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Perfluoroalkyl substance mixtures and cardio-metabolic outcomes in highly exposed male workers in the Veneto Region: A mixture-based approach.

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ABSTRACT

Background: Perfluoroalkyl substances (PFAS) have been consistently associated with cardio-metabolic traits. Occupational exposures to multiple PFAS with health outcomes have been poorly investigated. The aim of the present study was to examine these associations among former workers involved in PFAS production.

Methods: We considered 232 male ex-employees who had worked in a factory (Trissino, Veneto Region, Italy), which produced PFAS and other chemicals during 1968–2018. Out of twelve serum PFAS, only four (PFOA, PFOS, PFHxS, and PFNA) were quantifiable in at least 50% of samples. Non-fasting serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. The associations between serum PFAS mixture and considered outcomes were assessed through linear regression mixed models and Weighted Quantile Sum (WQS) regression, adjusting for potential confounders.

Results: PFOA was detected at the highest level, with a median concentration (in ng/mL) of 80.8 (min-max: 0.35–13,033), followed by PFOS (median: 8.55, min-max: 0.35–343), PFHxS (median: 6.8, min-max: 0.35–597) and PFNA (median: 0.8, min-max: 0.35–5). We observed that each A quartile increase in the WQS index was positively associated with the levels of TC (β: 8.41, 95% IC: 0.78–16.0), LDL-C (β: 8.02, 95% IC: 1–15.0) and SBP (β: 3.21, 95% IC: 0.82–5.60). No association of serum PFAS concentration on HDL cholesterol and DBP emerged. WQS analyses revealed a major contribution of PFNA and PFHxS for the cholesterol levels, although PFOA reported the highest concentration. PFOA and PFOS emerged as chemicals of concern regarding the association with SBP.

Conclusions: The results showed a clear association between serum PFAS levels and markers of cardiovascular risk and support the importance of clinical surveillance of cardiovascular risk factors in population with a high exposure to PFAS, especially in the occupational setting.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have become a serious global concern due to their ubiquitous presence in the environment. PFAS are used in a wide variety of commercial products and industrial applications since the 1940s and can be found in everyday household products (Glüge et al., 2020). Direct exposure to PFAS in humans can occur through drinking water, food, and air (Poothong et al., 2020; Seltenrich, 2020), but exposures at higher levels than the general population especially occur in populations living near facilities using PFAS and in workers (Winquist et al., 2013).

In recent years, a growing number of scientific reports has indicated a wide range of potential health effect of PFAS exposure in both humans and animals (ATSDR, 2021; EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel) et al., 2020; Fenton et al., 2021).

Increased levels of total and LDL cholesterol and high blood pressure

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are the most prevalent conditions increasing the risk of cardiovascular disease (CVD) (O'Donnell and Elosua, 2008) (O'Donnell and Elosua, 2008). To date, exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), has been evidently associated with altered cholesterol levels (EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel) et al., 2020), while the associations are less evident for other cardiovascular risk factors, as blood pressure (Min et al., 2012; Pitter et al., 2020d). Associations between PFAS concentrations and serum lipids have been evaluated among occupational exposed individuals, showing either significant and non-significant associations with cholesterol levels (Costa et al., 2009; Lin et al., 2020; Olsen et al., 2003; Olsen and Zobel, 2007; Sakr et al., 2007; Wang et al., 2012), whereas no study was conducted so far on the association with measured blood pressure levels in occupational setting. Furthermore, almost all the above-mentioned studies had measured only PFOA concentrations, despite the production and the consequent exposure usually covered a wide range of PFAS (Gibson et al., 2019).

Even if literature suggested that PFAS exposure increased the frequency of risk factors for CVD, studies that directly examined the relationship between PFAS and CVD incidence or mortality showed mixed findings (Anderson-Mahoney et al., 2008; Min et al., 2012; Shankar et al., 2012; Winquist and Steenland, 2014). Mortality studies based on occupational cohorts were limited, with small sample sizes and poor assessment of PFAS exposure (which for all is indirect), not providing a convincing evidence between exposure to PFAS and CVD mortality (Girardi and Merler, 2019; Lundin et al., 2009; Sakr et al., 2009; Steenland et al., 2015; Steenland and Woskie, 2012).

PFASs manufacturing facilities employed a complex industrial production process with the contemporary synthesis of several differentiated PFAS leading to exposure of mixture of PFAS, as demonstrated by biomonitoring data (Gibson et al., 2019). Associations of combined exposures to multiple PFAS with health outcomes through methods developed to specifically quantify the combined mixture effect have been poorly investigated in highly exposed populations, and no study is to the best of our knowledge conducted among workers.

In Veneto Region (North-Eastern Italy) the largest manufacturing plant in Europe (RIMAR MITENI; RM) produced PFAS, mainly PFOA and PFOS, from 1968 until 2014 which resulted in an elevated PFAS exposure of the workers as reported by the high concentrations recorded (Girardi and Merler, 2019). The subsequent environmental contamination of the soil and underground water involving the drinking water and, as a consequence, the population living the above area (about 300,000 people; Pitter et al., 2020c).

