval; CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PFDA, perfluorodecanoic acid; PFAS, per- and polyfluoroalkyl substances; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid; SD, standard deviation; SMC-C, Swedish Mammography Cohort-Clinical cohort; Trig, triglycerides.

Table 2. Multivariable-adjusted prospective associations between baseline PFAS plasma concentrations and subsequent risk of cardiovascular disease (CVD) in 1,528 men and women from two pooled Swedish cohorts, estimated using conditional logistic regression—PFHpA and PFOA results are from the 60YO cohort alone.

	Pooled cohorts ($n = 1{,}528$)					
			OR of incident CVD (95% CI)			
Exposure categories	Case/control (n/n)	Median (IQR) (mmol/L)[a]	Model 1	Model 2	Model 3	Model 4
$\sum$PFAS						
Tertile 1	270/278	−3.06 (−3.81, −2.34)	1.00	1.00	1.00	1.00
Tertile 2	253/275	−0.58 (−1.23, 0.10)	0.94 (0.74, 1.20)	1.03 (0.79, 1.34)	1.01 (0.77, 1.31)	1.05 (0.81, 1.37)
Tertile 3	176/276	3.46 (2.09, 5.70)	0.64 (0.49, 0.82)	0.70 (0.53, 0.93)	0.68 (0.51, 0.90)	0.73 (0.55, 0.97)
1-SD log	—	—	0.95 (0.92, 0.98)	0.97 (0.93, 1.00)	0.96 (0.93, 0.99)	0.97 (0.94, 1.00)
PFHxS						
Tertile 1	244/277	1.73 (1.44, 1.95)	1.00	1.00	1.00	1.00
Tertile 2	237/276	2.52 (2.32, 2.74)	0.98 (0.76, 1.25)	0.95 (0.72, 1.24)	0.92 (0.70, 1.20)	0.96 (0.73, 1.26)
Tertile 3	218/276	4.97 (3.20, 11.1)	0.87 (0.67, 1.13)	0.96 (0.72, 1.28)	0.94 (0.70, 1.25)	0.99 (0.74, 1.32)
1-SD log	—	—	0.93 (0.84, 1.04)	0.95 (0.85, 1.06)	0.94 (0.84, 1.06)	0.96 (0.85, 1.07)
PFHpA [b]						
Tertile 1	153/132	0.03 (0.02, 0.03)	1.00	1.00	1.00	1.00
Tertile 2	120/130	0.06 (0.05, 0.07)	0.79 (0.56, 1.12)	0.69 (0.46, 1.03)	0.64 (0.42, 0.97)	0.68 (0.45, 1.02)
Tertile 3	120/131	0.13 (0.10, 0.18)	0.78 (0.55, 1.11)	0.75 (0.50, 1.11)	0.68 (0.45, 1.03)	0.72 (0.48, 1.08)
1-SD log	—	—	0.93 (0.81, 1.08)	0.95 (0.81, 1.12)	0.94 (0.79, 1.11)	0.95 (0.80, 1.12)
PFOS						
Tertile 1	250/278	12.9 (10.0, 17.3)	1.00	1.00	1.00	1.00
Tertile 2	228/276	21.9 (17.1, 25.7)	0.89 (0.70, 1.15)	0.92 (0.70, 1.21)	0.86 (0.65, 1.14)	0.95 (0.73, 1.26)
Tertile 3	221/275	32.3 (24.8, 38.9)	0.87 (0.67, 1.13)	0.90 (0.68, 1.20)	0.87 (0.65, 1.16)	0.94 (0.71, 1.26)
1-SD log	—	—	0.89 (0.80, 0.99)	0.91 (0.81, 1.03)	0.89 (0.79, 1.01)	0.93 (0.82, 1.05)
PFOA [b]						
Tertile 1	135/131	3.41 (2.66, 3.94)	1.00	1.00	1.00	1.00
Tertile 2	142/131	5.25 (4.82, 5.70)	1.05 (0.76, 1.45)	1.14 (0.78, 1.65)	1.03 (0.70, 1.50)	1.17 (0.80, 1.70)
Tertile 3	116/131	7.63 (6.88, 9.18)	0.84 (0.58, 1.20)	0.90 (0.60, 1.37)	0.81 (0.52, 1.24)	0.93 (0.61, 1.42)
1-SD log	—	—	0.90 (0.77, 1.04)	0.91 (0.77, 1.08)	0.87 (0.73, 1.04)	0.92 (0.77, 1.09)
PFNA						
Tertile 1	260/279	0.51 (0.40, 0.60)	1.00	1.00	1.00	1.00
Tertile 2	234/276	0.81 (0.71, 0.93)	0.89 (0.71, 1.13)	0.88 (0.68, 1.14)	0.83 (0.64, 1.08)	0.88 (0.68, 1.14)
Tertile 3	205/274	1.28 (1.11, 1.55)	0.80 (0.62, 1.02)	0.91 (0.69, 1.20)	0.87 (0.66, 1.14)	0.93 (0.71, 1.23)
1-SD log	—	—	0.87 (0.78, 0.96)	0.91 (0.81, 1.02)	0.89 (0.79, 1.00)	0.92 (0.82, 1.03)
PFDA						
Tertile 1	256/285	0.20 (0.15, 0.26)	1.00	1.00	1.00	1.00
Tertile 2	254/269	0.31 (0.25, 0.38)	1.02 (0.80, 1.29)	1.17 (0.90, 1.52)	1.13 (0.87, 1.47)	1.19 (0.92, 1.55)
Tertile 3	189/275	0.52 (0.45, 0.66)	0.73 (0.56, 0.95)	0.81 (0.61, 1.08)	0.78 (0.58, 1.04)	0.86 (0.64, 1.15)
1-SD log	—	—	0.83 (0.75, 0.93)	0.89 (0.79, 1.00)	0.87 (0.77, 0.98)	0.90 (0.80, 1.02)
PFUnDA						
Tertile 1	288/294	0.16 (0.13, 0.19)	1.00	1.00	1.00	1.00
Tertile 2	236/260	0.27 (0.24, 0.31)	0.89 (0.70, 1.13)	0.99 (0.76, 1.29)	0.99 (0.76, 1.28)	1.01 (0.78, 1.32)
Tertile 3	175/275	0.48 (0.40, 0.60)	0.61 (0.47, 0.79)	0.76 (0.57, 1.02)	0.73 (0.54, 0.97)	0.80 (0.59, 1.07)
1-SD log	—	—	0.79 (0.71, 0.88)	0.86 (0.76, 0.97)	0.84 (0.74, 0.95)	0.87 (0.77, 0.99)

