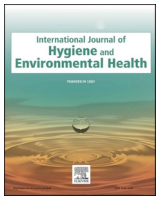
International Journal of Hygiene and Environmental Health 252 (2023) 114215

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh



Firefighters and the liver: Exposure to PFAS and PAHs in relation to liver function and serum lipids (CELSPAC-FIREexpo study)

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ARTICLE INFO

Keywords:
Firefighters
Occupational exposure
per- and polyfluoroalkyl substances/PFAS
Polycyclic aromatic hydrocarbons/PAHs
Mixture analysis
Liver function

ABSTRACT

Introduction: Firefighting is one of the most hazardous occupations due to exposure to per- and polyfluoroalkyl substances (PFAS) and polycyclic aromatic hydrocarbons (PAHs). Such exposure is suspected to affect the cardiometabolic profile, e.g., liver function and serum lipids. However, only a few studies have investigated the impact of this specific exposure among firefighters.

Methods: Men included in the CELSPAC-FIREexpo study were professional firefighters (n = 52), newly recruited firefighters in training (n = 58), and controls (n = 54). They completed exposure questionnaires and provided 1–3 samples of urine and blood during the 11-week study period to allow assessment of their exposure to PFAS (6 compounds) and PAHs (6 compounds), and to determine biomarkers of liver function (alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (BIL)) and levels of serum lipids (total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG)). The associations between biomarkers were investigated both cross-sectionally using multiple linear regression (MLR) and Bayesian weighted quantile sum (BWQS) regression and prospectively using MLR. The models were adjusted for potential confounders and false discovery rate correction was applied to account for multiplicity.

Results: A positive association between exposure to PFAS and PAH mixture and BIL (β = 28.6%, 95% CrI = 14.6–45.7%) was observed by the BWQS model. When the study population was stratified, in professional firefighters and controls the mixture showed a positive association with CHOL (β = 29.5%, CrI = 10.3–53.6%) and LDL (β = 26.7%, CrI = 8.3–48.5%). No statistically significant associations with individual compounds were detected using MLR.

Conclusions: This study investigated the associations between exposure to PFAS and PAHs and biomarkers of cardiometabolic health in the Czech men, including firefighters. The results suggest that higher exposure to a mixture of these compounds is associated with an increase in BIL and the alteration of serum lipids, which can result in an unfavourable cardiometabolic profile.

1. Introduction

Firefighting, as one of the most hazardous occupations, combines extreme physical and psychological demands, themselves potential risk factors, with the former including risk of acute trauma and exposure to both high temperatures and a complex mixture of hazardous pollutants released during fire suppression activities as well as from contaminated equipment and protective gear (Barros et al., 2021; Trowbridge et al., 2020). Previous studies have reviewed the increased incidence of cardiovascular disease (CVD) among firefighters (Soteriades et al., 2011,

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https://doi.org/10.1016/j.ijheh.2023.114215
Received 1 February 2023; Received in revised form 23 June 2023; Accepted 23 June 2023
Available online 5 July 2023

2019). The exposure of firefighters to per- and polyfluoroalkyl substances (PFAS) and polycyclic aromatic hydrocarbons (PAHs) may be of particular relevance due to recently reported associations between exposure to these compounds and adverse health outcomes in humans, many of which are related to CVD (Alshaarawy et al., 2016; Attanasio, 2019; Gallo et al., 2012a; Gleason et al., 2015a; Li et al., 2020a; Moorthy et al., 2015; Sakr et al., 2007a; Sakr et al., 2007b; Stanifer et al., 2018; Wagner et al., 2015; Xu et al., 2021; Yamaguchi et al., 2013).

PFAS are omnipresent and highly persistent synthetic chemicals extensively used for a variety of commercial and industrial applications due to their grease-, stain-, and water-repellent properties (Ho et al., 2022). Drinking water and diet have been identified as major sources of exposure in humans (Fenton et al., 2021). Firefighters are additionally exposed due to the application of PFAS in class B firefighting aqueous film-forming foams (AFFFs, used for extinguishing hydrocarbon-fuel and chemical solvent fires) and the use of PFAS-coated firefighting equipment (Khalil et al., 2022; Laitinen et al., 2014a; Pitter et al., 2020). Elevated levels of PFAS in firefighters' blood serum, especially perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS), have been reported in several studies (Dobraca et al., 2015; Jin et al., 2011; Laitinen et al., 2014a; Rotander et al., 2015a; Trowbridge et al., 2020).