The factory closed down any production on 2018, and the former workers entered in a health surveillance program built to monitor PFAS concentrations and potential adverse health effects. The aim of the present study was to examine the association between serum PFAS concentrations and PFAS mixture and various cardiometabolic traits (lipid profile parameters, blood pressure) among subjects employed at the RM factory recruited as part of a community-based health surveillance in Veneto Region, Italy (Pitter et al., 2020d).

2. Materials and methods

2.1. Participants and study design

Starting its production in 1968 in the municipality of Trissino (province of Vicenza, Veneto Region, Italy), the RIMAR-MITENI (RM) factory mainly manufactured perfluorinated alkylated substances (PFASs), fluoroaromatics (FA), and benzotrifluorides (BTF). While FA and BTF were produced for intermediate pharmaceutical applications, and agro-chemical products like herbicides, fungicides, and insecticides, PFASs were intermediates for industrial and consumer applications. PFOA was produced (1968–2014) from capryloyl chloride via electrochemical fluorination. In addition to PFOA, ammonia perfluorooctanoate (APFO), the potassium salt of PFOS (trade name RM95), perfluorooctane sulfonyl fluoride (PFOS; trade name RM90), and perfluorobutylsulfonyl fluoride (trade name RM60) were also produced (1968–2005). The end of production of long-chain PFASs was voluntary and preceded by few years the closure of the plant in November 2018. Production data were limited to the period 2001–2014: the annual production of PFOA and its ammonium salt was in average 250 tons, peaking at 460 tons in 2007, while the production of PFOS as liquid acid or its potassium, was lower with an average 36.6 tons per year in 2001–2011, peaking at 88 tons in 2004 (for more details see Girardi and Merler, 2019). The production of other PFAS started after the year 1968; to our knowledge the factory reported in 9 and 10.1 tons of PFHxS (trade name RM70) and its potassium salt for the year 2003 and 2004, respectively. No PFNA production has been documented since 2001. The production of short-chain PFAS mainly concerned the production of Perfluorobutanesulfonic acid (PFBS) and its potassic salt with an average production in the period 2001–2016 of 21.1 tons per year and a peak of 38.8 tons in the year 2015.

A health surveillance program was launched in January 2017 as a free population-based screening program offered by the regional Health Service to the residents of 30 municipalities (namely "Red Area") who were exposed to PFAS via contaminated drinking water for several decades (for more information please see Pitter et al., 2020c). The eligible population also included former employees in the RM factory, regardless of their residence in the Red Area. Participants completed an interview questionnaire assessing socio-demographic characteristics, personal health history and lifestyle habits. Non-fasting blood samples were obtained for the convenience of the participants throughout the course of the day when they visited the clinic.

The population investigated in the present study is a subgroup of the surveillance program target population, consisting of subjects previously employed at the RM factory (n = 288) who were enrolled during two distinct surveillance waves (from January to May 2018 n = 151; from January to February 2020 n = 137). The eligible population was different in the two recruitment periods, since the first one recruited only retired workers while, after the closure of the plant, the second one was extended to any remaining RM workers. Subjects eligible for the first period were invited also to the second one. In case of participation in both the surveillance waves, only data from the first recruitment period was considered for the analysis in the present study. Women (n = 53) were excluded from the analysis because of their low sample size and potential differences in metabolic pathways, in particular in relation to PFAS excretion.

Moreover, ex-workers with an age below 25 years old were excluded, since they had started working in the factory after the PFAS production ended (n = 3). A total of 232 subjects were included in the analyses.

2.2. PFAS quantification

Serum concentrations of twelve PFAS were quantified using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) [Prominence UFLC XR 20 (Shimadzu) coupled to API 4000TM LC-MS/MS System (Sciex)]: perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA). Additional information regarding PFAS measurement is provided elsewhere (Pitter et al., 2020c).

Details on the analytical method have been described in a previous paper (Pitter et al., 2020c). The limit of detection (LOD) for all PFAS was as low as 0.1 ng/mL and limit of quantification (LOQ) was 0.5 ng/mL. Concentrations below the LOQ were imputed by dividing the LOQ by the square root of 2 prior to statistical analysis. Four PFAS detected in more than 50% of participants were included in the main analyses, namely, PFOA (99.6%), PFOS (99.6%), PFHxS (98.7%) and PFNA (86.6%).

2.3. Outcome assessment

A single laboratory within the contaminated municipalities (Arzignano, Vicenza province) carried out the analyses of lipid parameters including total cholesterol (TC); high-density lipoprotein cholesterol (HDL-C); low-density lipoprotein cholesterol (LDL-C).

TC and HDL-C were measured by a direct enzymatic colorimetric assay using cholesterol esterase and cholesterol oxidase and units were recorded in mg/dL. LDL-C was calculated by the Friedewald equation when triglycerides were less than 400 mg/dL. The laboratory regularly follows an external quality assurance program.

