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Lipoprotein profiles associated with exposure to poly- and perfluoroalkyl substances (PFASs) in the EuroMix human biomonitoring study☆

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ABSTRACT

Exposure to per- and polyfluoroalkyl substances (PFASs) is associated with increased blood cholesterol. Although elevated cholesterol is a well-established risk factor for cardiovascular diseases (CVD), it is not clear whether PFASs affect this risk. Lipoprotein subclasses are emerging biomarkers for disease risk and lipoprotein profiling may provide an insight to physiological implications of PFAS exposure. We explored the association between serum PFAS concentrations and lipoprotein subclasses in a cross-sectional study. We determined the concentrations and lipid composition of the major subclasses of lipoproteins in plasma samples from 127 adult participants of the EuroMix human biomonitoring study by nuclear magnetic resonance (NMR). Serum concentrations of 17 PFASs showed a detection frequency between 30 and 100% and were included in further analyses. We examined the associations between PFAS concentrations and lipoprotein subclasses by linear mixed-effect regression models, adjusted for confounders. In the adjusted models, positive associations were found between several PFASs and cholesterol concentrations in large to medium sized HDL and medium sized LDL particles. We found a 4–12% increase in HDL cholesterol per interquartile range (IQR) increase for several PFASs. In women the associations with PFNA, PFUnDA, PFDoDA and PFOS were significant after adjustment for multiple comparisons. Similar magnitude of change was observed between longer chained PFASs and LDL cholesterol, and a few of these associations reached significance for cholesterol in large to medium LDL particle sizes in women. No significant associations with plasma triglycerides were observed. However, most PFASs tended to be associated with reduction in VLDL (very low-density lipoproteins) particle number and VLDL triglyceride. Findings from this exploratory study, suggest that background PFAS exposures influence particle size distributions and lipid composition of plasma lipoprotein subclasses, and that these effects may be more prominent in women. A two-points lipoprofiling for all subjects indicated both low intra-individual variability and good analytical reproducibility.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are highly persistent in the environment due the extreme stability of the carbon-fluoride bond. Widespread human exposure occurs through contaminated food and drinking water resulting in measurable concentrations in blood and other tissues among general populations worldwide. According to recent reviews, associations between several PFAS exposures and increased plasma total cholesterol concentration is a consistent finding (ATSDR, 2021; EFSA, 2018, 2020).

In experimental animals, disturbances in lipid metabolism is observed and has been attributed largely to PFAS activation of hepatic peroxisome proliferator-activated receptor alpha (PPARα) (Shipley et al., 2004), but other liver nuclear receptors might also be involved (Behr et al., 2020; Rakhshandehroo et al., 2010; Rosen et al., 2008). Most experimental studies in rodents have used high doses of PFASs and

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have reported reduced concentrations of serum cholesterol and triglycerides. However, some recent studies show the inverse effect in mice on a high fat or westernized diet (Rebholz et al., 2016; Schlezinger et al., 2020) suggesting an interaction with dietary factors. Of note, there are significant species difference affecting both lipid metabolism and PFAS toxicokinetics (Fragki et al., 2021) that complicates translation of rodent data to the human physiology.

In epidemiological studies of non-occupationally PFAS exposed populations, the evidence varies by lipid parameter. Most cross-sectional studies reported associations between PFASs and increases in total plasma cholesterol in the general populations (Dong et al., 2019; Eriksen et al., 2013; Fisher et al., 2013; Geiger et al., 2014; Graber et al., 2019; Jain and Ducatman, 2019; Lin et al., 2019; Liu et al., 2018; Mora et al., 2018; Nelson et al., 2010; Skuladottir et al., 2015; Spratlen et al., 2020; Zeng et al., 2015), as well as in highly exposed populations (Canova et al., 2020; Dalla Zuanna et al., 2021; Frisbee et al., 2010; Li et al., 2020; Starling et al., 2014; Steenland et al., 2009). Most longitudinal studies are in agreement (Fitz-Simon et al., 2013; Lin et al., 2019; Maisonet et al., 2015; Winquist and Steenland, 2014). Nevertheless, there is still inconsistency in the findings with a few reports of inverse and null results from longitudinal (Donat-Vargas et al., 2019; Manzano-Salgado et al., 2017; Mora et al., 2018; Tian et al., 2021; Winquist and Steenland, 2014) and cross-sectional (Chateau-Degat et al., 2010; Dalla Zuanna et al., 2021; Nelson et al., 2010) studies covering both occupational and non-occupational exposures.

Elevated low-density lipoprotein cholesterol (LDL-C), the main component of total cholesterol in humans, elevated circulating concentration of triglycerides, and a lower concentration of high-density lipoprotein cholesterol (HDL-C) are thought to play central roles in atherogenesis and are associated with increased risk of cardiovascular disease (CVD) (Emerging Risk Factors Collab, 2009). The epidemiological evidence of a positive association between PFAS exposure and LDL-C is largely in agreement with the consistent findings for TC (EFSA, 2020), while for HDL-C data are less consistent. The controversy increases when exploring the association between total serum triglyceride and PFAS concentrations, with several positive (Canova et al., 2020; Geiger et al., 2014; Lin et al., 2020; Lin et al., 2019; Manzano-Salgado et al., 2017; Spratlen et al., 2020; Steenland et al., 2009; Zeng et al., 2015), and as many inverse (Chateau-Degat et al., 2010; Donat-Vargas et al., 2019; Li et al., 2020; Liu et al., 2018; Mora et al., 2018; Spratlen et al., 2020; Tian et al., 2021) and null associations (Fisher et al., 2013; Fitz-Simon et al., 2013; Frisbee et al., 2010; Jain and Ducatman, 2019; Maisonet et al., 2015; Mora et al., 2018; Starling et al., 2014).

Recent development in high throughput technology, using NMR lipidomics enables the quantification of serum and plasma lipoprotein subclasses that are the carriers of circulating cholesterol and triglycerides (Dona et al., 2014; Jiménez et al., 2018; Monsonis Centelles et al., 2017; Soininen et al., 2015). Currently there are several groups active in subclasses lipoprofiling based on a few commercial NMR solutions (Garcia et al., 2015; Jiménez et al., 2018; Llauradó et al., 2019; Soininen et al., 2015; Tzoulaki et al., 2019) It has become evident that lipoprotein subclass characterisation can provide more significant information on CVD risk than measurement of traditional lipid parameters alone (Joshi et al., 2020; Pappa et al., 2020; Santos et al., 2020; Tzoulaki et al., 2019). Several profiles of lipoprotein subfractions have emerged as non-atherogenic, as for example a profile of low triglyceride content in VLDL particles and high triglyceride content in LDL particles, and an increase in the size of LDL particles resulting in less small-LDL particles (Barona and Fernandez, 2012; McCullough et al., 2011). Diet, age, sex, medication and clinical conditions are considered main factors contributing to variations in lipoprotein subfractions, while this is still an area of active investigation (Amigo et al., 2020; Barona and Fernandez, 2012; Garcia et al., 2015; McCullough et al., 2011; Santos et al., 2020). The association between PFAS exposure and the specific lipoprotein subfractions changes that could influence the CVD risk are largely unknown.