PAHs consist of two or more fused benzene rings and are generated by the incomplete combustion of organic matter (Kim et al., 2013). The main exposure routes are the inhalation of polluted air or cigarette smoke, the ingestion of contaminated food, or dermal absorption (Li et al., 2020b). Several biomonitoring studies reported increased internal exposure among firefighters after firefighting activity compared to controls, even when using self-contained breathing apparatuses (SCBAs) for protection against the inhalation of airborne contaminants (Banks et al., 2021; Ekpe et al., 2021; Fent et al., 2020; Rossbach et al., 2020), suggesting dermal absorption as the most relevant route for PAH uptake (Andersen et al., 2018; Fent et al., 2019; Rossbach et al., 2020; Wingfors et al., 2018) as well as inhalation uptake due to SCBAs removal during the overhaul stage at the fire incident site (Banks et al., 2021; Baxter et al., 2014).

The liver, with its central role in the metabolism of xenobiotics, is considered to be the main target organ of both PFAS and PAHs. Epidemiological studies suggest associations between PFAS exposure and altered levels of biomarkers of liver function (e.g., liver enzymes, bilirubin, and serum lipids). However, reported associations are often inconsistent and cause-and-effect relationships have not yet been established (Costello et al., 2022; Darrow et al., 2016; Gallo et al., 2012a; Gleason et al., 2015a; Lin et al., 2010; Omoike et al., 2021; Salihovic et al., 2018; Stratakis et al., 2020). A high abundance of cytochrome P450 in the liver is responsible for the oxidation of PAHs, resulting in a complex mixture of hydroxylated metabolites (OH-PAHs), which are excreted predominantly through urine (Oliveira et al., 2020; Weyand and Bevan, 1986) and used as biomarkers of PAH exposure. Exposure to PAHs was also associated with altered liver biomarkers (Alshaarawy et al., 2016; Brucker et al., 2014; Wang et al., 2019). Abnormal liver function (altered levels of liver enzymes and bilirubin) and dyslipidaemia (altered levels of serum cholesterol, low-density lipoprotein, or triglycerides) are considered risk factors for developing CVD (Choi et al., 2018; Ekstedt et al., 2015; Ismaiel and Dumitraşcu, 2019; Soderberg et al., 2010).

Monitoring exposure among firefighters is challenging due to the unpredictability and specificity of particular firefighting activities, resulting in exposure to many chemicals in multiple exposure pathways. Many studies monitoring exposure both on- and off-duty are available (Banks et al., 2021; Ekpe et al., 2021; Rossbach et al., 2020; Rotander et al., 2015b; Trowbridge et al., 2020; Wingfors et al., 2018), though only a minority of these consider multiple exposures (Bessonneau et al., 2021; Fent et al., 2020; Laitinen et al., 2012; Park et al., 2015). A more consistent assessment of the simultaneous exposure to a wide range of chemicals is essential for the evaluation of potential health effects. Only a few studies have considered the simultaneous assessment of both exposure and effect biomarkers in firefighters (Andersen et al., 2018; Bessonneau et al., 2021; Oliveira et al., 2020) and, to the best of our knowledge, none of them have focused on the liver and lipidic health or simultaneous exposure to PAHs and PFAS. Therefore, this study aimed to assess the effects of exposure to PAHs and PFAS on liver function and serum lipid profile with a special focus on firefighters at different professional stages.

2. Materials and methods

2.1. Study population

The study population is described in detail in Říhářková et al. (2023). Briefly, between 2019 and 2020, a total of 166 participants were recruited for the CELSPAC-FIREexpo study, a collaborative research project with the aim of assessing firefighters' exposure to PAHs and PFAS while firefighting and training, and determining chemical and biochemical biomarkers of exposure and its effects. Participants were divided into 3 sub-cohorts: newly recruited firefighters before professional training for active participation in responses to incidents ("NEW FF"; n = 58), professional firefighters actively participating in responses to incidents ("PROF"; n = 52), and a control group of non-firefighters ("CTRL"; n = 54). PROF and NEW FF were recruited by the chief accredited project deputy in the Training Center of the Fire Rescue Service in Brno (Czech Republic). Controls were recruited at the Faculty of Sport, Masaryk University, Brno (Czech Republic). The participants were men (until August 2022 no female professional firefighters were actively participating in responses to incidents), either firefighters (active or enrolled in training) or physically active men (for the control group), who were 18–35 years old and non-smokers with no chronic diseases. Two participants withdrew from the study before completion. Therefore, a total of 164 participants, who had answered questionnaires and for whom information with respect to exposure and biochemical analyses was complete, were included in the present study. The study was approved by the ELSPAC Ethics Committee in 2019, and all participants gave their written informed consent.

