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Plasma levels of per- and polyfluoroalkyl substances (PFAS) and cardiovascular disease – Results from two independent population-based cohorts and a meta-analysis

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ABSTRACT

Background: Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals that have been linked to increased cholesterol levels and thus may have a role in the development of cardiovascular disease (CVD).
Objectives: To investigate associations between PFAS exposure and incident CVD (a combined CVD end-point consisting of myocardial infarction, ischemic stroke, or heart failure) in two independent population-based cohorts in Sweden. In addition, we performed a meta-analysis also including results from previous studies.
Methods: In 2,278 subjects aged 45–75 years from the EpiHealth study, the risk of incident CVD in relation to relative plasma levels of perfluorohexanesulfonic acid (PFHxS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) was investigated. Associations between plasma levels of six PFAS and incident CVD were also examined in the PIVUS-study (n = 1,016, all aged 70 years). In addition, a meta-analysis was performed including three previous prospective studies, together with the results from the present study.
Results: There were no overall statistically significant associations between levels of the different PFAS and incident CVD, neither in EpiHealth nor in PIVUS. However, there was a significant sex interaction for PFOS in EpiHealth (p = 0.008), and an inverse association could be seen only in men (Men, HR: 0.68, 95 % CI: 0.52, 0.89) (Women, HR: 1.13, 95 % CI: 0.82, 1.55). A meta-analysis of five independent studies regarding PFOA and incident CVD showed a risk ratio (RR) of 0.80 (CI: 0.66, 0.94) when high levels were compared to low levels.
Conclusions: This longitudinal study using data from two population-based cohort studies in Sweden did not indicate any increased risk of incident CVD for moderately elevated PFAS levels. A meta-analysis of five independent cohort studies rather indicated a modest inverse association between PFOA levels and incident CVD, further supporting that increasing PFAS levels are not linked to an increased risk of CVD.

1. Introduction

Cardiovascular diseases (CVDs) are one of the main causes of morbidity and mortality globally, with 17.9 million people dying each year, and more than 80 % of CVD deaths are due to myocardial infarction, stroke and heart failure. CVDs can be prevented to a great extent if we live in adherence to lifestyle guidelines involving diet, exercise, and avoidance of smoking. Thus, the largest part of the etiology can be explained by non-genetic features. Although the aforementioned factors are the most well-known risks of developing CVDs, there is now growing evidence that exposure to environmental contaminants could play a role in risk of CVD development (Al-Kindi et al. 2020; Burroughs Pena and Rollins, 2017).

Per- and polyfluoroalkyl substances (PFAS) are man-made, very persistent chemicals produced in high volumes and used in a variety of applications worldwide. Due to their dirt- and water-repellent properties, PFAS are used in food packaging coatings, firefighting foams, cosmetics, textile impregnation, electroplating, medical utensils, cleaning

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supplies and personal care products (Glüge et al. 2020). Humans are primarily exposed to PFAS via diet, including migration from food packaging, cookware and drinking water, which has been shown to be contaminated with PFAS in many places worldwide. In a recent report by the Swedish Society for Nature Conservation it is stated that around 50 % of the population of Sweden is/has been exposed to PFAS-contaminated water (Conservation 2022). PFAS can be detected in almost all individuals, including pregnant women and children in different populations around the world (Fromme et al. 2009; Göckener et al. 2020; Kato et al. 2011; Liu et al. 2022; Manzano-Salgado et al. 2016; Mørck et al. 2015; van Beijsterveldt et al. 2022; Zhao et al. 2012), thus highlighting the importance of investigating potential health effects related to PFAS exposure.

In 2020, the European Commission asked EFSA to assemble a scientific evaluation on the human health risks of the presence of PFAS in food items. In the report, EFSA concludes that there is clear epidemiological evidence for an association between PFAS exposure and increased cholesterol levels in humans, but that there is insufficient evidence for a causal link of an increased risk of CVD (Chain EPanel oCitF et al., 2020). However, epidemiological data on PFAS exposure and CVD is rather limited and for the most part conducted solely on perfluorooctane sulfonic acid (PFOS) and/or perfluorooctanoic acid (PFOA) in studies of a cross-sectional nature.

The primary aim of the present study was to investigate incident CVD risk (a combined CVD end-point consisting of myocardial infarction, ischemic stroke, or heart failure) in relation to moderately elevated levels of PFAS in two population-based cohorts of middle-aged and elderly women and men (The Epihealth study and PIVUS study, Uppsala, Sweden). In the PIVUS study we also performed a supportive analysis of associations between levels of six PFAS and subclinical markers of CVD. Additionally, we aimed at performing a meta-analysis, including the results from the present study, together with data from previously published studies investigating associations between PFAS levels and incident CVD.

2. Material and methods

2.1. The ethical statement

This study was approved by the regional ethics review board at Uppsala University and all the participants provided their written informed consent upon participation.

2.2. Cohorts

Two independent population-based Swedish cohort studies were used in the present study. In both cohorts, individuals with a history of heart failure, stroke and myocardial infarction at baseline were excluded from the analyses. The Epidemiology for Health (EpiHealth) cohort, consisting of men and women (45–75 years old) from the general population of Sweden, was carried out, using the same protocol, in two Swedish cities, Uppsala and Malmö. In brief, participants were randomly selected (from 2011 to 2018) from the population registries of the two cities, and about 25,000 individuals took part (response rate 20 %). The present study included a random subset of 2,278 individuals from the Uppsala site in whom data has been collected on both plasma PFAS and incident CVD over nearly 10 years. More details on the cohort have been described previously (Lind et al. 2013).