Note: Adjusted ORs (95% CIs) of incident CVD (myocardial infarction or stroke) are presented according to the PFAS tertiles as well as by 1-SD increment in natural log-transformed plasma PFAS concentrations (ng/mL). Model 1: adjusted for matching factors (sex, age, sampling date). Model 2: additionally adjusted for education, BMI, diabetes, hypertension, family history of CVD, smoking habits, physical activity, and healthy diet score. Model 3: additionally adjusted for LDL (19 observations deleted due to missing LDL). Model 4: additionally adjusted for HDL and triglycerides. Individual PFAS were standardized (rescaled with mean = 0 and SD = 1) and summed (ΣPFAS). —, not applicable; 60YO, Cohort of 60-year-olds; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid; SD, standard deviation; SMC-C, Swedish Mammography Cohort-Clinical cohort.
[a]The ΣPFAS score are standardized values.
[b]Estimated from the 60YO cohort alone.

infarction and ischemic stroke in random-effects meta-analyses showed an overall similar pattern of nonsignificant inverse associations, although slightly more inconsistencies between cohorts were found (Figures 3 and 4; Tables S8 and S9). There was no indication of interactions with BMI ($p_{\text{interaction}} > 0.1$, Table S6).

Discussion

In this large prospective nested case–control study, despite statistically significant cross-sectional associations between PFAS and increased total and LDL cholesterol among the controls, we observed overall null associations between PFAS and risk of CVD, myocardial infarction, and stroke. If anything, these associations displayed an inverse tendency.