2.2. Study design

The complete design of the CELSPAC-FIREexpo study based on 3 subcohorts (NEW FF, PROF, and CTRL) is described in detail in Říhářková et al. (2023). In this study, a reduced dataset was used for statistical analyses due to the specificity of the biomarkers included (Fig. 1). Upon inclusion in the study, all participants filled out exposure questionnaires about lifestyle and dietary factors possibly contributing to PFAS exposure (wearing GoreTex or eVent clothing, work in the ski service sector, skiing activities, source of drinking water, use of dental floss, and blood donation), PAH exposure (former smoking habit, years since quitting smoking, exposure to fire, type of heating at home), or exposure to both PFAS and PAH (length of firefighting career if relevant, diet and frequency of consumption of relevant foods and supplements). The questionnaires also included information on the presence of acute or chronic infectious disease, the participant's subjective health assessment, and employment (Table S1).

Participants from NEW FF were completing a 15-week initial professional training programme prior to becoming active firefighters; hence, they followed a specific experimental design. In phase 1 (weeks 1–5), NEW FF provided a morning void urine sample and fasting blood sample for the further analysis of exposure biomarkers (PFAS, OH-PAHs). Phase 2 (week 6) corresponded with the time 4 h after the firefight training in an indoor environment. In this phase, NEW FF again provided urine and non-fasting blood samples for the analysis of exposure biomarkers (PFAS, OH-PAHs). In phase 3 (week 10), which corresponded with the period 1 week after training with AFFFs (considered as PFAS exposure) and 4 weeks after phase 2, NEW FF once more provided