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study is a longitudinal investigation performed in Uppsala, Sweden. At the age of 70, 1,016 subjects were investigated at baseline during the period 2001–2004 (50 % were women). The participants were invited to undergo follow-up examinations at age 75, (n = 822), and at 80 years (n = 603). During the first 5 years of the study, 52 individuals died and 142 withdrew. During the next 5 years, 106 individuals passed away and 113 subjects withdrew. Plasma PFAS analyses were performed at all three time points for all study participants. At all three time points traditional CVD risk factors were measured, and an echocardiogram and a carotid ultrasound were recorded. More detailed information on the study population can be found in Lind et al. 2005 (Lind et al. 2005).

2.3. Covariates

Across both cohorts, weight and length of the participants were measured by a research nurse at the laboratory and BMI was calculated as weight in kilograms divided by the square of body height in meters (kg/m^2).

In EpiHealth, all participants filled in a web-based questionnaire about medical and family history and symptoms as well as lifestyle factors, including diet. Participants also reported medication usage, leisure time, and physical activity in five levels from low (level 1) to strenuous physical activity (level 5). Finally, they also stated age, sex, alcohol intake (drinks per week), education length (up to 9 years, 10–12 years, or > 12 years), and smoked years in life.

In PIVUS, a questionnaire regarding socioeconomic status, medical history, physical activity, smoking habits and regular medication was filled out by all participants.

2.4. PFAS analyses

In Epihealth, blood was prepared into plasma, serum and whole blood (for later DNA extraction) and stored at −80 °C in a biobank facility for later PFAS analysis. PFAS levels could be detected in > 95 % of the study population. PFAS (perfluorohexane sulfonate (PFHxS), PFOA and PFOS) were analyzed in plasma by non-targeted metabolomics (Metabolon Inc, Morrisville, NC; UAS). After the analysis, the PFAS values were normalized and expressed in relative concentrations. Since both the targeted and the non-target approach to measure PFAS was applied in the PIVUS study at the age of 80 years (n = 601), we used these data to validate the non-targeted measurements in the EpiHealth study. As could be seen in the **supplemental Figs. 1–3**, there was an excellent agreement between the methods for PFHxS and PFOA (Spearman rho = 0.93 and 0.93), while for PFOS the agreement was less good (rho = 0.80). As could be seen in the Bland-Altman plots in **supplemental Figs. 1–3**, the differences between the two measurements increased with increasing mean levels of all three evaluated PFAS.

In the PIVUS study, blood serum and plasma were collected in the morning (8–10 am) after an overnight fast and then stored in freezers (−70 °C) until later analysis. The current study evaluated six PFAS for which > 75 % of the study population showed measurable levels above the lower limit of detection (LOD); PFHxS, PFOA, linear isomer of PFOS, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA). PFAS levels were analyzed by UPLC-MS/MS as previously described (Salihovic et al. 2013). The method detection limits (MDLs) for all three investigations ranged from 0.01 to 0.18 ng mL^{-1} depending on the analyte. PFAS values below LOD were replaced by LOD/$\sqrt{2}$.

2.5. CVD end-point

Data from the Swedish registers of mortality and in-hospital care were used to follow up incident cases of CVD in both the EpiHealth and PIVUS cohorts. In this study we used a combined CVD end-point consisting of myocardial infarction (ICD-10 code I21), ischemic stroke (I63), and heart failure (I50 and I11.0). Both fatal and non-fatal events were included in the combined end-point.

2.6. Subclinical markers of CVD

In the PIVUS study, a two-dimensional echocardiography examination was performed with an Acuson XP124 cardiac ultrasound unit

(Acuson, California, USA). The subclinical markers of CVD were assessed only at 80 years and below follows information on how each subclinical marker was assessed:

A 2.5 MHz transducer was used for the majority of the examinations. Left ventricle (LV) dimensions were measured with M−mode on-line from the parasternal projection, using a leading-edge-to-leading-edge convention. Measurements included left atrial diameter (LA) and left ventricular diameter in end-diastole (LVEDD). Left ventricular mass (LVM) was determined from the Penn conversion. LVM was then indexed for height$^{2.7}$ to obtain left ventricular mass index (LVMI).

Left ventricular (LV) volumes were calculated according to the Teichholz formula (7*D3/(2.4 + D), where D is the diameter of the LV in systole and diastole, respectively, and from that ejection fraction (EF) was calculated as: (LVEDV-LVESV)/LVEDV. In addition, the E/A ratio (EARATIO) was calculated as the ratio of the early to atrial inflow velocity in the left ventricle during diastole evaluated by pulsed Doppler. Finally, the isovolumetric relaxation time (IVRT) was obtained as the time between the closing of the aortic valve and the opening of the mitral valve using pulsed Doppler. Details on the myocardial ultrasound measurements has been published previously (Andren et al. 1996).