In the present cross-sectional study, we explored the association between serum PFAS concentrations and plasma levels of lipoprotein particle subclasses, as measured by NMR in a well characterised adult human biomonitoring study cohort in Norway.

2. Methods

2.1. Study population and blood sample collection

The human biomonitoring study is part of the EU funded project EuroMix "European Test and Risk Assessment Strategies for Mixtures", as described in detail by Husøy and co-workers (Husoy et al., 2019). Established at the Norwegian Institute of Public Health (NIPH), the study is characterizing exposure to environmental chemicals from various sources during two full days, with a mean interval of 18 days (range: 9–36 days). Participants were recruited among employees from governmental institutes and authorities, and universities in the counties of Oslo and Akershus in Norway between September 2016 and November 2017. The study recruited 144 participants (44 males aged 25–72 years and 100 females aged 24–72 years) who completed day one, and 140 (43 males and 97 females) of these participants completed both full 2-day collections. The participant's reported their age, weight, height, education and smoking status in questionnaires, as well as their consumption of foods and drinks in a semi-quantitative food frequency questionnaire (FFQ). The participants reported no sickness during the week before the first study day, to avoid any effects of recent illness. Our study population includes the 127 participants of the EuroMix study, with complete information (Supplementary Table S1). For all 127 participants, both Day 1 and Day 2 plasma samples were analysed by NMR. The study was approved by the Regional Committees for Medical and Health Research Ethics (REK ID no 2015/1868) and all the participants provided their written informed consent.

A blood sample of 70 mL per participant per study day was collected in the morning at the end of each 24 h registration period, when the participants came to deliver the urine samples. All samples were immediately handled and aliquoted into smaller quantities and stored at −80 °C to avoid unnecessary sample handling, and repeated thawing and freezing.

2.2. Analysis of poly- and perfluoroalkyl substances

Twenty five PFASs were analysed in this study comprised of five perfluoroalkyl sulfonates: perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluoroheptanesulfonate (PFHpS), perfluorooctanesulfonate (PFOS), perfluorodecanesulfonate (PFDS) and three perfluoroalkyl sulfonamides (FOSAs): perfluorooctanesulfonamide (PFOSA), N-methyl perfluorooctanesulfonamide (MeFOSA), and N-ethyl perfluorooctanesulfonamide (EtFOSA), as well as ten perfluoroalkyl carboxylates: perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluoro octanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA), perfluorotetradecanoate (PFTeDA) and four polyfluoroalkyl phosphate esters (PAPs): 6:2 polyfluoroalkyl phosphate monoester (6:2PAP), 8:2 polyfluoroalkyl phosphate monoester (8:2PAP), 6:2 polyfluoroalkyl phosphate diester (6:2diPAP), 8:2 polyfluoroalkyl phosphate diester (8:2diPAP), as well as three perfluoroalkyl phosphonates (PFPAs): perfluorohexylphosphonate (PFHxPA), perfluorooctylphosphonate (PFOPA), perfluorodecylpho sphonate (PFDPA) (Supplementary Table S2). More details in the analytical methods are given by Poothong et al., (2017). In brief, 50 μL of serum was added to a 2 mL centrifuge tube, and then 90 μL of a 5 ng/mL internal standard solution and 90 μL of methanol were added. To precipitate the proteins, the sample tubes were mixed on a whirl mixer and centrifuged for 40 min at 14,000 rpm at 20 °C. The supernatant was transferred to a 250 μL polypropylene vial, and then 80 μL of the sample

was injected in an online-SPE-UHPLC-MS/MS system as previously described (Poothong et al., 2017). PFAS measurements were only performed on serum samples collected at Day 1 since the biological half-lives of the most abundant PFASs in humans is so long that no significant changes were expected on Day 2. The EuroMix cohort has no specific occupational or environmental exposure sources and thus represents a population with a "background" exposure level dominated by dietary sources.

2.3. NMR-determined lipids and lipoprotein main fractions and subfractions

Volumes of 1.5 mL of blood plasma have been aliquoted immediately after blood centrifugation and stored at −80 °C until further analysis as previously described (Husoy et al., 2019). The cryovials have been kept at −80 °C until 30–60 min before NMR analysis. For series of several samples, two cryovials have been moved to room temperature every hour and allowed to defreeze for about 20–30 min. A mixture of 400 μL plasma and 400 μL of 5 mM sodium 3-(trimethylsilyl)-[2,2,3,3-d_4]-1-propionate (TSP) in $Na_2HPO_4/NaN_3/D_2O$ buffer (Bruker Biospin) was gently homogenized in a blood rotator (VWR). 600 μL of the homogenized solution have been transferred into a 5 mm Wilmad 507 NMR tube and loaded into the NMR sample changer. The operator's performance and the NMR tubes quality was assessed as we previously described (Stavarache et al., 2022). The NMR experiments have been performed with an Avance III HD 600 NMR spectrometer (Bruker Biospin, Rheinstetten) operating at 600.12 MHz for ^{1}H nuclei, equipped with a 5 mm inverse detection (BBI) probe with z-axis gradients. Five types of NMR experiments have been recorded at 300.0 K (±0.05 K) with pulse sequences and parameters as delivered with Bruker Biospin IVDr methods V.1.0 (Monsonis Centelles et al., 2017), i.e. ^{1}H NMR spectrum with an ERETIC type of signal as quantitation reference, J-Resolved 2D spectrum for signal assignments, CPMG spectrum for suppression of lipids, 1D Diffusion filtered spectrum for suppression of low molecular weight metabolites, and ^{1}H Gradient profile for quality control of the sample preparation (sample homogeneity, shim, and tube quality). Before each series of plasma samples, a set of three Bruker QC standard samples have been recorded in order to assess (and when necessary to correct) the compliance with temperature stability, water suppression quality, and ERETIC quantitation accuracy. The IVDr raw NMR data have been processed with the commercial Bruker Biospin B.I.LISA methods and models (Jiménez et al., 2018; Monsonis Centelles et al., 2017). The full set of 114 lipoprotein parameters included Total Triglycerides, Total Cholesterol, Total Apo-A1, Total Apo-A2, Total Apo-B100, ApoB100/ApoA1, Total Particle number, LDL/HDL-cholesterol ratio, HDL-Apo-A1 (and subfractions 1–4), HDL-Apo-A2 (and subfractions 1–4), HDL-Cholesterol (and subfractions 1–4), HDL-Free cholesterol (and subfractions 1–4), HDL-Phospholipids (and subfractions 1–4), HDL-Triglycerides (and subfractions 1–4), LDL-Apo-B100 (and subfractions 1–6), LDL-Cholesterol (and subfractions 1–6), LDL-Free cholesterol (and subfractions 1–6), LDL-Phospholipids (and subfractions 1–6), LDL-Particle number (and subfractions 1–6), LDL-Triglycerides (and subfractions 1–6), IDL (intermediate density lipoproteins)-Apo-B100, IDL-Cholesterol, IDL-Free cholesterol, IDL-Phospholipids, IDL-Particle number, IDL-Triglycerides, VLDL-Apo-B100 (and subfractions 1–5), VLDL-Cholesterol (and subfractions 1–5), VLDL-Free cholesterol (and subfractions 1–5), VLDL-Phospholipids (and subfractions 1–5), VLDL-Particle number (and subfractions 1–5), VLDL-Triglycerides (and subfractions 1–5) (Jiménez et al., 2018). All the analysed lipids and lipoproteins main fractions and subfractions are presented in Supplementary Table S3.