Although in our study the median PFAS levels were not particularly high [e.g., PFOS levels approximated the previously established lower bound benchmark doses (Dong et al. 2019)], we still observed statistically significantly associations for $\sum$PFAS, PFOS, PFNA, PFDA, and PFUnDA with higher total- and LDL cholesterol. There were no significant associations for PFHxS, PFHpA and PFOA, possibly due to lower potency of the shorter chain lengths (PFHxS and PFHpA) (Wolf et al. 2008) or due to lower power (PFOA) given that this was assessed only in the 60YO cohort. Our findings align with several risk assessments (ATSDR 2021; EFSA CONTAM et al. 2018; IARC 2018), reviews (Steenland et al. 2020; Sunderland et al. 2019), and other large studies showing positive associations (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009). In contrast to these potential atherogenic associations observed, we found associations with lower triglycerides and higher HDL. Interestingly, stronger associations were found for the newer, less abundant and longer chain PFAS compounds (PFNA, PFDA, and

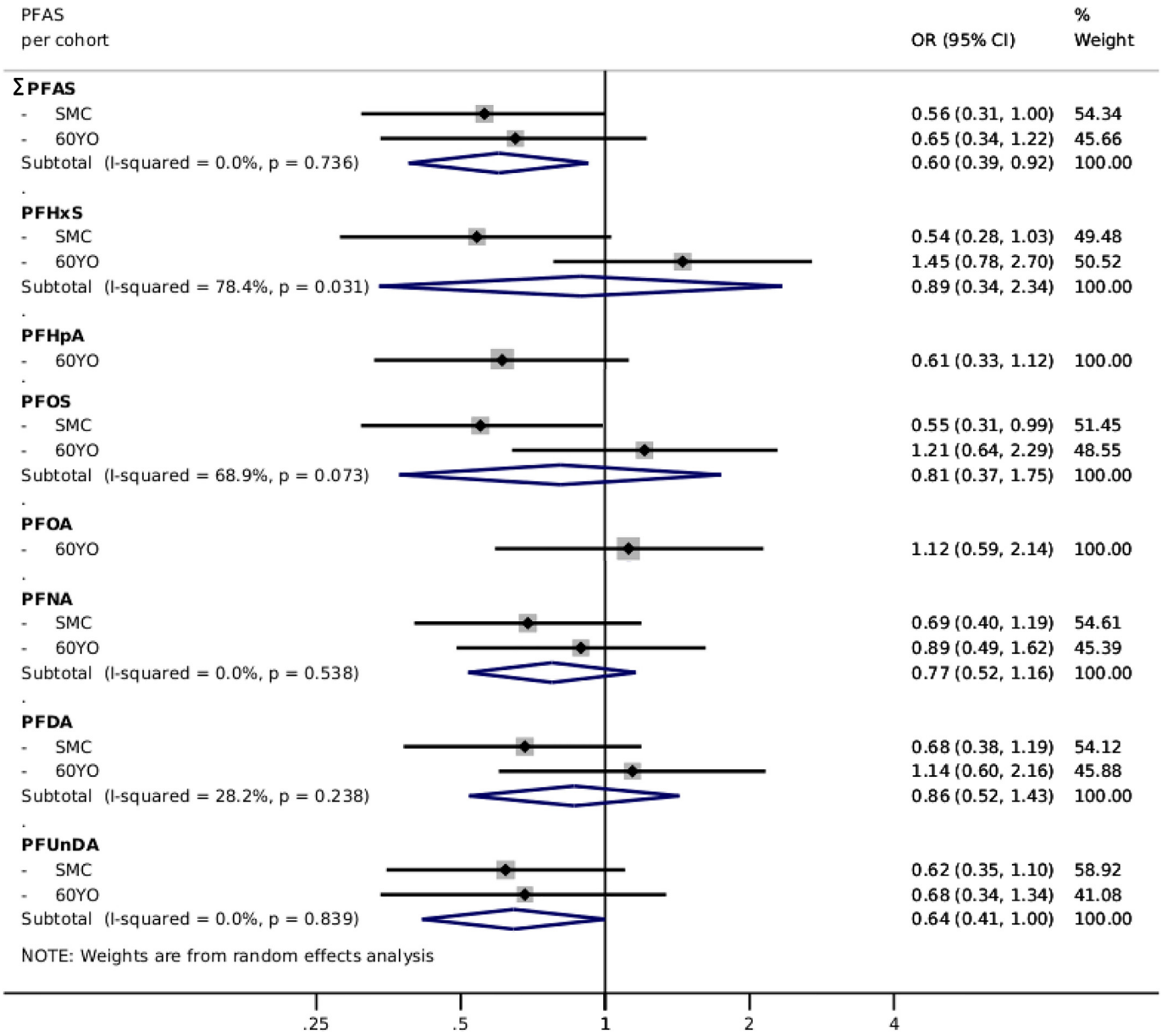

Figure 3. Multivariable-adjusted risk of myocardial infarction, presented as pooled ORs (95% CIs) from two Swedish cohorts (SMC-C: $n = 398$ and 60YO: $n = 422$) using random effects meta-analysis, comparing the third tertile of each PFAS with the first tertile—PFHpA and PFOA results are from the 60YO cohort alone. Estimations adjusted according to Model 2: sex, age, sampling date, education, BMI, diabetes, hypertension, family history of CVD, smoking habits, physical activity, and healthy diet score. Individual PFAS were standardized (rescaled with mean = 0 and SD = 1) and summed (ΣPFAS). Note: 60YO, Cohort of 60-year-olds; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid; SD, standard deviation; SMC-C, Swedish Mammography Cohort-Clinical cohort.