Blood pressure (BP) was measured by trained nurses with participants first sitting at rest for at least 5 min, according to the European Society of Hypertension recommendations. A validated semi-automatic sphygmomanometer with an appropriate cuff size for the arm circumference was used.

Medical history data were collected directly from participants by trained nurses via structured software-based questionnaire using in-person interviews at the study enrolment.

2.4. Covariates

The structured interview was aimed to collect information about residential history, education, occupation, dietary habits, smoking habits, alcohol consumption, physical activity, family and personal history of disease, medications, reproductive history, and self-reported height and weight.

In the analysis we considered information on age, education level, smoking habits, self-reported history of certain medical diseases, currently used medication and alcohol consumption.

Age was subdivided in quartiles (32–49, 50–58, 59–65, 66–84), while education level was categorized in low (middle school or lower), medium (high school) and high educational level (university degree or higher). Smoking status was subdivided into current smokers and non-smokers, and alcohol consumption was categorized in 0, 1–6, 7+ alcohol units (AU) per week. We also considered the period of recruitment in binary form (2018 and 2020).

The set of covariates was defined based on our previous works (Canova et al., 2020; Pitter et al., 2020d), where the minimally sufficient sets of variables were selected from available variables, based on the literature and through the construction of a directed acyclic graphs. Due to the smaller sample size of the analyzed population we reduced the set of potential confounders previously considered (dietary variables, physical activities were not considered).

The questionnaire also included items on personal health history ("Which diseases do you suffer from?") and medications ("Do you take any medication on a regular basis?" "If yes, which medications do you take?"). 60 participants reported use of lowering cholesterol medications (specifically statins), and this characteristic "dyslipidemia" was included in models of lipids. Self-reported "hypertension" was defined as reporting a diagnosis of hypertension or use of antihypertensive medications (n = 85). Presence of dyslipidemia or diagnosis of hypertension was included in the corresponding models, where appropriate in terms of variable selection; in addition, people with this diagnosis were excluded in the sensitivity analyses (see next section).

2.5. Statistical analysis

The serum concentrations of PFAS are expressed as Geometric Mean (GM), arithmetic mean (Standard Deviation, SD) and percentiles. Because of the non-normal distribution of the serum PFAS levels we adopted a natural log-transformation (ln-PFAS). Spearman's rank correlation (ρ) was used to evaluate pair-wise correlations between the PFAS isomers.

2.5.1. Single PFAS models

We ran single-chemical linear regression mixed models (LMMs) in order to account for the two recruitment periods. Each regression aimed to examine associations between each single serum PFAS isomer and health parameters. We also evaluated the effect of serum PFAS concentrations categorized into quartiles (using the first quartile as reference) and of an interquartile range (IQR) increment, using delta method to derive confidence intervals.

All analyses were adjusted for the following set of categorical covariates: age-group, education level, smoking habit, alcohol consumption, dyslipidemia or hypertension presence as previously defined, for the analyses related to lipids and blood pressure respectively. We choose the regression model with a random intercept on the recruitment period, after a comparison among models with different types of random component (no random effects, random intercept on the recruitment period and/or slope on the PFAS concentrations), and with a linear model in which the recruitment period was included as covariate, through the minimization of the Bayesian Information Criterion (BIC) index.

For supplementary analyses, each single-chemical analysis was also stratified by recruitment period, running both two separate linear regression models and an overall model including an interaction term between recruitment period and ln-PFAS concentration. In addition, because of the presence of outliers and/or influential observations, we performed a sensitivity analysis to assess the robustness of the results, based on a robust alternative to the LMMs (Koller, 2016). A second sensitivity analysis conducted excluding subjects with dyslipidemia (n = 60) and hypertension (n = 85), for each outcome of interest.

2.5.2. Weighted quantile sum (WQS) regression models

To investigate associations between the mixture of four most represented PFASs (PFOA, PFOS, PFHxS, and PFNA) and each cardiometabolic outcome, we applied a Weighted Quantile Sum (WQS) regression model (Carrico et al., 2015). WQS regression summarizes the overall exposure to the mixture by creating a weighted linear index of correlated predictors (in terms of quantiles from each component) that are weighted according to their strength of association with the outcome of interest. We scored PFAS in quartiles and set an a priori cut-point for identifying important toxic compartment of a mixture, as a weight $\geq 1/p = 1/4 = 0.25$, i.e., weights that exceed the case of uniform weights. Results were reported by the estimated coefficients and its relative 95% Coefficient Interval (CI).

All analyses were performed using the statistical software R (R Core Team, 2016). We employed "lme4" and "robustlmm" packages to run LMMs and its robust alternative respectively, and "gWQS" package to conduct WQS. The statistical significance was set at 0.05.