Carotid artery ultrasound was performed using a 10 MHz probe investigating both arteries and the common carotid artery distensibility (CCAdist) was evaluated. Intima-media thickness (IMT) was measured over a 10 mm distance proximal of the bifurcation in the far wall by a semi-automated software. In the same segment, the gray scale of the intima-media complex was determined (IMGSM). The mean value of the two arteries was used for both indices. Details on the carotid artery ultrasound measurements can be found in (Lind et al. 2007).

2.6.1. Relevance of the subclinical markers for the combined CVD outcome

The echocardiographic indices of systolic and diastolic function, as well as LVEDD and LA are mainly related to future heart failure. An increased LVMI is also a risk factor for future heart failure, but also makes the myocardium more susceptible to an ischemic insult. Distensibility of the CCA is a marker of stiff arteries being a hallmark of patients to develop a stroke. IMT and IM-GSM are two dimensions (thickness and lipid content) of the structure of the arteries, being of major importance for atherosclerosis formation.

2.7. Literature search for meta-analysis

Epidemiological articles published before December 1, 2022 were identified via PubMed and search components included were “PFAS,” “PFCs,””CVD,” “Cardiovascular disease,” “endocrine disrupting chemicals.” In addition, many individual PFAS chemicals were also included in the search, such as “PFOS,” “PFOA” etc. In total, seven publications were found that had reported data on associations between PFAS levels and CVD risk in humans. Studies were included in the meta-analysis of the present study when they met all of the following criteria: a) original full paper that presented unique data that could be used in the meta-analysis setting; b) exposure to PFAS; c) longitudinal/follow-up design (incident CVD).

2.8. Statistical analyses

STATA16.1 was used for the calculations (Stata inc., College Station, TX, USA). Due to the skewed distributions and in order to obtain all PFAS on the same scale, all PFAS were subjected to inverse rank normalization prior to analyses.

2.8.1. PFAS vs incident CVD

In both EpiHealth and PIVUS, Cox proportional hazard analysis was applied when relating PFAS levels to incident CVD (a combined CVD end-point consisting of myocardial infarction, ischemic stroke, or heart failure). The exposures, as well as the traditional risk factors were all on a continuous scale, except sex, diabetes and smoking. Two degrees of adjustments were performed. First, adjustment for age and sex was made. Second, additional adjustment for traditional CVD risk factors was done; systolic blood pressure, diabetes, smoking, low-density lipoprotein (LDL)-and high-density lipoprotein (HDL)-cholesterol, and BMI. All available PFAS were evaluated one by one in both of the studies. For Epihealth, we also performed another analysis where we did not include LDL- and HDL-cholesterol and BMI as confounders due to their previous reported links to PFAS (Dunder et al. 2022; Inoue et al. 2020) (see **Fig. S4** for the directed acyclic graph (DAG).

The only difference between the two studies regarding this analysis plan was that the baseline variables were measured just once in EpiHealth, while the same measurements of both exposures and confounders were measured on three occasions in PIVUS; ages 70, 75 and 80 years. Therefore, the follow-up period in PIVUS was split into three time periods and thereby data from all three examinations could be used in the analysis (Cox proportional hazard analysis with updated covariates). The proportional hazard assumption was checked by dividing the exposures by the median and inspected with Kaplan-Meier curves for proportional hazards, which was fulfilled in the analyses.

Since sex-dependent and non-monotonic responses have previously been reported for environmental contaminants, including PFAS, we also included an interaction term between PFAS and sex and a squared term for the different PFAS in each statistical model.

2.8.2. PFAS vs subclinical markers of CVD

The subclinical markers of CVD were inverse rank normalized before use. One linear regression model was carried out for each combination of a PFAS and a subclinical marker using data from individuals of 80 years of age in PIVUS only. The models were adjusted only for age and sex.

2.8.3. Meta-analysis

PFOA was the PFAS that was available in the largest number of studies, and it was therefore selected for the meta-analysis. The least adjusted estimate for the highest (quartile or quintile) vs lowest level of PFOA from each study was used in the inverse-variance weighted (IVW) random-effect meta-analysis. The data used from the EpiHealth and PIVUS studies were based on highest vs lowest quartile.

3. Results

Basic characteristics of the two independent samples are given in Table 1. In EpiHealth, after excluding 64 individuals with prevalent CVD at baseline, 2,278 persons were at risk during a median follow-up of 8.6 years (max 9.6 years, 18,852 person-years at risk (PYAR)), and the incident rate was 5.7/1000 PYAR. During that period 107 individuals suffered from CVD (a combined CVD end-point consisting of myocardial infarction, ischemic stroke, or heart failure). The estimated relative plasma PFAS concentrations (ng/mL) were the following: PFOS: 8.2; PFOA: 2.2; PFHxS: 5.5.

In PIVUS, after excluding 147 individuals with prevalent CVD (myocardial infarction, ischemic stroke, or heart failure)at baseline, 870 subjects were at risk during a median follow-up of 15.0 years (max 15.9, 10,666 PYAR), and the incident rate was 20.8/1000 PYAR. During that period 222 persons suffered from a CVD. Mean plasma levels of the six PFAS investigated at 80 years were the following: PFHxS: 7.5; PFOS: 9.4; PFOA: 2.8; PFNA: 1.1; PFDA: 0.40; PFUnDA: 0.42.