2.4. Statistical analysis

PFASs concentrations in serum were described and compounds with detection frequencies (DF) < 30% were not included in further statistical analysis (n = 8 PFASs). For the compounds detected in more than 30% of the samples, those with 30% ≤ DF < 60% were used as binary variables ("detected/not detected", n = 4 PFASs). For compounds with 60% ≤ DF < 100%, values below the level of detection (LOD) were imputed using singly imputed values by a quantile regression approach (n = 5 PFASs). Since all lipid parameters were repeatedly measured in blood samples collected within a 9–36 day interval (Day 1 and Day 2) we calculated the intraclass correlation (ICC) coefficients, as an estimate of inter-individual variation of the lipid parameters. The small intra-individual variability for the two-points comparison indicated no significant lifestyle or health status changes and also confirmed the good quality of samples and reproducibility of the NMR analytical method (Supplementary, Figure S1).

We used linear mixed-effect regression models, adjusted for confounders, to investigate the association between serum PFAS concentrations and plasma lipid profile, with random intercept by participant id, by using the repeated measurements of the plasma lipids at Day 1 and Day 2. All models were adjusted for participant's age (years), sex (men/women), body mass index (BMI, in kg/m^2) and smoking status (never smoke; current smoker; occasional smoker; quit smoking ≤5 years ago; quit smoking >5 & ≤10 years ago; quit smoking >10 years ago). Given that 97% of our study participants had high education, we did not adjust our models for educational status, due to lack of variability.

All PFASs, except those that were categorical (below or above LOD), and all lipid parameters were log transformed (base 10) to reach normal distribution. We estimated the percentage (%) change in each lipid parameter per interquartile range (IQR) increase in each of the PFAS congeners by the following formula: ((IQR^beta)-1)*100, where beta is the beta coefficient of each model (Barrera-Gomez and Basagana, 2015). For the models of PFAS that were included in the analysis dichotomized, beta coefficients (and 95%CIs) were exponentiated (base 10) to produce the ratio of the geometric mean (GM) of lipid concentrations for values above LOD in respect to the GM of values bellow LOD.

In the adjusted model, p-values <0.05 were considered significant (significance level α = 0.05). Because multiple testing can be an issue, we have also reported the corrected p-value (q-value) of the estimated associations after correction for multiple comparisons with a false discovery rate controlled at <10% (Benjamini and Yekutieli, 2005).

Further, we examined the interaction with i) sex, by including an interaction term between PFAS and sex. Given the strong interactions by sex, all the final models include the interaction term between PFAS and sex.

We examined the association of exposure to PFAS mixture with the lipid parameters by Bayesian Kernel Machine Regression (BKMR) models. All our models were adjusted for the same set of confounders as the linear mixed models. BKMR is a non-parametric flexible modelling approach that can accommodate for correlation, non-linearity and interaction effects when estimating the exposure (PFAS mixture)-response associations (Bobb et al., 2015).

The main models are described as:

$$Y_i = h[\mathrm{PFOA}, \mathrm{PFNA}, \mathrm{PFDA}, \mathrm{PFUnDA}, \mathrm{PFDoDA}, \mathrm{PFTrDA}, \mathrm{PFBS}, \mathrm{PFHxS}, \mathrm{PFHpS}, \mathrm{PFOS}, \mathrm{PFDS}] + \beta z_i + e_i,$$

where Y_i is each of the lipid parameters for each participant i, h[] is the high-dimensional exposure-response function which can incorporate both non-linear relationships and interactions among exposures and is estimated using a Gaussian kernel machine representation. Further, z_i is a vector of covariates and β their associated regression coefficients and $e_i \sim N(0,\sigma 2)$. PFAS were log-transformed for the BKMR analyses and BKMR was fitted using the Markov chain Monte Carlo algorithm with 10,000 iterations. Once fitted, BKMR provides a posterior inclusion probability (PIP) for each of the exposures, which constitutes a measure of the relative importance of each exposure within the *h* function to the overall mixture effects. Note that PIPs are not constrained to sum to 1.

Credible intervals obtained from the BKMR model incorporated the additional uncertainty due to estimation of a high-dimension set of exposures and accounting for multiple-testing penalty. We further estimated the overall joint effect of exposure to the PFAS mixture by providing an estimate of the change in the outcome when the PFAS mixture exposure is increasing up to 95th percentile, compared to holding all PFAS at their 25th percentile (reference level). We also explored the sex differences by stratification in the BKMR analyses.

Statistical analyses were performed using Stata statistical software (release 14; Stata Corporation) and R (version 3.2.2; R Development Core Team).

3. Results

3.1. Sex differences in PFAS concentrations and lipoprotein particle profile

In our study population, men had higher median weight, height, and BMI than women, and half of the men were defined as overweight (Supplementary Table S1). Our study population (n = 127) did not differ with regard to age, weight, height, as well as sex, education level, BMI status and smoking status distribution, compared to the total EuroMix population (n = 144) (Supplementary Table S1).

Out of the 25 PFASs measured, eight PFAS were detected in less than 30% of the samples and were excluded from further data analyses; namely EtFOSA, MeFOSA, PFPeA, PFHxA, 6:2 PAP, 8:2 PAP, PFOPA, PFDPA (Table 1 and Supplementary Table S2). Four PFASs, PFHpA, PFTeDA, 8:2 diPAP and PFHxPA, were detected in more than 30% but less than 60% of the samples. For several PFASs, men had higher serum concentrations than women, and this difference was statistically significant for PFHxS, PFHpS, PFOS, PFOA and PFNA (Table 1 and Supplementary Table S2).

Total triglyceride and TC concentrations were higher in men than in women ($p < 0.001$ and $p < 0.01$, respectively), and men had higher LDL, IDL and VLDL particle numbers, $p < 0.001$ (Table 2 & Supplementary Table S3). In contrast, HDL-C and HDL phospholipid levels were higher in women than in men, $p < 0.001$. Even though men had higher levels of LDL-particles and LDL-C, these differences appeared only for the medium to small LDL-particles (Supplementary Table S3). Overall, we observed high ICCs (ICC≥0.80) for all lipid parameters measured twice within a ~2-week interval, while relatively lower ICCs (0.50 ≤ ICC≤0.60) were found for VLDL5 parameters (Table 2 & Supplementary Table S3).

3.2. PFAS concentrations and lipid profiles in main lipoprotein particle classes

By applying linear mixed-effect regressions models, we found a general tendency of most PFCAs to be associated with increased HDL and LDL cholesterol and decreased VLDL particle number and VLDL triglyceride (Fig. 1A). More specifically, for each IQR increase in all PFCAs concentrations, except PFOA, we found a 5–11% increase in HDL-C, with significant increases associated with PFNA and PFDoDA. Similar positive associations were found for HDL free cholesterol (9–19% increase) and HDL phospholipids (5–9% increase) (Supplementary Table S4). The associations between PFAS concentrations and LDL-cholesterol did not reach statistical significance (Fig. 1B).