3. Results

The study population included 232 male workers (mean age: 57.3 years; SD: 10.6, range: 32–84) almost equally recruited among the two surveillance waves (Table 1). General characteristics and health outcomes of participants are reported in Table 1. Dyslipidemia and hypertension were reported by about a quarter and a third of the sample, respectively. Of the 12 PFAS measured, 4 were detected in more than 85% of participants (Table 2): PFOA was detected at the highest level (GM 87.4 ng/mL), followed by PFHxS, PFOS and PFNA (GMs 30.0, 15.6, and 1.03 ng/mL respectively). The median serum concentration for TC, HDL-C, LDL-C, SBP, DBP were 197, 50 and 121 mg/dL for lipid parameters, 130 and 80 mmHg for blood pressure parameters.

Moderate correlations were observed between the four PFAS (Table S1). PFOA and PFNA were most strongly correlated (ρ = 0.53), followed by PFOA and PFHxS (ρ = 0.40); the correlation of PFOS with the two compounds was weaker (ρ = 0.36 with PFNA, ρ = 0.18 with PFHxS).

A ln-increase in PFOS and PFNA was most strongly associated with

Table 1
Distributions of covariates in the study population (n = 232).

Characteristics	Mean (SD)	Min-Max	Median (Q1-Q3)
Age (years)	57.32 (10.57)	32–84	58 (49–65)
		N	**%**
Education level			
Elementary/Middle school		94	40.52%
Highschool		113	48.71%
University		25	10.78%
Smoke habit			
No		195	84.05%
Yes		37	15.95%
Alcohol intake (AU per week)			
0		55	23.71%
(1–6)		58	25.00%
>7		119	51.29%
Dyslipidemia (Medication)			
No		172	74.14%
Yes		60	25.86%
Hypertension (Medication, or self-reported disease)			
No		147	63.36%
Yes		85	36.64%
Recruitment period			
2018		120	51.72%
2020		112	48.28%

Table 2
Distributions of serum PFASs concentrations (ng/mL), serum lipids concentrations (mg/dL) and blood pressure (mmHg) in the study population (n = 232).

Variables	Mean (SD)	GM	Min-Max	Median (Q1-Q3)	<LOQ
PFAS					
PFOA	624.74 (1584)	87.4	0.35–13033.3	80.75 (14.88–469.55)	0.43%
PFOS	15.62 (31.28)	8.91	0.35–343	8.55 (4.95–15.93)	0.43%
PFHxS	29.99 (75.63)	7.99	0.35–597.5	6.8 (2.4–21)	1.29%
PFNA	1.03 (0.79)	0.84	0.35–5	0.8 (0.5–1.3)	13.36%
Outcomes					
Total C	197.1 (40.05)		86–298	197 (172.8–223)	
HDL-C	50.6 (12.08)		26–83	50 (42–59)	
LDL-C	121.5 (38.27)		20–221	121 (98–145.8)	
SBP	126.3 (11.73)		90–160	130 (120–131.2)	
DBP	81.11 (8.78)		55–105	80 (80–85)	

TC with an increase of 7.26 mg/dL (CI 2.04–12.5) and 10.7 mg/dL (CI 2.77–18.6) respectively (Table 3), and LDL-C with increases of 5.90 mg/dL (CI 0.97–10.8) and 7.32 (CI -0.19 to 14.83). PFOA and PFHxS showed much smaller associations though in the same direction. There was no evidence of any associations with HDL-C.

Trends of SBP were similar across PFAS, with coefficients reaching statistical significance for PFOA and PFOS: 1.04 mmHg (CI 0.32–1.77) and 2.58 mmHg (CI 0.97–4.18) respectively (Table 3). Associations between DBP and PFAS did not reach the statistical significance.

Analyses of TC and LDL-C all suggest a fairly monotonic increase across quartiles of each PFAS. In every case the coefficient for the highest quartile is the largest. As for the overall associations with ln-PFAS the strongest effects are for PFOS and PFNA. Subjects in the highest PFOS quartile had a 17.0 and 16.8 mg/dL increase in TC and LDL-C than subjects in the lowest quartile, while subjects with highest PFNA quartile had an increase of 20.6 and 15.9 mg/dL in TC and LDL-C levels (than those in the 1st quartile, Table 3). Regarding SBP, analyses by quartile were not so neatly monotonic, and the strongest effects were evident for PFOA and PFOS's highest quartile, of 7.26 and 4.51 mmHg respectively. All four PFAS, both in their categorical and continuous version, didn't reveal any association with the remaining two outcomes (HDL-C and DBP). Full models showing the effect of all covariates included in the models of the association between each PFAS and TC and SBP are presented in the supplementary material (Table S2 for TC; Table S3 for SBP).

Descriptive statistics on covariates, PFASs and cardiometabolic outcomes, stratified by recruitment period, are presented in Table S4. When we examined associations according to the recruitment period (Table S5), results were not different to the combined analyses, but due to the sample size reduction most of the linear coefficients lost their significance. Period of recruitment did not significantly modify the association between all four PFAS and cardiometabolic outcomes (all interaction terms *p-values* were >0.05).

Robust LMM analysis reported consistent results, except for a slight decrease in the magnitude of the effect of PFAS exposures on the cardiometabolic outcomes (Table S6). In contrast, the exclusion of subjects with dyslipidemia or hypertension affected the significance of the estimates (the sample size decreased to n = 172 and n = 147 for subjects without dyslipidemia and hypertension, respectively), but not the direction of the observed association (Table S7).