3.1. PFAS levels vs incident CVD in EpiHealth and PIVUS

No overall statistically significant associations could be seen between relative levels of the three analyzed PFAS and incident CVD in EpiHealth or between the six analyzed PFAS and incident CVD in PIVUS after full adjustment for age, sex, smoking, LDL-cholesterol, HDL-cholesterol, systolic blood pressure, BMI and diabetes in Cox proportional hazard analyses (Fig. 1) ($p > 0.05$). However, a significant multiplicative sex-

Table 1
Basic characteristics of the two independent samples.

	EpiHealth (n = 2,278)	PIVUS (n = 1,016) at age 70 years	PIVUS (n = 603) at age 80 years
Age	60.9 (8.4)	70.0 (0.2)	80.1 (0.2)
Female sex (%)	50.5	50.1	50.4
Systolic blood pressure (mmHg)	134.6 (17.2)	149.0 (22.1)	146.4 (19.2)
LDL-cholesterol (mmol/L)	3.9 (0.9)	3.5 (0.8)	3.4 (0.8)
HDL-cholesterol (mmol/L)	1.5 (0.4)	1.5 (0.4)	1.4 (0.4)
Diabetes (%)	9.4	8.1	11.2
Smoking	6.6 (8.8) years smoked	11 % current smokers	3.1 % current smokers
Body mass index (kg/m^2)	26.5 (3.8)	27.0 (4.3)	26.8 (4.3)
Left atrial diameter (mm)	NA	NA	42.2 (6.6)
Ejection fraction (%)	NA	NA	64.8
Left ventricular mass index (g/m$^{2.7}$)	NA	NA	45.1 (12)
Intima media thickness (mm)	NA	NA	0.95 (0.2)
Echogenicity of the carotid artery wall	NA	NA	59.8 (15.4)

Means and (SD) or proportion are given. NA = Not assessed, LDL = Low-density lipoprotein, HDL = High-density lipoprotein.

interaction could be observed for PFOS in EpiHealth (p = 0.008), with an inverse association being significant in men only after full adjustment (Men: HR: 0.68, 95 % CI: 0.52, 0.89, Women: HR: 1.13, 95 % CI: 0.82, 1.55) (Fig. 2).

Removal of LDL- and HDL-cholesterol and BMI as confounders in the models (based on the DAG presented in **Figure S4**) did not change the estimates, neither in EpiHealth nor in PIVUS, in any important direction compared when these variables were included in the models. In EpiHealth for example, the estimates remain almost identical to before (now HR: 0.91, 95 % CI: 0.75, 1.12 for PFHxS, HR: 0.85, 95 % CI: 0.70, 1.04 for PFOS and HR: 0.90, 95 % CI: 0.74, 1.09). Across both cohorts, the results did not reveal any significant multiplicative sex interactions or non-monotonic dose responses (quadratic term for the PFASs) in the associations between levels of the different PFAS and incident CVD (data not shown).

3.2. *PFAS exposure vs subclinical markers of CVD in PIVUS*

In a supportive analysis, levels of PFHxS, PFOA and PFOS were analyzed against nine different subclinical markers of CVD in individuals aged 80 years within the PIVUS study. When using linear regression models, the analysis revealed four significant associations with $p < 0.05$ (Fig. 3). Both PFHxS and PFOA were significantly inversely associated with IVRT, while only PFHxS was significantly associated with EF and IM-GSM (**Table S1**).