Regarding triglycerides, PFOA had the strongest inverse association with total triglycerides and per IQR increase in PFOA, total triglycerides and VLDL triglycerides were reduced by 23% (95%CI = −38, −4) and 27% (95%CI = −47,2), respectively (Fig. 1A & Supplementary Table S4). However, none of the observed associations in the overall sample analysis were significant after adjusting for multiple comparisons.

When we examined the associations by sex, significant results were observed in women and several of these associations persisted after adjustment for multiple comparisons (q-value≤0.10). More specifically, in women we found 12% (95%CI = 5,19), 8% (95%CI = 3,14), 12%

Table 1
Poly- and perfluoroalkyl substances (PFASs) concentrations (ng/mL) in serum from Norwegian men and women (n = 127).

	Men (n = 38)				Women (n = 89)			
	DF (%)	Median	25th percentile	75th percentile	DF (%)	Median	25th percentile	75th percentile
Perfluoroalkyl sulfonates (PFSAs)								
PFBS	76%	0.23	0.16	0.27	65%	0.18	<LOD	0.25
PFHxS	100%	1.13[a]	0.87	1.57	100%	0.66	0.48	0.88
PFHpS	100%	0.29 [a]	0.19	0.37	100%	0.15	0.10	0.18
PFOS	100%	9.35 [a]	5.68	11.61	100%	4.66	3.33	6.53
PFDS	97%	0.06	0.03	0.07	88%	0.05	0.03	0.07
Perfluoroalkyl carboxylates (PFCAs)								
PFPeA	29%	<LOD	<LOD	0.10	15%	<LOD	<LOD	<LOD
PFHxA	0%	<LOD	<LOD	<LOD	0%	<LOD	<LOD	<LOD
PFHpA	55%	0.17	<LOD	0.24	61%	0.18	<LOD	0.25
PFOA	100%	1.95 [a]	1.32	2.66	100%	1.50	0.99	2.20
PFNA	100%	0.88 [a]	0.60	1.40	100%	0.76	0.56	1.03
PFDA	100%	0.43	0.27	0.56	100%	0.40	0.31	0.49
PFUnDA	100%	0.32	0.21	0.45	100%	0.34	0.20	0.44
PFDoDA	100%	0.05	0.04	0.07	100%	0.05	0.04	0.08
PFTrDA	82%	0.08	0.05	0.13	82%	0.10	0.06	0.17
PFTeDA	24%	<LOD	<LOD	<LOD	32%	<LOD	<LOD	0.07
Polyfluoroalkyl phosphate esters (PAPs)								
6:2 PAP	16%	<LOD	<LOD	<LOD	16%	<LOD	<LOD	<LOD
8:2 PAP	8%	<LOD	<LOD	<LOD	12%	<LOD	<LOD	<LOD
6:2 diPAP	82%	0.04	0.02	0.09	73%	0.03	<LOD	0.05
8:2 diPAP	58%	0.02	<LOD	0.04	54%	0.02	<LOD	0.04
Perfluoroalkyl sulfonamides (FOSAs)								
PFOSA	66%	0.01	<LOD	0.01	65%	0.01	<LOD	0.01
MeFOSA	0%	<LOD	<LOD	<LOD	2%	<LOD	<LOD	<LOD
EtFOSA	0%	<LOD	<LOD	<LOD	0%	<LOD	<LOD	<LOD
Perfluoroalkyl phosphonates (PFPAs)								
PFHxPA	50%	0.02	<LOD	0.06	55%	0.05	<LOD	0.07
PFOPA	0%	<LOD	<LOD	<LOD	0%	<LOD	<LOD	<LOD
PFDPA	0%	<LOD	<LOD	<LOD	0%	<LOD	<LOD	<LOD

[a] P-value<0.05 for Mann–Whitney non-parametric test for the differences between sex.

Table 2
Lipid and lipoprotein concentrations overall and by subfractions, in serum samples Norwegian men and women collected at Day1 of the EuroMix study (n = 127).

	Men (n = 38)		Women (n = 89)				
	Mean	(SD)	Mean	(SD)	ICC [a]	(95%	CI)
Lipid profile (mg/dL)							
Total Triglycerides	126.1	(67.8)	85.5	(47.5) [b]	0.80	(0.73	0.85)
Total Cholesterol	213.6	(39.9)	194.8	(33.6) [b]	0.87	(0.82	0.91)
Total Apo-A1	140.6	(16.7)	163.9	(21.3) [b]	0.89	(0.84	0.93)
Total Apo-A2	33.5	(3.8)	34.5	(5.1)	0.80	(0.73	0.86)
Total Apo-B100	88.7	(24.8)	71.9	(18.9) [b]	0.91	(0.88	0.94)
Total HDL-Cholesterol	57.8	(10.4)	70.9	(13.3) [b]	0.93	(0.88	0.95)
Total LDL-Cholesterol	124.5	(30.1)	104.8	(27.1) [b]	0.83	(0.76	0.88)
Total IDL-Cholesterol	11.6	(6.4)	6.8	(4.4) [b]	0.88	(0.83	0.92)
Total VLDL-Cholesterol	22.0	(11.7)	12.5	(7.0) [b]	0.78	(0.69	0.85)
Cholesterol concentrations by lipoprotein subfractions (mg/dL)							
High Density Lipoproteins (densities)							
HDL1 (1.063–1.100 kg/L)	16.0	(6.0)	25.5	(11.2) [b]	0.97	(0.94	0.98)
HDL2 (1.100–1.112 kg/L)	8.2	(2.3)	11.2	(3.0) [b]	0.93	(0.89	0.95)
HDL3 (1.112–1.125 kg/L)	9.7	(1.8)	12.0	(1.9) [b]	0.86	(0.81	0.90)
HDL4 (1.125–1.210 kg/L)	22.2	(3.8)	21.1	(4.7)	0.83	(0.77	0.88)
Low Density Lipoproteins (densities)							
LDL1 (1.019–1.031 kg/L)	22.1	(6.4)	21.5	(5.5)	0.76	(0.65	0.84)
LDL2 (1.031–1.034 kg/L)	19.7	(6.8)	20.3	(6.2)	0.73	(0.63	0.81)
LDL3 (1.034–1.037 kg/L)	17.5	(7.3)	16.8	(6.1)	0.68	(0.58	0.76)
LDL4 (1.037–1.040 kg/L)	17.4	(7.4)	12.7	(8.6) [b]	0.76	(0.69	0.83)
LDL5 (1.040–1.044 kg/L)	20.8	(9.1)	12.8	(7.5) [b]	0.88	(0.83	0.92)
LDL6 (1.044–1.063 kg/L)	26.4	(12.2)	20.1	(7.0) [b]	0.83	(0.76	0.88)
Very Low Density Lipoproteins (densities: 0.950 - 1.006 kg/L)							
VLDL1	7.3	(5.4)	3.0	(3.4) [b]	0.79	(0.72	0.85)
VLDL2	3.4	(2.3)	1.6	(1.2) [b]	0.71	(0.60	0.80)
VLDL3	3.8	(2.3)	1.9	(1.5) [b]	0.73	(0.62	0.81)
VLDL4	5.0	(2.7)	3.4	(1.7) [b]	0.69	(0.57	0.79)
VLDL5	1.6	(0.7)	1.5	(0.5)	0.62	(0.50	0.72)

[a] Intra-class Correlation Coefficient (ICC) for repeated measurements in serum samples collected at Day 1 and Day2.
[b] p-value <0.05 for differences between men and women.