In the WQS analysis, the weighted quantile sum indices for mixtures showed a positive association with TC, LDL-C and SBP (Table 4). One-quartile increase in weighted quantile sum index was associated with 8.41 mg/dL (CI: 0.78–16.0) increased in TC and of 8.01 mg/dL (CI: 1–15.04) in the LDL-C concentration (Table 4). Finally, for every quartile increase in the WQS index, the level of SBP increased 3.21 mmHg (CI: 0.82–5.6). No significant effect was detected between the mixture of chemicals and HDL-C and DBP (−0.37 mg/dL, CI: 2.59-1.86; 0.64 mmHg, CI: 1.16-1.44). We identified PFHxS and PFNA as contributing the most to the weighted quantile sum index for TC (weights: 0.38 and 0.48) and LDL-C (weights: 0.25 and 0.65) (Table 4); PFOA and PFOS had the lowest weights for both TC and LDL-C. PFOS showed the highest weight for SBP (weight: 0.56), followed by PFOA, also identified as a chemical of concern, with contributing weight that exceeded the 25% threshold (weight: 0.31).

4. Discussion

The aim of this study was to evaluate the cross-sectional association between serum PFAS levels and cardiometabolic parameters in an occupational population of 232 males who had worked in the most important PFAS manufacturing factory (RIMAR-MITENI) in Europe during a period of active PFAS production. We observed that the current serum PFOS and PFNA concentrations were the most associated with total cholesterol and LDL cholesterol. Current PFOA and PFOS concentrations, on the other hand, were positively associated with systolic blood pressure. There was no evidence that any of the PFAS were positively, or negatively, associated with HDL cholesterol or diastolic blood pressure. We also observed a positive significant association of exposure to combined index of the mixture of four PFAS with TC, LDL-C and SBP among our population, using WQS regression.

We detected PFOA, PFOS, PFHxS and PFNA in most occupational workers included in the study, among whom PFOA showed the highest concentrations, as expected given that it was the most produced PFAS in the RM factory (Girardi and Merler, 2019). The PFOA concentrations in exposed workers were higher compared to those detected in the surrounding highly exposed community (with a 58.3 ng/mL median concentration in males aged 20–39 (Canova et al., 2020)) and in the general population (median concentration of 1.64 ng/mL, measured on Veneto citizens with background levels of exposures (Ingelido et al., 2018)). On the other hand, PFOA was found at much higher levels in previous studies on active occupationally exposed populations (Costa et al., 2009; Wang et al., 2012), but our investigation included ex-workers investigated in period some years later than the peak production. Other PFAS

Table 3
Association between PFAS (ln ng/mL) and cardiometabolic parameters from random intercept models on the recruitment period adjusted by several covariates, β coefficients for ln-transformed PFAS and PFAS quartiles and 95% Confidence Intervals (95% CI).