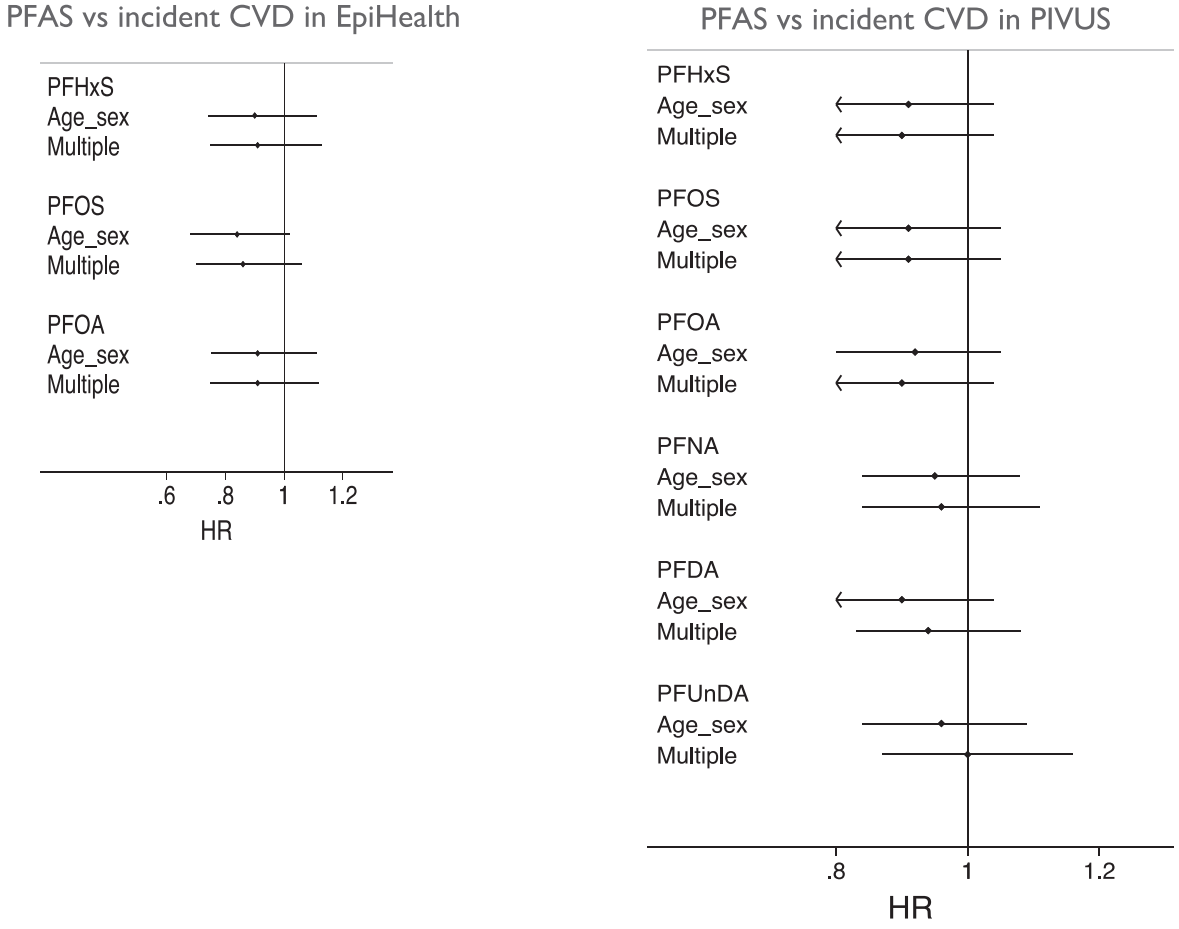

Fig. 1. Forest plot of associations between PFAS levels and incident cardiovascular disease (CVD) in the EpiHealth and PIVUS cohorts. Multiple represents multivariable adjustment for the following covariates: systolic blood pressure, diabetes, smoking, low-density lipoprotein (LDL)-and high-density lipoprotein (HDL)-cholesterol, and BMI. HR = Hazard ratio for a one SD change in PFAS, PFDA = perfluorodecanoic acid, PFNA = perfluorononanoic acid, PFHxS = perfluorohexanesulfonic acid, PFOA = perfluorooctanoic acid, PFOS = perfluorooctane sulfonic acid, PFUnDA = perfluoroundecanoic acid.

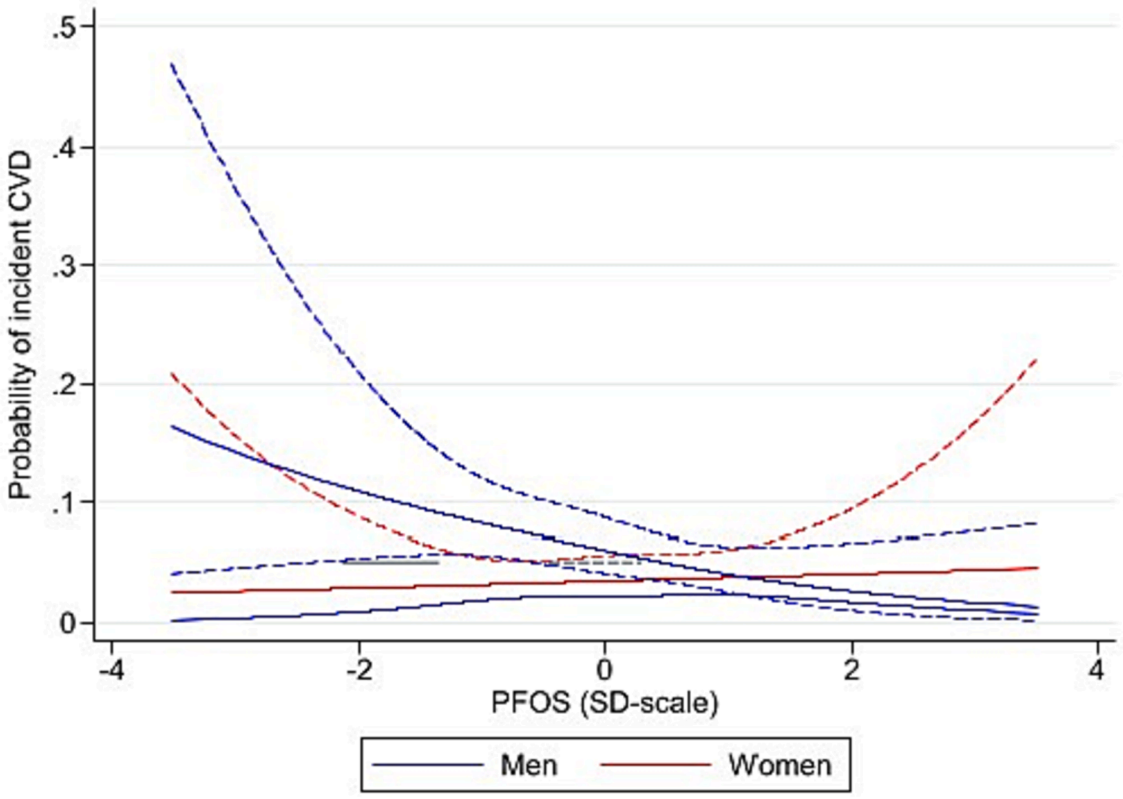

Fig. 2. The probability (and 95 % confidence interval) for incident CVD in EpiHealth was calculated for the distribution of perfluorooctane sulfonic acid (PFOS) on a standard deviation (SD) scale (n = 2,278). Analysis was performed separately on men and women due to the significant sex-interaction. The probability is given as a solid line, while the lower and upper 95 % CI are given as dashed lines. Results in men are given in blue and women in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Meta-analysis