(95%CI = 6,18) and 10% (95%CI = 4,18) increases in HDL-C per IQR increase in PFNA, PFUnDA, PFDoDA and PFOS, respectively (Supplementary Table S4). The reduction in VLDL particle number was significant for PFNA in women (Supplementary Table S4). In addition, PFNA, PFDoDA and PFOS were associated with increases in total apolipoprotein A1 (apoA1), as well as with HDL phospholipids. Increases in apolipoprotein A2 (apoA2) were associated with PFHpS and PFOS, whereas no significant associations with total apolipoprotein B (apoB) or LDL particle number were observed (Supplementary Table S4).

The ratio between LDL and HDL cholesterol and the apoB:apoA1 ratio are regarded as more sensitive markers of CVD risk than LDL-C concentrations (Walldius and Jungner, 2006). In our study, the LDL-C: HDL-C and the apoB:apoA1 ratios were mostly unaltered by PFAS exposures (Supplementary Table S4).

By applying BKMR analyses, we observed that PFAS mixture exposure was directly associated with HDL-C and inversely associated with VLDL triglycerides (VLDL-TG) (Table S7 & Fig. 2). For HDL-C the association was stronger and, in a dose-response manner, while the inverse association with VLDL-TG was seen only in high PFAS mixture exposure levels (≥75th percentile). More specifically, when exposure of the PFAS mixture was in the 95th percentile vs 25th percentile, the increase in HDL-C was 12.2 mg/dL (95% CI = 5.3, 19.1 mg/dL) and the decrease in VLDL-TG was −26.3 mg/dL (95% CI = −50.2, −2.4 mg/dL) (Table S7). Regarding the contribution of individual PFAS in the identified mixture, PFDoDA was the main contributors for HDL-C and PFOA, PFNA, PFUnDA and PFDoDA for VLDL-TG (Table S6). We did not observe an association between PFAS mixture exposure and LDL-C. After performing stratified analysis by sex, we found similar associations between PFAS mixture exposure and HDL-C in women but no association in men (Table 2 & Fig. 1B & C). The association with VLDV-TG was attenuated in the stratified analysis.

3.3. PFAS concentrations and HDL subfractions

Four HDL subclasses were defined based on the NMR analysis and numbered HDL1-4 according to size with HDL1 being the largest. The increase in HDL-C associated with the long-chained PFCAs was mostly due to increases in the two largest HDL particles (Supplementary Table S5), but the associations were not significant after correcting for multiple comparisons. Similar tendencies were observed for men and women.

Focusing on women, we found that the PFCAs were associated with increased cholesterol concentrations in the HDL subfractions within the range of 7–21% in HDL1, 10–19% in HDL2, 5–9% in HDL3 and 0–7% in HDL4 (Fig. 3A). The strongest associations observed were between PFNA and PFDoDA and HDL-C. By applying linear mixed-effect regression models, we found a 20% (95% CI = 4, 39), 18% (95% CI = 7, 30), and 9% (95% CI = 3, 15) increase in cholesterol levels within HDL1, HDL2 and HDL3 subfractions, per IQR increase in PFNA, respectively (Supplementary Table S5). After adjusting for multiple comparisons, positive associations remained between PFOA, PFNA, PFUnDA and PFDoDA with HDL1, HDL2 and/or HDL3 cholesterol.

Among the PFSAs, PFOS was associated with 14% (95% CI = −1, 32), 15% (95% CI = 5, 27), 10% (95% CI = 5, 16) and 5% (95% CI = −3, 13) increase in the cholesterol concentrations of each HDL subfraction, from 1 to 4 (Fig. 3B). The associations with HDL2 cholesterol and HDL3 cholesterol remained significant after adjustment for multiple comparisons.

3.4. PFAS concentrations and LDL subfractions

For the LDL particles, six subclasses were defined based on the NMR analysis and numbered LDL1-6 according to size. In the total population, the long-chained PFCAs, PFNA, PFUnDA and PFDoDA were associated with increases in LDL2 and LDL3 cholesterol and phospholipid parameters, while in men associations were found between PFNA and PFUnDA with LDL2 particle number, cholesterol and phospholipid parameters (Supplementary Table S5). None of these associations were significant after correction for multiple comparisons.

In women, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA were associated with a 14–32% and 14–33% increase in the LDL2-C and particle number (Fig. 4A and Supplementary Table S5), but these associations