PFUnDA), which may be related to differences in potency (Bijland et al. 2011; Buhrke et al. 2013).

PFAS-related molecular mechanisms underlying altered lipid metabolism are not yet clarified. Proposed pathways include increased cholesterol absorption or synthesis, impaired mobilization, reduced reverse transportation (Fletcher et al. 2013), reduced turnover to bile acids (Behr et al. 2020), and sterol imbalance (Monroe and Dobs 2013). These processes may be impacted through PFAS-activation of transcription factors, such as PPARα (Bijland et al. 2011), constitutive androstane receptor (CAR) (Abe et al. 2017), Pregnane X Receptor (PXR) (Bijland et al. 2011), and endocrine receptor α (ERα) (Benninghoff et al. 2011). Evidence for these pathways mainly comes from *in vitro* or animal studies that have shown hypocholesteremia and reduced triglycerides upon PFAS exposure (Bijland et al. 2011). Apart from our observed lower triglyceride levels with increasing PFAS, an overall hypocholesteremia in animal data contrasts with the elevated cholesterol observed in many epidemiological studies, including this one. Discrepancies in doses or differences in physiology may explain interspecies differences (Bjork and Wallace 2009; Golforoush et al. 2020; Lau et al. 2007). Furthermore, diet may play a modifying role given that hypercholesteremia has been shown in animals maintained on a high-fat diet (Rebholz et al. 2016). In humans, obesity has been shown to modify the associations between PFAS and blood lipid levels (Jain and Ducatman 2019), and we also found stronger associations for LDL and apoB