PFAS	Quartile	TC		HDL-C		LDL-C		SBP		DBP	
		β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[b]	95% CI	β[b]	95% CI
PFOA	Q1 [0.354,14.9]	212.63		53.11		131.77		119.14		75.35	
	Q2 (14.9,80.8]	5.16	[-9.92; 20.25]	−2.23	[-7.08; 2.61]	5.62	[-8.6; 19.85]	2.71	[-1.99; 7.41]	1.62	[-1.93; 5.16]
	Q3 (80.8470]	3.87	[-10.73; 18.47]	−1.46	[-6.16; 3.24]	8.39	[-5.31; 22.1]	3.85	[-0.65; 8.35]	3.45	[0.02; 6.89]
	Q4 (470,13,000]	12.38	[-2.76; 27.52]	−3.85	[-8.72; 1.03]	8.40	[-5.81; 22.61]	**7.26**	[2.66; 11.86]	1.45	[-2.09; 4.99]
	per ln-ng/mL	1.83	[-0.54; 4.19]	−0.39	[-1.15; 0.38]	0.97	[-1.26; 3.21]	**1.04**	[0.32; 1.77]	0.14	[-0.42; 0.69]
	per IQR	6.31	[-1.88; 14.50]	−1.33	[-3.97; 1.31]	3.36	[-4.35; 11.07]	**3.60**	[0.86; 5.86]	0.48	[-1.41; 2.37]
PFOS	Q1 [0.354,4.95]	207.27		51.40		127.32		120.44		76.73	
	Q2 (4.95,8.55]	7.11	[-7.58; 21.79]	−4.48	[-9.2; 0.24]	12.50	[-1.3; 26.29]	1.83	[-2.81; 6.48]	0.87	[-2.63; 4.36]
	Q3 (8.55,15.9]	12.12	[-1.85; 26.1]	0.75	[-3.74; 5.24]	11.39	[-1.76; 24.54]	2.13	[-2.29; 6.54]	1.05	[-2.27; 4.37]
	Q4 (15.9343]	**17.04**	[2.8; 31.27]	−0.33	[-4.9; 4.24]	**16.79**	[3.37; 30.21]	**4.51**	[0.09; 8.93]	0.63	[-2.69; 3.96]
	per ln-ng/mL	**7.26**	[2.04; 12.48]	0.83	[-0.87; 2.53]	**5.90**	[0.97; 10.83]	**2.58**	[0.97; 4.18]	−0.16	[-1.39; 1.06]
	per IQR	**8.49**	[2.39; 14.58]	0.97	[-1.02; 2.96]	**6.89**	[1.12; 12.66]	**3.01**	[1.13; 4.89]	−0.19	[-1.63; 1.25]
PFHxS	Q1 [0.354,2.4]	210.75		50.99		130.58		119.97		75.69	
	Q2 (2.4,6.8]	8.11	[-7.32; 23.55]	0.71	[-4.28; 5.71]	8.49	[-6.08; 23.06]	0.48	[-4.36; 5.32]	−0.57	[-4.14; 2.99]
	Q3 (6.8,21]	5.52	[-9.83; 20.88]	−0.26	[-5.23; 4.71]	6.52	[-7.95; 20.99]	5.08	[0.28; 9.89]	**4.28**	[0.73; 7.83]
	Q4 (21,598]	14.22	[-1.11; 29.55]	−0.21	[-5.17; 4.76]	11.80	[-2.66; 26.26]	3.93	[-0.85; 8.71]	0.46	[-3.08; 4]
	per ln-ng/mL	2.98	[-0.22; 6.19]	−0.17	[-1.21; 0.87]	2.18	[-0.83; 5.2]	0.96	[-0.04; 1.96]	0.21	[-0.55; 0.96]
	per IQR	6.47	[-0.46; 13.40]	−0.37	[-2.62; 1.88]	4.74	[-1.81; 11.29]	2.08	[-0.09; 4.25]	0.45	[-1.21; 2.11]
PFNA	Q1 [0.354,0.5]	209.31		50.03		131.63		120.85		76.84	
	Q2 (0.5,0.8]	9.39	[-4.56; 23.33]	1.45	[-3.08; 5.97]	8.49	[-4.7; 21.67]	0.64	[-3.82; 5.1]	0.72	[-2.63; 4.07]
	Q3 (0.8,1.3]	5.46	[-7.7; 18.62]	−0.62	[-4.89; 3.65]	2.28	[-10.25; 14.8]	3.96	[-0.23; 8.15]	1.17	[-1.98; 4.32]
	Q4 (1.3,5]	**20.64**	[7.23; 34.04]	3.99	[-0.36; 8.34]	**15.88**	[3.19; 28.56]	2.86	[-1.38; 7.1]	0.34	[-2.85; 3.53]
	per ln-ng/mL	**10.68**	[2.77; 18.6]	2.28	[-0.29; 4.84]	7.32	[-0.19; 14.83]	2.20	[-0.31; 4.71]	0.33	[-1.55; 2.22]
	per IQR	**10.21**	[2.64; 17.78]	2.17	[-0.28; 4.62]	7.00	[-0.17; 14.17]	2.09	[-0.31; 4.49]	0.32	[-1.48; 2.12]

The first β coefficient for each PFAS is the predicted values of each outcome for the 1st percentile (quartile) of the PFAS distribution.
[a] Adjusted by age, smoke habit, alcohol, education level, dyslipidemia.
[b] Adjusted by age, smoke habit, alcohol, education level, hypertension.

Table 4
Associations between WQS regression index and Serum Lipids (mg/dL), and blood pressure (mmHg). WQS regression model weights of each PFAS component, for each outcome.

Outcome	TC	HDL-C	LDL-C	SBP	DBP
β[a] (95% CI)	**8.41** [0.78; 16.03]	−0.37 [-2.59; 1.86]	**8.02** [1; 15.04]	**3.21** [0.82; 5.6]	0.64 [-1.16; 2.44]
PFAS	**Weigths**				
PFOA	0.07	0.03	0.04	**0.31**	**0.43**
PFOS	0.08	0.24	0.07	**0.56**	**0.25**
PFHxS	**0.38**	0.1	**0.25**	0.06	0.2
PFNA	**0.48**	**0.64**	**0.65**	0.08	0.12

[a] β represents the increase of cholesterol level and blood pressure associated with a quartile increase in the WQS index. In bold weights that exceed the case of uniform weights ($\geq$(number of chemicals)$^{-1}$ = 0.25).

concentrations on previous occupational exposed populations were mostly not available, with the exception of a US study which reported a median measured serum PFOA level of 113 ng/mL (Steenland et al., 2015).

Although the literature on the effect of single PFAS and cardiometabolic outcomes is substantial, few previous studies have examined this relationship in an occupational setting (Costa et al., 2009; Olsen et al., 2003, 2012; Olsen and Zobel, 2007; Sakr et al., 2007; Wang et al., 2012), and none of them considering the combined effects of the exposure to multiple PFAS.