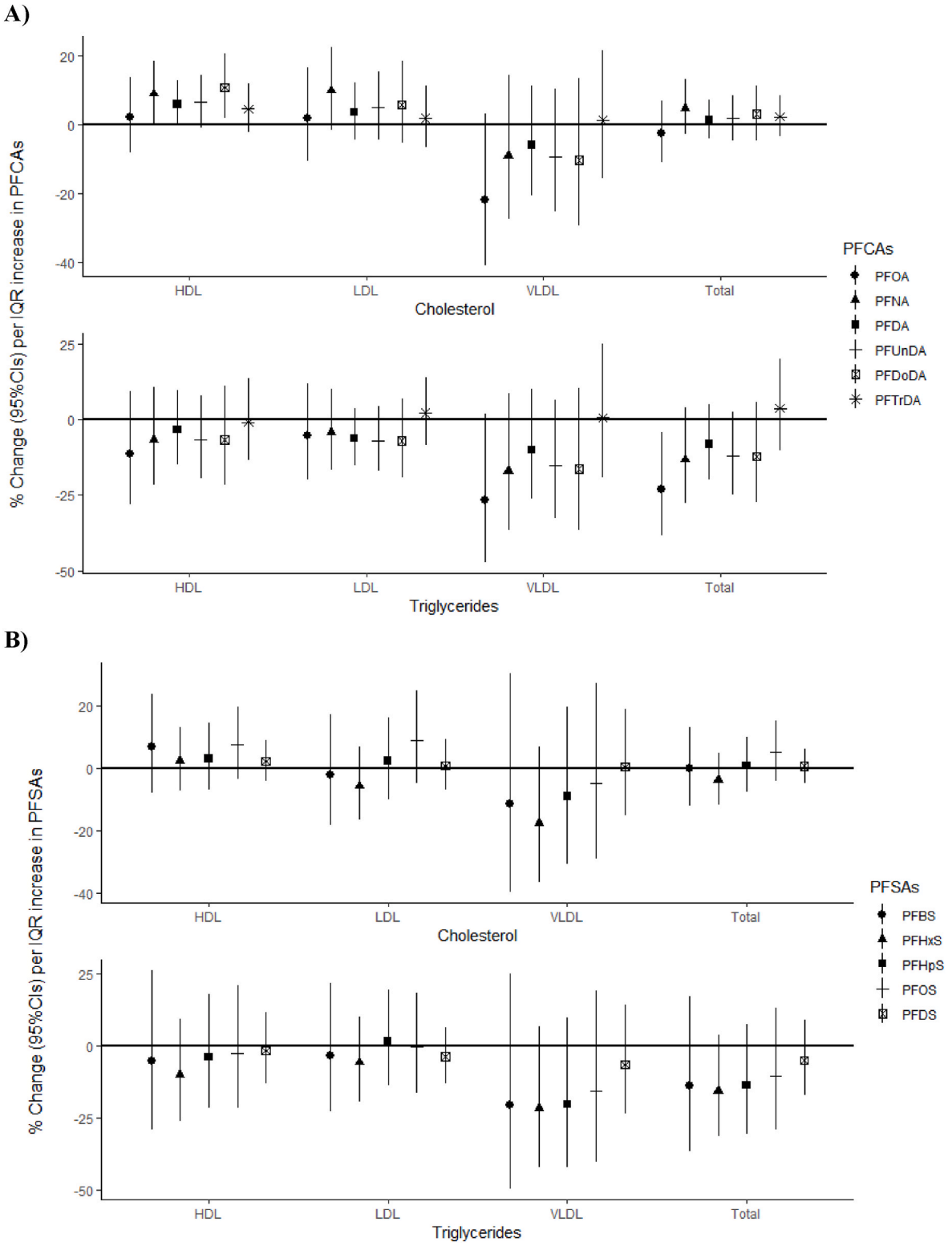

Fig. 1. Adjusted percentage change (and 95%CI) of concentrations for total and main lipid (cholesterol and triglycerides) fractions, per 10% increase in A) PFCA and B) PFSA concentrations. All models are adjusted for participants' age, sex, BMI and smoking status, and the interaction term between PFAS and sex (n = 127).

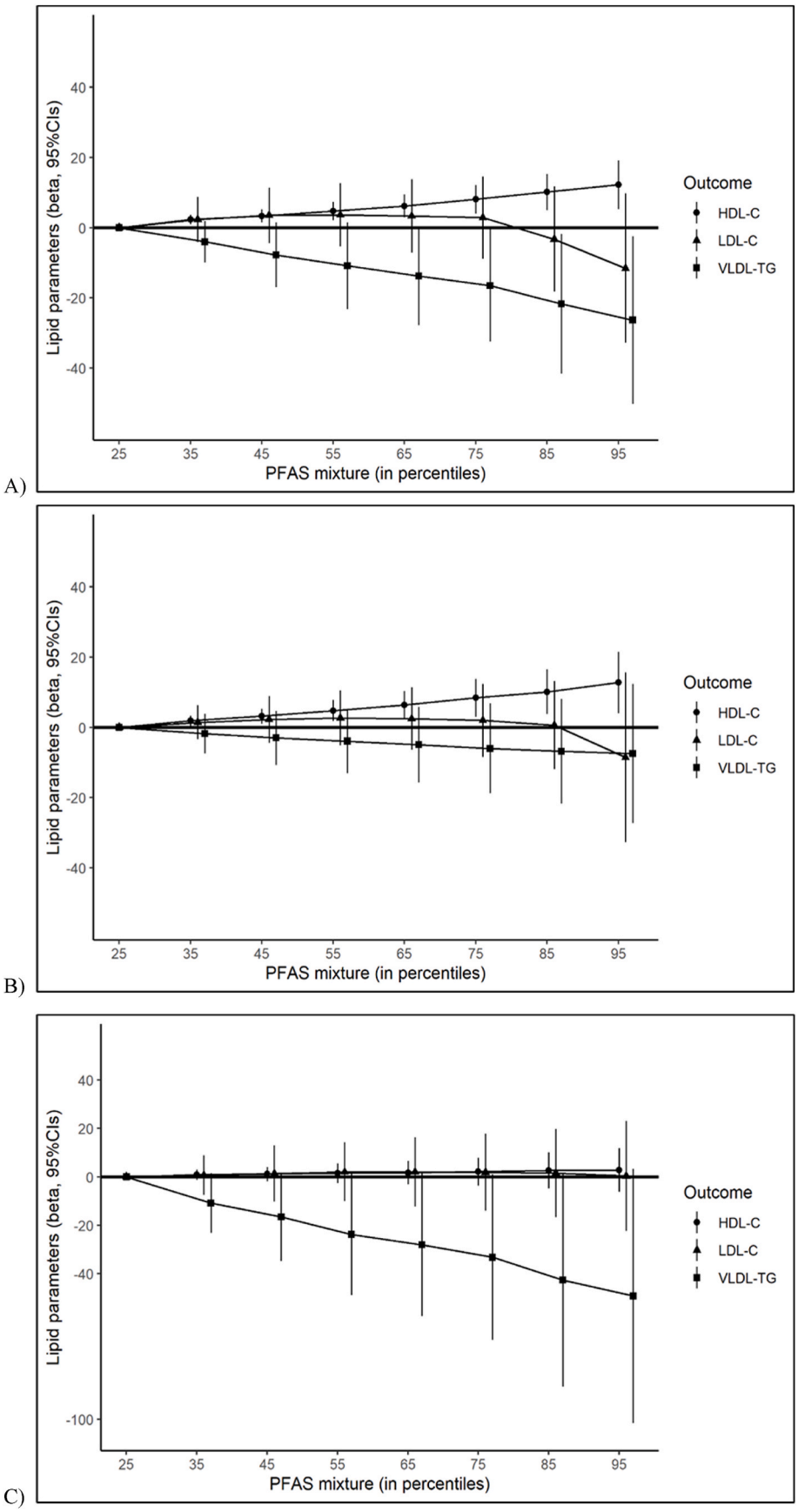

Fig. 2. Joint effect (h(z), 95% CIs) of the PFASs mixture on the lipid parameters by increasing PFAS mixture levels (from 25th to 95th percentile), compared to low PFASs mixture (reference: 25th percentile), using Bayesian kernel machine regression (BKMR) model, overall and stratified by gender. Mixture effects shown for, A) all participants, B) women and C) men.