In our PubMed search of previously published epidemiological studies investigating associations between PFAS levels and CVD, we found in total seven articles (**Table S2**). However, only four studies presented data on incident CVD, and one of those only presented data on the less commonly evaluated PFHpA. Therefore, we proceeded with the inverse-variance weighted random-effect meta-analysis including the three studies that all have data on PFOA measurements and also added data from PIVUS and EpiHealth.

As can be seen in Fig. 4, all five studies showed an estimate below one, and the overall estimate in the meta-analysis was 0.80 (CI: 0.66, 0.94, p = 0.023). The heterogenicity (I^2) between studies was 0 %.

4. Discussion

4.1. Main findings

Our two population-based longitudinal studies including more than 3,000 adults combined, did not reveal any overall significant associations between moderately elevated PFAS levels and incident CVD over 8–15 years of follow-up, neither in middle-aged individuals in the EpiHealth study nor in elderly subjects in the PIVUS study. However, we did observe a sex interaction for PFOS, and an inverse association could be seen between PFOS levels and incident CVD for men in EpiHealth. Furthermore, a meta-analysis of these two studies together with three already published studies displayed an inverse relationship between PFOA levels and incident CVD. Moreover, in a supportive analysis, weak cross-sectional associations could be seen between levels of PFHxS and PFOA and nine subclinical markers of CVD in elderly individuals among the PIVUS study.

4.2. Comparison with previous studies

The relationship between PFAS levels and dyslipidemia has been studied extensively, and the majority of data shows positive associations between PFAS and cholesterol levels (Batzella et al. 2022; Dunder et al. 2022; Ho et al. 2022; Li et al. 2020; Sakr et al. 2007). However, many PFAS have been related to elevated levels of both HDL- and LDL-cholesterol, but the net effect of such a dual increase in HDL and LDL on atherosclerotic diseases is not stated. Based on these findings, it is evident that additional studies on PFAS and CVD are needed. It is very common in cardiovascular research to use a combined end-point of myocardial infarction, stroke and heart failure as in the present study. This combined end-point definition has been used in both drug intervention trials as well as in epidemiology. At first sight, heart failure might not be seen as an atherosclerotic disease as myocardial infarction and ischemic stroke, but about half of all heart failure cases have been proceeded by a myocardial infarction and LDL-cholesterol is has been found to be a causal risk factor also for heart failure using Mendelian randomization analysis (Lind et al. 2021).

Much of the research on PFAS levels and CVD has been conducted in population studies in the USA in which self-reported outcomes were used, and the analyses have often been conducted in a cross-sectional fashion. In one study of NHANES data (1999–2014) including 10,859 individuals with self-reported cardiovascular events, total PFAS levels were positively associated with CVD (an increase of nearly 45 % in odds ratio was observed for the higher quartiles), independently of traditional cardiovascular risk factors. In addition, levels of some individual PFAS were positively related to individual CVD events such as congestive heart failure and coronary heart disease (Huang et al. 2018). Similarly, by examining 1,216 participants in NHANES cycles 1999 to 2003, Shankar et al. reported that levels of PFOA were positively related to

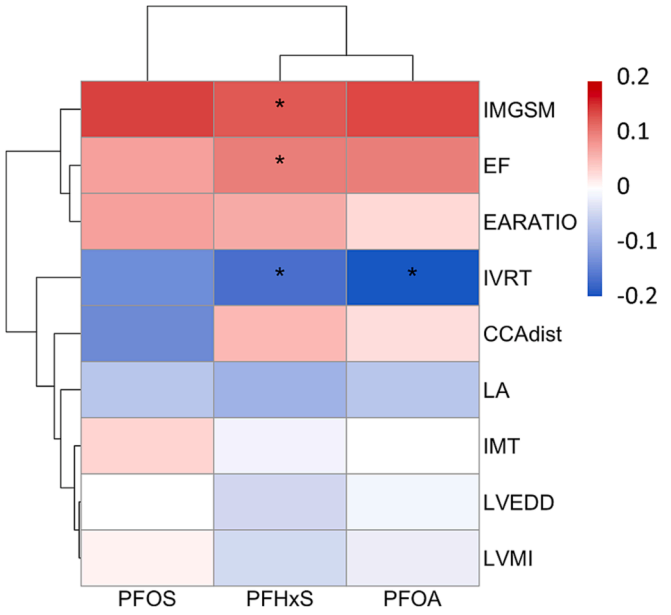

Fig. 3. Heat map of associations between perfluorooctane sulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanoic acid (PFOA), and subclinical markers of CVD in individuals of 80 years of age from the PIVUS cohort. Both subclinical markers of CVD and PFAS were evaluated on an SD scale. Coloring represents the strength of the beta values in a linear regression analysis. * denotes associations with $p < 0.05$. CCAdist = Common carotid artery distancibility, EARATIO = E/A-ratio, EF = ejection fraction, IMGSM = ecogenicity of the intima-media complex, IMT = intima-media thickness, IVRT = isovolumetric relaxation time, LA = left atrial diameter, LVEDD = left ventricular diameter in end diastole, LVMI = left ventricular mass index.