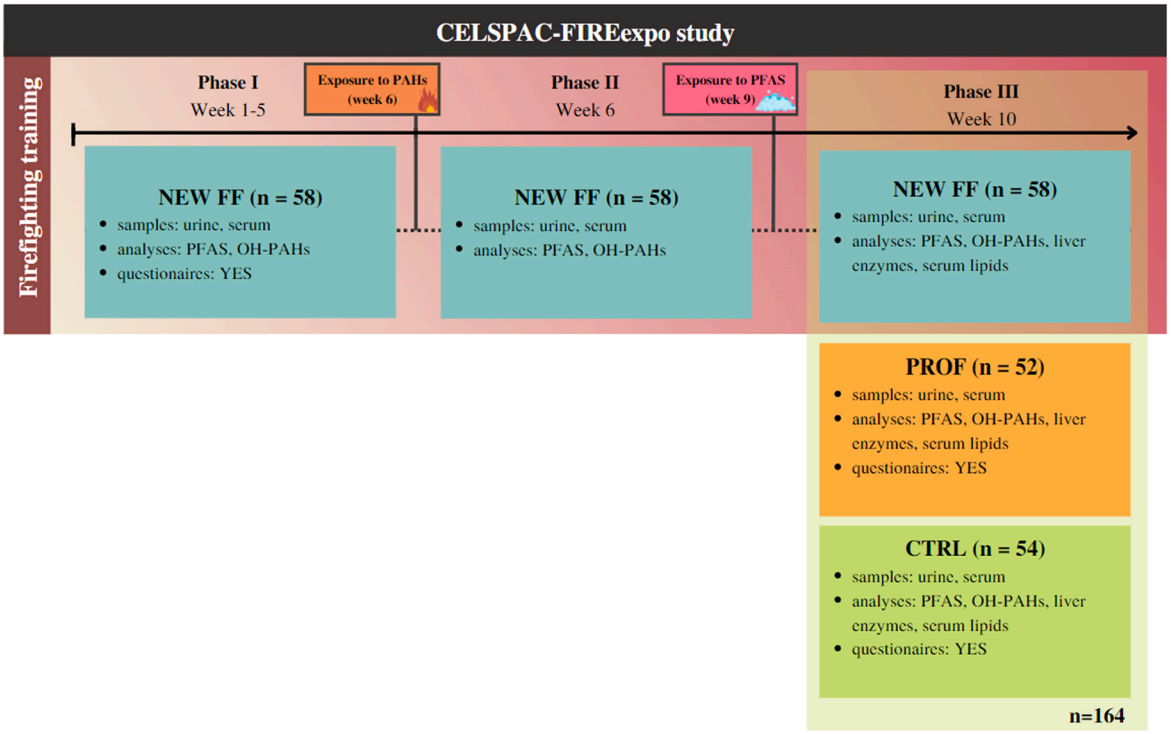

Fig. 1. Design of CELSPAC-FIREexpo study consisting of 3 sub-cohorts: new firefighters in training ("NEW FF"), professional firefighters ("PROF"), and controls ("CTRL"). In this study, NEW FF were monitored 3 times (correspondingly with phases 1, 2, and 3) throughout their firefighting training, while PFOF and CTRL provided just one set of samples.

fasting blood samples and morning void urine for the analysis of biomarkers of exposure (OH-PAHs, PFAS) and also biomarkers of liver function and serum lipids (biochemical analyses). A detailed description of the training activities and the equipment of trainees is provided in the supplementary material. Participants from the PROF- and CTRL-subcohorts provided a single sample of morning void urine and fasting blood, which was used for the analyses of both exposure biomarkers and biomarkers of liver function and serum lipids.

2.3. Blood and urine collection

Blood samples were collected by medical personnel in an operational ambulance. Urine samples were collected at the workplace by own urine collection following the instruction of medical personnel. In phases 1 and 3, morning void midstream urine was sampled, along with venous blood on an empty stomach. In phase 2, the sampling of morning void urine and venous blood on an empty stomach was not possible due to training schedule.

Venous blood for serum isolation was sampled in 7.5 mL S-Monovette® tube containing the Z-gel clotting activator. Each participant provided approximately 40 mL of midstream urine, which was collected in a 50 mL centrifuge tube. Both the venous blood and urine samples were immediately transported to laboratories in a cooling box set at 8 °C.

Once the clot had formed in the venous blood tube, it was centrifuged at 2500×*g* and 20 °C for 10 min. Subsequently, 0.5 mL aliquots were separated and placed into 1.2 mL cryotubes, which were then gradually frozen and stored in a biobank facility at −80 °C for further analyses of the biomarkers and biochemical analysis. Similarly, the urine samples in 50 mL centrifuge tubes were divided into 1 mL aliquots in 1.2 mL cryotubes, frozen gradually, and stored in a biobank facility at −80 °C until further analyses.

2.4. Determination of OH-PAHs and PFAS

A total of 6 PFAS in blood serum (perfluorohexane sulfonate [PFHxS], perfluorooctanoate [PFOA], perfluorooctane sulfonate [PFOS], perfluorononanoate [PFNA], perfluorodecanoate [PFDA] and perfluoroundecanoate [PFUnDA]) and 6 hydroxylated PAH metabolites (OH-PAHs) (1-hydroxynaphtalene [1-OH-NAP], 2-hydroxynaphtalene [2-OH-NAP], 2-hydroxyfluorene [2-OH-FLU], 3-hydroxyfluorene [3-OH-FLU], 1-hydroxypyrene [1-OH-PYR] and 2/3-hydroxyphenanthrene [2/3-OH-PHEN]) in urine were measured. Samples were analysed at RECETOX (Brno, Czech Republic) following the methods described in detail elsewhere (Říhářková et al., 2023).

OH-PAHs were analysed using the modified CDC method 6705.02 (CDC's National Center for Environmental Health (NCEH), n.d.). Briefly, 500 μL of each urine sample were transferred to a 96-well plate and β-glucuronidase solution and internal standards in hydrolysing buffer were added into each well. All components were then mixed and incubated at 55 °C for 2 h. Samples were extracted using SPE plate Oasis HLB (60 mg) and analysed using an Agilent 1200 series liquid chromatography (HPLC) system with analyte detection performed on AB Sciex Qtrap 5500 tandem mass spectrometer (MS) operating in negative electrospray ionization (ESI) mode. Regrading PFAS analysis, modified CDC method 6304.04 was used (CDC's National Center for Environmental Health (NCEH), 2013). Each serum sample was transferred to a 96-well plate (Phenomenex, USA) and internal standards and acetonitrile with an addition of 1% formic acid were added to each sample. Samples were then mixed, filtered, transferred to glass vials, and evaporated to the last drop, after which methanol and ammonium acetate (1:1) were added. Then, the samples were analysed using Qtrap 5500 LC-MS/MS system (ABSciex, CA, USA) with ESI. The mobile phases were methanol with 5 mM ammonium acetate in water (55:45, component A) and methanol (component B). Gradient elution was used. Laboratory and method performances were successfully verified by participation in third-party proficiency testing (ICI-EQUAS, OSEQA). List of chemicals and information regarding QA/QC is available in supplementary

material.