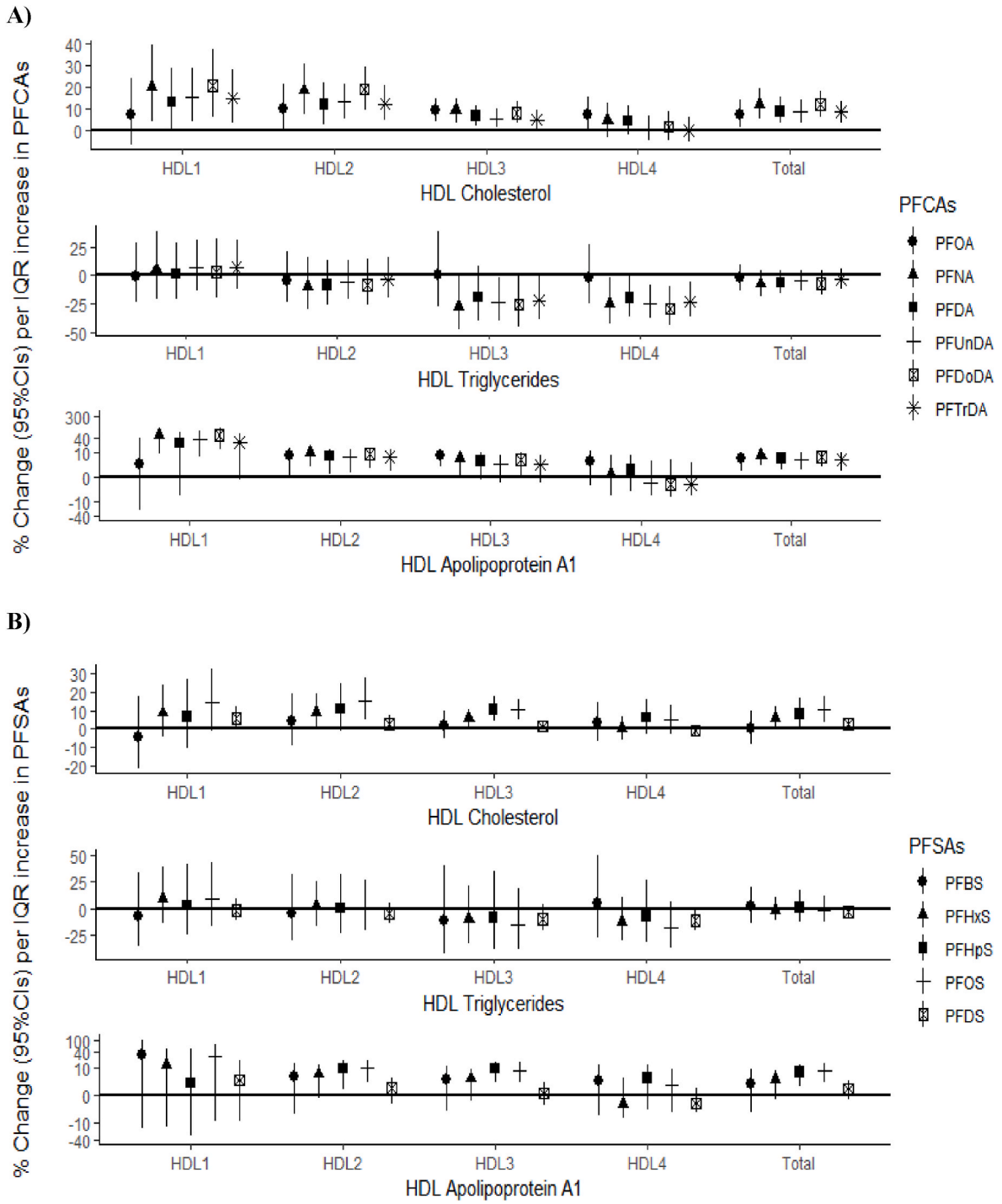

Fig. 3. Adjusted percentage change (and 95% CI) of HDL subfractions concentrations, per 10% increase in A) PFCA and B) PFSAs concentrations, in women. All models are adjusted for participants' age, BMI and smoking status (n = 89).

were mostly non-significant.

For most PFSAs, a tendency of increases in cholesterol in the larger LDL particles were observed (Fig. 4B), but the associations were not significant. Interestingly, one IQR increase in PFHpS and PFOS was associated with 14% and 11% increase in the cholesterol concentrations of the small dense LDL6 subfraction (Supplementary Table S5), but these associations were not significant after correcting for multiple comparisons.

Of note, similar associations as those observed between the small LDL6 and PFASs in women, were not observed in men.

3.5. PFAS concentrations and VLDL subfractions

The tendency of an association between most of the PFASs with reduced levels of VLDL components was observed in both men and women. The % change was more marked in the large than in the small VLDL particles. In women, the inverse association between PFNA and PFDoDA and VLDL1 triglycerides remained significant after correction for multiple comparisons (Supplementary Table S5).

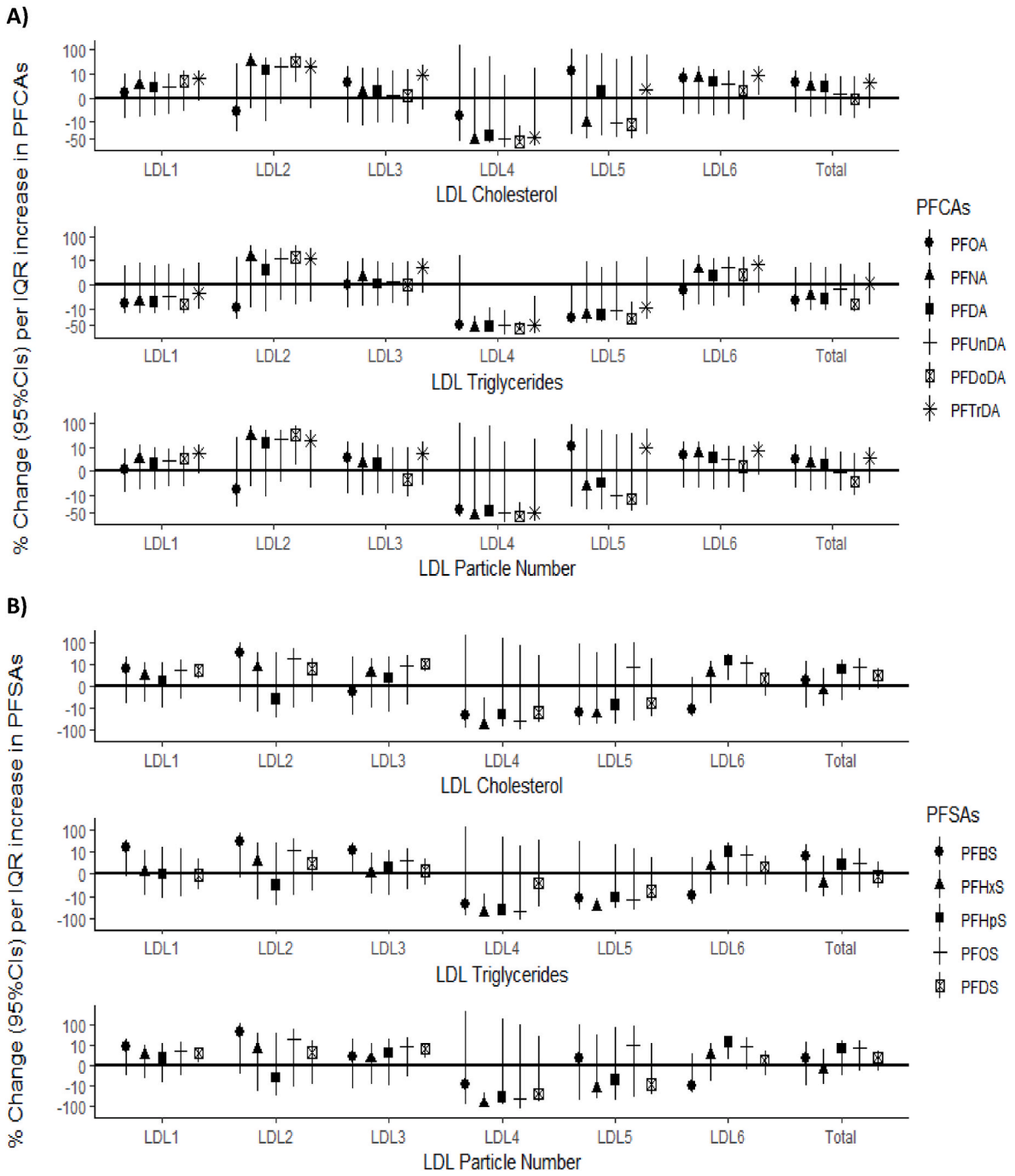

Fig. 4. Adjusted percentage change (and 95%CI) of the cholesterol and triglyceride concentrations by LDL subfraction, per IQR increase in A) PFCA and B) PFSAs concentrations, in women. All models are adjusted for participants' age, BMI and smoking status (n = 89).