self-reported CVDs (coronary heart disease or stroke) and peripheral artery disease, after adjustment for multiple traditional risk factors. The risk for CVD was doubled and increased by 75 % for peripheral artery disease among individuals in the highest quartile, when compared to the lowest quartile (Shankar et al. 2012).

Although early cross-sectional studies could give an indication of associations, longitudinal outcome studies with incident cases are needed. In a literature search, we could find only 4 longitudinal studies that have investigated the relationship between PFAS exposure and CVD. Mattsson *et al.* investigated levels of eight PFAS and found a significant positive relationship only between PFHpA and incident coronary heart disease for the 3rd and 4th quartile compared to the lowest quartile. However, the authors conclude that this could be a chance finding, given that PFHpA has a similar chemical structure as the other PFAS included in the study (Mattsson et al. 2015). Another large study (N = 32,254) of a community living around a chemical plant did not find any evidence of an association between estimated (modeled) PFOA concentrations and stroke incidence (Simpson et al. 2013) or coronary artery disease (Winquist and Steenland, 2014). Finally, in a recently published nested-control study eight PFAS were measured in plasma and included incident myocardial infarction and stroke cases with matched controls from two Swedish cohorts. The results showed that PFAS, although related to increased cholesterol levels, were not associated with an increased risk of myocardial infarction, stroke, or their composite endpoint (Schillemans et al. 2022).

As the previously published data on incident CVD showed inverse, but not (statistically) significant, associations for PFOA, and we obtained similar non-significant results in PIVUS and EpiHealth, we decided to perform a meta-analysis of data from five independent studies. This meta-analysis clearly showed a negative association between PFOA and incident CVD. This is an interesting finding that potentially could represent a positive action of PFOA. It should however be borne in mind that some studies in the meta-analysis used measured levels while others used estimated levels where PFAS concentrations were modeled. Also, it is important to highlight that the outcome is not uniformly specified across the studies. In some cases, the outcome is a composite of 2 or more CVDs, while in other cases the outcome was stroke or coronary heart disease. Thus, based on these studies with different designs regarding both exposure of PFOA and the outcome CVD we do not want to convey the message that increasing PFOA levels could be beneficial for heart health, but rather that more studies in this field are warranted with harmonized CVD end-points and measurements of PFAS to see if this potential positive effect of PFOA could be replicated when more stringent criteria regarding both exposure and outcome are applied. In addition, data on other PFAS than PFOA are needed to obtain a picture on how PFAS as a chemical class could affect CVD. Therefore, our results do not conclude that the outcome CVD should be deleted from future risk assessment of PFAS, but rather that our finding in the meta-analysis is of interest and therefore well deserves future attention.

4.3. Sex-specific effects

Previous studies have indicated that there is a sex difference when it comes to elimination of PFAS, probably due to female reproductive biology (menstruation, pregnancy, lactation, and menopause), (Fletcher et al. 2013; Ingelido et al. 2010; Knox et al. 2011; Mogensen et al. 2015; Rickard et al. 2022) but also analyte differences in chain length or mode of action among PFAS themselves (Pizzurro et al. 2019). It has also been reported that PFAS could have an effect on sex hormones, (Joensen et al. 2012; Nian et al. 2020) even in children (Lopez-Espinosa et al. 2016). Therefore, we always investigate potential sex-specific effects in our studies. In the present study, we observed a sex-specific inverse association between PFOS and incident CVD in middle-aged men in the EpiHealth study, but not in the elderly individuals in the PIVUS study. We have previously reported sex-specific relationships in the Epihealth study regarding diabetes (Dunder et al. 2023) and in the PIVUS study regarding atherosclerosis (Lind et al. 2017) and diabetes (Dunder et al. 2023).

4.4. Subclinical markers of CVD

In a supportive analysis in the PIVUS study, we intended to investigate whether subclinical markers of CVD were related to PFAS levels in a fashion congruent with the findings regarding incident CVD. If so, the longitudinal findings would be strengthened. The findings were in agreement, but only weak relationships could be seen between subclinical markers of CVD and PFAS. We observed no indications that PFAS levels should be linked to poor cardiovascular performance in the elderly. To our knowledge there are not many previously published studies on the association between PFAS exposure and subclinical markers of CVD. In PIVUS we have previously reported cross-sectional relationships between PFAS exposure and left ventricular geometry. Inverse relationships were found between PFNA, PFDA and PFUnDA, and relative wall thickness of the heart. In addition, elevated levels of PFNA were associated with increased LVEDD (β: 1.01 (95 % CI: 0.43, 1.58, $p < 0.001$) (Mobacke et al. 2018). Further, in longitudinal analyses of PIVUS data we have seen that the change in plasma levels of 6 of 8 PFAS over 10 years are related to increase in IMT, which indicates that PFAS might interfere with the atherosclerotic process (Lind et al. 2018). Another population-based cross sectional study of Taiwanese adolescents and young adults has previously reported that carotid intimia-media thickness (CIMT) increased across increasing quartiles of PFOS (0.434 mm, 0.446 mm, 0.458 mm, 0.451 mm; P for trend < 0.001), while a negative association could be seen between PFUnDA and CIMT (Lin et al. 2013).