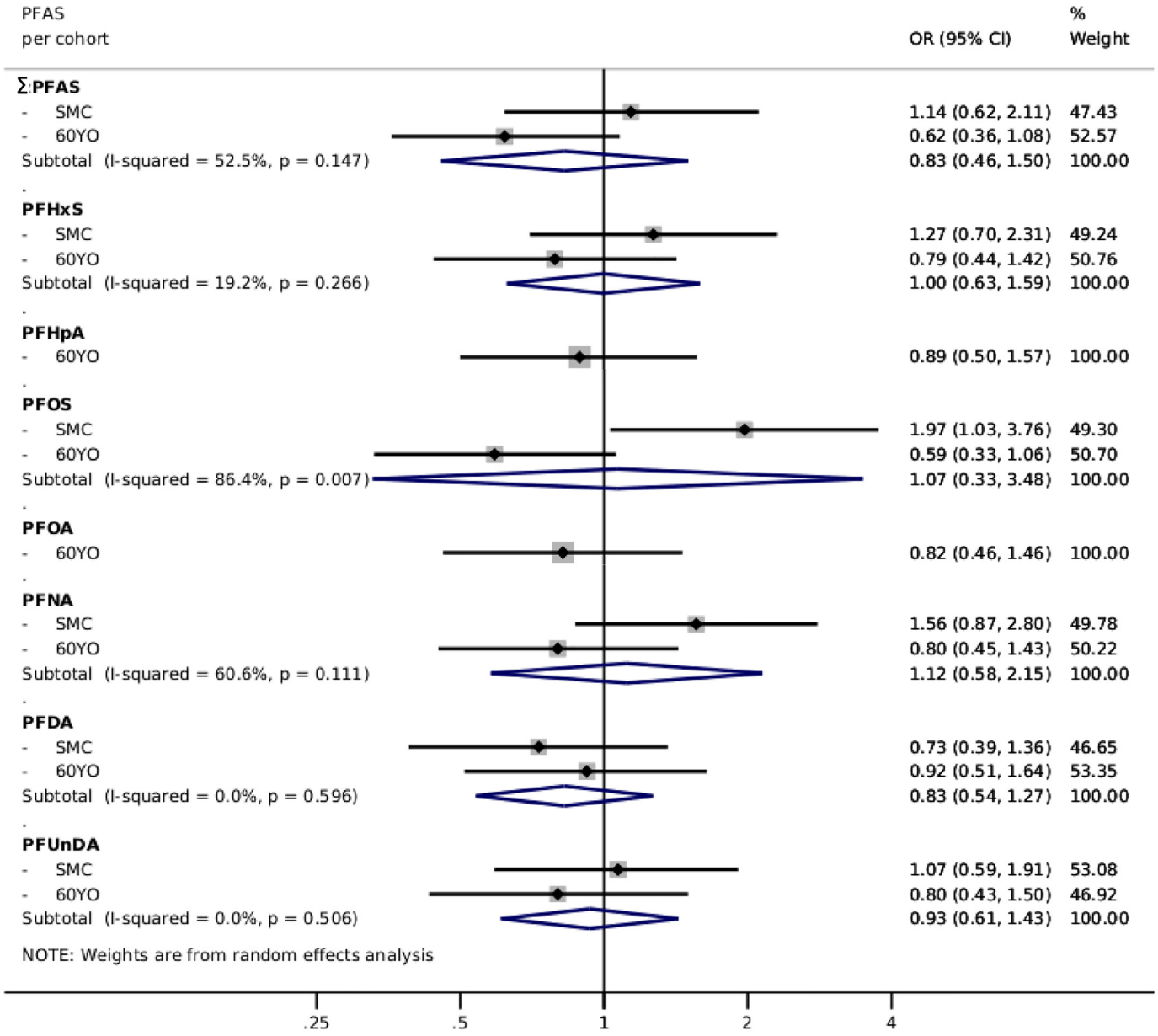

Figure 4. Multivariable-adjusted risk of stroke, presented as pooled ORs (95% CIs) from two Swedish cohorts (SMC-C: $n = 344$ and 60YO: $n = 364$) using random effects meta-analysis, comparing the third tertile of each PFAS with the first tertile—PFHpA and PFOA results are from the 60YO cohort alone. Estimations adjusted according to Model 2: sex, age, sampling date, education, BMI, diabetes, hypertension, family history of CVD, smoking habits, physical activity, and healthy diet score. Individual PFAS were standardized (rescaled with mean = 0 and SD = 1) and summed (ΣPFAS). Note: 60YO, Cohort of 60-year-olds; BMI, body mass index; CI, confidence interval; CVD, cardiovascular disease; OR, odds ratio; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid; SD, standard deviation; SMC-C, Swedish Mammography Cohort-Clinical cohort.

among overweight and obese participants. This suggests that obese individuals are more susceptible to alterations in lipid metabolism, potentially due to liver steatosis (Bagley et al. 2017; Jain and Ducatman 2019). However, cautious interpretation is recommended because stratification by baseline BMI could introduce (collider stratification) bias (Inoue et al. 2020).

Evidence for a causal relationship between LDL cholesterol and CVD is strong (Prospective Studies Collaboration et al. 2007; Cholesterol Treatment Trialists' Collaborators et al. 2012), particularly for myocardial infarction (Yusuf et al. 2020), mainly owing to the role of blood lipids in atherosclerosis (Geovanini and Libby 2018). In addition, HDL cholesterol and triglycerides may be relevant, although the evidence for causality is less conclusive (Emerging Risk Factors Collaboration et al. 2009; Farnier et al. 2021; Musunuru and Kathiresan 2016; Schwartz et al. 2012). Thus, the impact of PFAS on cholesterol levels may translate into increased CVD risk. However, this study showed null associations with an inverse tendency for PFAS and CVD. Our findings align with another study with retrospectively modeled PFOA and prospective coronary artery disease (retrospective self-reported diagnosis verified by medical records) performed on workers and residents in the Mid-Ohio Valley C8 cohort (Winquist and Steenland 2014), with a smaller prospective nested case–control study (Mattsson et al. 2015), and with a large cross-sectional study on self-reported diagnosis of stroke using data from the C8 cohort (Hutcheson et al. 2020). In contrast, a cross-