4. Discussion

In this cross-sectional study, we explored associations between serum PFAS concentrations and subclasses of lipoprotein particles in the human biomonitoring study EuroMix. While the majority of epidemiological studies performed to date, show a positive association between the main PFASs found in human serum and serum total cholesterol (ATSDR, 2021; EFSA, 2020), there are very few data available on potential associations with lipoprotein subclasses and content. Recent reviews, report that major serum PFAS exposure levels are associated with total serum cholesterol with somewhat weaker support for a direct association with LDL-C and weaker still for HDL-C (Andersen et al., 2021; ATSDR, 2021; EFSA, 2020).

After adjusting for identified confounders, we found significant associations between several PFASs and increased HDL-C for both men and women. The associations were particularly strong for the longer chained PFCAs with the large to medium sized HDL-C particles showing the largest change in concentration. No change in the cholesterol level of the small HDL particles was observed. Furthermore, a slight decrease in the HDL-TG:HDL-C ratio was revealed, especially for the longer chained PFCAs, suggesting that not only the particle size distribution, but also the lipoprotein lipid composition was affected.

BKMR modelling showed that the PFAS mixture was dose-dependently associated with increased HDL-C in the overall population and in females. Although the associations between PFAS exposures and HDL-C is variable across studies, several epidemiological studies

report direct associations with both LDL-C and HDL-C (Canova et al., 2020; Chateau-Degat et al., 2010; Dalla Zuanna et al., 2021; Frisbee et al., 2010; Geiger et al., 2014; Li et al., 2020; Lin et al., 2020; Liu et al., 2018; Mora et al., 2018; Starling et al., 2014). It seems likely that both the level of the individual PFASs and the general health and/or dietary status of the individuals affect the lipoprotein particle distribution and lipid content. This lends some support from a recent animal study in which the effects of PFAS on cholesterol homeostasis is reported to differ depending on diet (Rebholz et al., 2016; Schlezinger et al., 2020). Importantly, the EuroMix cohort represents people with background PFAS exposure dominated by dietary sources. It has been shown that intake of fatty fish influences serum lipoprotein profiles (Hustad et al., 2021). In the EuroMix study, serum concentrations of PFDS, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA were associated with fish consumption, with the strongest association observed for PFDS and PFUnDA (Thépaut et al., 2021). Similarly, marine food consumption has repeatedly been directly associated with concentrations of several PFASs in Norwegian and European populations (Augustsson et al., 2021; Hansen et al., 2016; Haug et al., 2010; Papadopoulou et al., 2017; Rylander et al., 2009; Thépaut et al., 2021) (Falandysz et al., 2006; Papadopoulou et al., 2019). Thus, when considering associations between serum PFASs and HDL particle concentrations, both the environmental sources of the substances and the health status of the individuals may be of importance.

In addition to increases in HDL-C, there was a tendency of increased LDL particle number and LDL-C associated with PFAS exposure. The ratios between apoB/apoA1 and LDL-C/HDL-C showed modest changes, reflecting that PFAS exposures was associated with an increase in both HDL and LDL cholesterol. The increases in LDL-C occurred mainly in the large to medium sized LDLs. Recent data indicate that larger LDL particles are less strongly related to CVD risk than small dense LDL particles (Ference et al., 2017). The BKMR modelling did not show a significant association between the PFAS mixture and LDL-C.

In our data, a general reduction in VLDL-particle number and an accompanying reduction in VLDL-lipids including VLDL-TG was suggested in the adjusted model and was significant for PFNA in women. The BKMR mixture analysis suggested a reduction in VLDL-TG at higher PFAS exposure levels in the overall population. Other studies have reported both positive and inverse associations between PFASs and TG (EFSA, 2020) and the data appear complex.

Lipoprotein particles are very heterogeneous in their structure, size and function. It is now recognised that HDL functionality, in the sense of their efficiency for reverse cholesterol transport, can be impaired in chronic inflammatory disease states. HDL-functionality and hence their CVD protective effects depend in part on their protein content (Chiesa and Charakida, 2019). Interestingly, one study has reported associations between PFOA serum concentrations and apoC3 containing lipoprotein particles in humans (Liu et al., 2020). ApoC3 inhibits lipoprotein lipase and hepatic lipase activities and elevated apoC3 is suggested to be a risk factor for CVD (Crosby et al., 2014; Jensen et al., 2012) underlining the heterogeneity of lipoprotein particle subclasses.

Although relatively small sample size, the strength of the EuroMix biomonitoring study is the uniform high quality of the biological samples and the detailed exposure from a fairly homogeneous population. To date there are very few epidemiological studies describing potential effects of PFASs on major lipoprotein subclasses and their content. We also provide data on some of the less explored PFASs. A limitation of the data is that although we have described changes in distribution of the subpopulations of lipoprotein particles, we have not measured their functionality. However, the data support that background PFAS exposures is associated with increased plasma cholesterol and further suggest that this effect is quite prominent for HDL-C/apoA1 at low to moderate PFAS exposure levels in a healthy population. The data indicate that the increase in cholesterol is distributed in the medium to large HDL particles.

5. Conclusion

Background PFAS serum concentrations were associated with altered lipoprotein subclass profiles in a generally healthy and homogeneous Norwegian population. The strongest associations identified were between several of the long chained PFCAs and increased cholesterol in large and medium sized HDL-particles in women. The change in cholesterol distribution among the LDL-particles appeared more complex, but the most significant increases were found among the LDL1-LDL3 fraction and in particular among the PFCAs. Furthermore, a general reduction in large VLDL particle number and lipid content was suggested. This exploratory study suggests that PFAS serum concentrations are associated with both lipoprotein particle subclass distributions and particle content at background PFAS exposure levels.

Author contributions

Papadopoulou E: Data curation, Formal analyses, Writing – original draft preparation, writing– review and editing, Visualization. **Nicolescu A**: Investigation, analyses, writing– review and editing. **Haug LS**: Methodology, Writing – original draft, writing– review and editing. **Husøy T**: Conceptualisation, Resources, Writing – original draft, writing– review and editing, Project administration. **Deleanu C**: Conceptualisation, Methodology, Resources, Writing – original draft, writing– review and editing. **Dirven HAAM**: Conceptualisation, Resources, Writing– review and editing, Funding acquisition. **Lindeman B**: Conceptualisation, Writing – original draft preparation, writing– review and editing, Supervision.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119664.

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