Some of those previous studies looking at the relationship between serum PFOA and lipids, supported our findings. Sakr (Sakr et al., 2007) reported a statistically significant association between PFOA and total cholesterol, in a longitudinal study based on 454 workers (1 ppm increase in serum PFOA was associated with a 1.06 mg/dL increase in total cholesterol). Similarly, Costa (Costa et al., 2009) reported a weak but significant association of total cholesterol with PFOA in 53 workers followed for 30 years in a medical surveillance plan (1 μg/mL increase in PFOA associated with a 0.028 mg/dL increase in total cholesterol). However, neither of them found evidence of an association between PFOA and HDL-cholesterol. On the other hand two other studies reported a negative association between PFOA and HDL-C (Olsen and Zobel, 2007; Wang et al., 2012). Conversely, other studies found no evidence of PFOA associations with serum total cholesterol (Olsen et al., 2003, 2012; Olsen and Zobel, 2007) or LDL (Olsen and Zobel, 2007; Sakr et al., 2007; Wang et al., 2012). Only two studies investigated the relationship between serum lipids and PFOS concentration (Olsen et al., 2003, 2012), showing no significant association with both TC and LDL. Moreover, no association was found between estimated historical PFOA serum levels and incidence of self-reported hypertension and hypercholesterolemia in Steenland and colleagues' large occupational study on 3713 workers at the DuPont Washington Works facility (Steenland et al., 2015).

A possible explanation for some of these different findings may relate to non-linearity in the exposure-response relationship. In fact, studies that encompass very wide exposure ranges it has been observed the incremental effect of PFOA into cholesterol level is steeper in the low exposure range up to 50 ng/nL than in the higher ranges, which is more typical in occupational exposure settings (Canova et al., 2020; Frisbee et al., 2010; Steenland et al., 2009) as well as in the present study. Conversely, all the other 3 PFAS reported a lower level of concentrations and partially explained the patterns observed.

Conversely, there was no previous published study on the association between PFAS concentrations and blood pressure in occupationally exposed individuals. Available data derived from studies on populations with background levels of exposure (Liao et al., 2020; Lin et al., 2019; Ma et al., 2019; Min et al., 2012) or on highly exposed individuals (Bao et al., 2017; Pitter et al., 2020d), still relatively limited and with contradictory results. An increase in SBP was often associated with higher concentrations of PFOA (Liao et al., 2020; Lin et al., 2019; Min et al., 2012), PFOS (Bao et al., 2017; Min et al., 2012) and PFHxS (Min et al., 2012); while a positive association between PFAS and DBP was found only for PFOS (Liao et al., 2020; Ma et al., 2019) and PFNA (Min et al., 2012).Findings from a large cross-sectional study on the exposed community of young adults in the Veneto Region (Pitter et al., 2020d),

displayed a clear positive association between serum PFAS concentrations and blood pressure levels, especially among males. The present study on a small population of male past workers is consistent with the above-mentioned results from the general population and thus lends support to the association between serum PFAS and SBP in males. The magnitude of the effect per ln-increase was modest, albeit greater than that observed in the general population. Since the serum PFOA distribution was the most skewed toward high values, subjects in the highest quartile (470-13,000 ng/ml) had a considerable predicted increase of SBP of more than 7 mmHg. However, even smaller increases of SBP may be of clinical significance at the individual level, especially in individuals with the concomitant presence of other cardiovascular risk factors or comorbidities, pushing their global cardiovascular risk upwards. Also, from a public health point of view, even a modest shift of the population's SBP distribution towards higher levels determines a correspondent excess of potentially amenable cardiovascular events (Hardy et al., 2015; Lewington, 2002).

Comparing our results across single-chemical linear and WQS regression, some similarities and differences emerged. First, both models suggested that higher exposure to some PFAS was associated with higher level of TC, LDL-C and SBP, though the strength of the association varied by model employed. Second, we identified PFNA and PFHxS as the most important contributor in the observed associations with TC and LDL-C. Using WQS though, PFOS was no longer identified as *chemical of concern* for lipid parameters, conversely from what single-pollutant analysis suggested. The correlation between PFNA and PFOS could explain why PFOS weights in lipids were lower than expected. Instead, PFOA and PFOS have exceeded the threshold of 25% when related to SBP, consistent with what was found with LMMs.

Considering the association with lipid parameters, PFNA appeared to have a greater contribution than PFOA and PFOS. This unexpected results can be explained by the limited range of variation of this PFAS respect to the other PFAS, a longer persistence in the body and potentially, a greater intrinsic toxicological power (Gleason et al., 2015). A partial support of this latter hypothesis, can be found in experimental in vitro studies considering a series of PFASs in cultured cells, where PFNA was the most potent activator of human and mouse peroxisome proliferator-activated receptor-alpha (PPAR-α), a nuclear receptor believed to be involved in many of the toxic effects caused in general by PFAS (Louisse et al., 2020). This latter is probably not the only route that guide the reported metabolic alteration. However, a particular attention has to be paid in the interpretation of the effects resulting by the analysis of PFAS mixture, given the different toxicological effect of each single component (Ojo et al., 2020).