sectional study using the National Health and Nutrition Examination Surveys cohort based on self-reported diagnosis of five different CVD outcomes found that several PFAS exposures (among which were PFOS and PFNA) associated with increased CVD (Huang et al. 2018), another cross-sectional study found a positive association between PFOA and self-reported CVD (Shankar et al. 2012), and an ecological study in the Veneto Region in Italy found increased rate ratios for myocardial infarction (Mastrantonio et al. 2018).

The present study found a tendency for inverse associations of PFAS with CVD risk, which was unexpected because of the consistent evidence for an association with PFAS and elevated cholesterol, as reviewed by the European Food Safety Agency (EFSA CONTAM et al. 2018). It is possible that the LDL increase associated with PFAS exposure was not enough to increase the risk for CVD. On the other hand, we hypothesize that the PFAS-associated higher HDL and apoA1 as well as lower triglyceride levels may negate the detrimental effect of the PFAS-associated higher LDL levels on CVD risk. In addition, PFAS associations with apoB, a better marker for atherosclerosis risk than LDL (Sniderman et al. 2003), were weaker. However, inclusion of lipids (LDL or HDL and triglycerides) in the CVD analysis models did not change PFAS associations. Furthermore, diet or environmental factors can covary with PFAS and alter blood lipid levels, for example, fish is the main source of PFAS among regular consumers of fish (Bjorke-Monsen et al. 2020; Domingo and Nadal 2017; Vestergren et al. 2012) and fish consumption associates with higher HDL cholesterol levels in the blood (Alhassan et al. 2017) and thus could confound PFAS–HDL and PFAS–CVD relationships (Leung Yinko et al. 2014). However, associations remained similar after adjustment for diet. Another possible explanation for the slightly reduced CVD risk is the potential of PFAS to diminish the immune response (Grandjean et al. 2017; Salihovic et al. 2020), possibly through PPARα activation (DeWitt et al. 2009), given that inflammation aggravates atherosclerotic plaque formation and rupture (Geovanini and Libby 2018).

Limitations and Strengths

Our analyses were limited by the PFOA and PFHpA contamination of blood samples in the SMC-C, and we cannot exclude that small effects on CVD risk were missed owing to limited power or low PFAS levels. However, our sample size was relatively large and lower PFAS levels allow for drawing inference for the general population. The generalizability of the study is limited to seniors, this is, nevertheless, the most critical group for CVD. There may be potential residual or unmeasured confounding. Confounding by PFAS binding to blood lipoproteins is unlikely because PFAS bind mainly to albumin with little affinity for lipoproteins (Forsthuber et al. 2020). However, given the cross-sectional nature of our lipid analyses, we could not establish the temporal relation or make causal inferences for the associations between PFAS and lipid fractions, and there may be reverse causality for PFAS and cholesterol through shared excretion in the bile (EFSA CONTAM et al. 2018; Zhao et al. 2015). Last, PFAS-related elevated cholesterol may result in increased lipid-lowering medication use during follow-up, subsequently lowering the risk of CVD. However, cholesterol levels did not differ strongly between cases and controls at baseline. Important strengths are the prospective design; long follow-up for risk of CVD; and that PFAS concentrations may well reflect the long-term exposure, given that high intra-class correlation coefficients have been shown over a 10-y period (Donat-Vargas et al. 2019); measurement of several commonly occurring PFAS in blood plasma; as well as robust case selection from register linkages, which limit the possibility for reverse causality and exposure and outcome misclassification. In addition, elaborate questionnaires and visit information allowed for adjustment of many potentially important covariates.

Conclusions

We confirmed PFAS cross-sectional associations with elevated cholesterol, which has been indicated as one of the main adverse outcomes of exposure to PFOS and PFOA. However, this did not translate into increased CVD risk in our study.

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