4.5. Strengths and limitations

The strengths of the present study include a long follow-up time in a

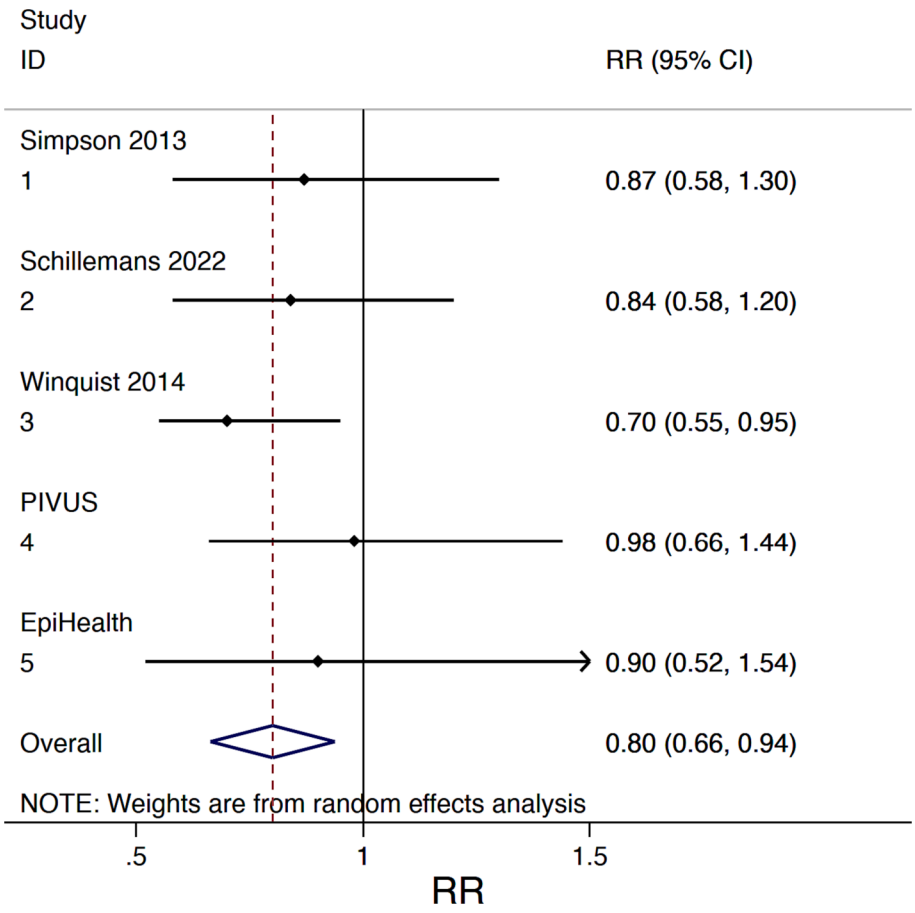

Fig. 4. Meta-analysis with overall risk ratio (RR) of five independent studies investigating PFOA levels and incident CVD.

fairly large sample from two different population-based cohort studies with measurements of several PFAS and incident cases of CVD. We were also able to present a supportive analysis of subclinical markers of CVD in PIVUS.

One limitation of the study is that we only had a relative quantification of PFAS levels in EpiHealth, while in PIVUS we had absolute PFAS concentrations. Nonetheless, a relative quantification does not influence the associations, although it does limit our ability to directly compare the level of PFAS exposures in EpiHealth with PIVUS and other studies. When the non-targeted measurements were compared with targeted measurements for the three PFAS evaluated in both study samples (Figs. S1-S3), there was an excellent agreement between the methods for PFHxS and PFOA (Spearman rho = 0.93 and 0.93), while for PFOS the agreement was less good (rho = 0.80). However, the results for PFOS in EpiHealth was very similar to the other two PFAS and also very similar to the results for PFOS in PIVUS (being evaluated with a targeted approach. Thus, it is therefore less likely that the less good agreement seen for PFOS compared to the other PFAS would have introduced any major bias in the present study. Lastly, both cohorts include elderly and middle-aged individuals residing exclusively in Sweden, a fact that limits the generalizability to other age and ethnic groups.

4.6. Conclusions

In conclusion, we did not observe any overall significant associations between PFAS exposure and incident CVD (a combined CVD end-point consisting of myocardial infarction, ischemic stroke, or heart failure) in the two population-based cohort studies. However, we did observe a significant sex interaction, and an inverse association was seen between PFOS levels and incident CVD in middle-aged men in the EpiHealth study. The meta-analysis of five independent cohort studies showed an overall moderate inverse association between PFOA levels and incident CVD, further supporting that increasing PFAS levels are not linked to an increased risk of CVD.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108250.

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