In the current state of knowledge, the mechanisms underlying the association between PFAS and serum lipids are not completely understood (Fragki et al., 2021). For example, PFAS can bind to serum lipoproteins, however, the percentage of PFAS bound to serum lipoproteins is low (Butenhoff et al., 2012). Another possible contribution to this association may be a confounding mechanism that affects serum levels of both PFAS and lipids: since PFAS levels may alter entero-hepatic circulation, and consequently the activity of bile acids transporters, a reduction of serum cholesterol levels can be the ending result (Zhou et al., 2017).

Biological mechanisms linking PFAS exposure to hypertension are not clearly established (Liao et al., 2020). One indirect route might be mediated by lipid changes, as increases of lipid level can lead to pressure alterations as well as increases of reaction oxygen species (Wielsøe et al., 2015) which in turn is a key mechanism of arterial damage and endothelial dysfunction. However, the Pearson's correlation coefficient between TC and SBP in the studied population was low (0.07). Furthermore, adjusting for total cholesterol did not change the observed association between PFAS mixture and systolic blood pressure (2.72 mmHg with 95% CI: 0.32–5.11). In addition PFASs have also been reported to affect endothelial barrier function by regulating connections (Liu et al., 2018) which is in turn related to hypertension (Konukoglu and Uzun, 2016).

The main strength of the present study consisted in investigating the effect of PFAS concentration on multiple cardiometabolic outcomes, not merely lipids, in an occupational exposure setting, considering a multi-chemicals specific approach. In fact, the application of WQS regression allowed us to account for the correlation among these chemicals and estimate the combined effect of a mixture of PFAS concentration. However, WQS regression scores exposures in quantiles, assuming linear exposure-outcome associations and a first-order approximation of non-additivity. Therefore, estimates of average weights may be influenced by WQS constraints and the discretization of the chemicals, leading to discrepancies with single chemical models.

Weaknesses include limited sample size and the cross-sectional nature of the study, which limited our ability to determine temporality of exposures in relation to the outcomes. Furthermore, we cannot rule out potential residual confounding from uncontrolled factors, included dietary habits and other non-measured occupational exposures co-occurring with PFAS exposure. However the reported associations were strong enough with respect to the potential effect of unconsidered confounders (Tozzi et al., 2019). We noticed a negative effect of age on total cholesterol, which is the opposite of what happens among the general population. Despite the exclusion of subjects taking lipid-lowering medication and the estimation of a model stratified by recruitment period, the negative effect of age on the total cholesterol remained. This unexpected result is probably due to the cross-sectional design of the study and the presence of unobserved factors which altered the inclusion probability in this occupational cohort, possibly due to health-related differential retirement and loss from the cohort among older workers.

Respect to previous works based on data from the Veneto surveillance plan (Canova et al., 2020; Pitter et al., 2020c), in the current study the main analysis included participants under lipid lowering medication or participants with self-reported diagnosis of hypertension or under treatment with antihypertensive medications; however sensitivities analyses confirmed the reported association.

The workers included in the analysis represent a significant fraction of all workers who ever worked at RM factory (33%, 232 out of 706 workers (Girardi and Merler, 2019)). Unfortunately, since we considered only living people with a voluntarily adhesion to the surveillance program, it was not possible to evaluate the effect of possible selection bias.

At the time of writing, this past worker population is now being recruited for a second blood sample, to quantify the decline of blood PFAS concentrations. These repeated measures provide an opportunity to estimate the half-life of these compounds among people previously exposed in occupational settings and to assess longitudinally whether and by how much the fall in serum PFAS levels is associated with a concomitant improvement of biomarkers as observed for lipids in the C8 study (Fitz-Simon et al., 2013).

5. Conclusions

In the present study we found that some PFAS exposures, have a significant strong association with cardiometabolic parameters in the RM factory employees. The mixture of the four PFAS found with higher concentrations in blood samples, was positively associated with TC, LDL-C and SBP. Altogether the results of this study showed a clear association between serum PFAS levels and markers of cardiovascular risk and support the importance of clinical surveillance of cardiovascular risk factors in population with a high exposure to PFAS, especially among those exposed in occupational settings.

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Authors contributions

Erich Batzella: Conceptualization, Data curation, Methodology, Writing - original draft, Paolo Girardi: Conceptualization, Writing - original draft, Methodology, Pitter Gisella: Conceptualization, Project administration, Resources, Funding acquisition, Writing - review & editing, Russo Francesca: Project administration, Resources, Funding acquisition, Writing - review & editing, Filippo Da Re: Project administration, Resources, Funding acquisition, Writing - review & editing, Fletcher Tony: Methodology, Supervision, Writing - review & editing, Canova Cristina: Conceptualization, Methodology, Resources, Supervision, Writing - original draft.

Ethics

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Regional (Veneto Region) Ethics Committee (24 maggio 2017 prot. n. 203,638).

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.envres.2022.113225.

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