2.5. Biochemical measurements and specific gravity

The levels of alanine aminotransferase (ALT, in μkat/L), gamma-glutamyl transferase (GGT, in μkat/L), aspartate aminotransferase (AST, in μkat/L), alkaline phosphatase (ALP, in μkat/L), and total bilirubin (BIL, in μmol/L) in blood serum were considered as markers of liver function. Indicators of blood lipids included total cholesterol (CHOL, mmol/L), low-density lipoprotein (LDL, mmol/L), and triglycerides (TG, mmol/L). All markers were measured spectrophotometrically with an Alinity c instrument (©Abbott, Illinois, U.S.A). The specific gravity of urine samples (SG) was measured by a handheld refractometer (Atago PAL-10S).

2.6. Statistical analysis

The concentrations of PFAS and OH-PAHs below LOQ were imputed using maximum likelihood multiple estimation based on the observed values and an expected log-normal distribution (Lubin et al., 2004). SG-standardized concentrations of OH-PAHs in urine (based on Eq. S1 in SI), serum concentrations of PFAS, and all biochemical measurements were $\log_2$ transformed and IQR standardized to approach normality and reduce the influence of outliers. Spearman correlation coefficients were calculated to estimate correlations between the concentrations of PFAS and OH-PAHs as well as between the biochemical measurements. Differences in internal exposure and biochemical parameters between the study sub-cohorts were investigated using ANOVA/Kruskal-Wallis ANOVA with Tukey/Wilcox post hoc tests and $\chi 2$ test with post hoc tests.

Firstly, using individual multiple linear regression models (MLR), associations between each biomarker of exposure (considered individually) and each biomarker of liver function or serum lipids were examined cross-sectionally including all participants (PROF, CTRL, and NEW FF from phase 3). For each chemical compound, results are expressed as the relative change in the median of liver or serum lipids biomarker for a doubled concentration of OH-PAHs or PFAS in urine or serum, respectively. Secondly, associations between the OH-PAHs and PFAS mixture and biomarkers of liver function and serum lipids were assessed on the same dataset using Bayesian weighted quantile sum (BWQS) regression. BWQS is a novel approach that extends original weighted quantile sum (WQS) regression, which is designed to estimate the effect of a mixture of correlated chemicals by creating a single score summarizing overall exposure while accounting for the individual contributions of mixture components using weights. BWQS overcomes certain limitations of WQS, especially the requirement of the *a priori* selection of the directionality of the coefficients associated with the mixture, which improves the statistical power and stability of the estimates (Colicino et al., 2020; Maitre et al., 2022; Pedretti and Colicino, 2021). In addition, prospective associations were examined in the NEW FF sub-cohort by studying the effect of exposure measured in the 1st and 2nd phases of firefighting training on liver and serum lipids biomarkers measured in the third phase using MLR. All models were adjusted for the same set of confounders, identified on the basis of *a priori* knowledge and a directed acyclic graph (DAG) approach (Fig. S1) (Shrier and Platt, 2008): age (in years), body mass index (BMI, in [kg/m^2]), previous smoking (yes/no), length of firefighting career (in years) and study sub-cohorts (NEW FF/PROF/CTRL; except the prospective model for NEW FF).

The CELSPAC-FIREexpo study population was already quite specific and homogenous – it comprised men of similar ages from the same geographical region; hence, in this study, just a few sensitivity analyses were performed. To explore associations in the subpopulations with potentially different vulnerabilities, the dataset was stratified into 3 study sub-cohorts (NEW FF, PROF, and CTRL) and MLRs were performed. To evaluate the robustness of the associations with the mixture, the BWQS regression model was run for 3 population subsets – each time excluding one sub-cohort (subset *A* – professional FF and new FF; subset *B* – new FF and controls; subset *C* – professional FF and controls). Moreover, BWQS regression models for separate mixtures of PFAS and OH-PAHs were additionally run. To account for multiple testing in the case of linear regression models, correction for multiple comparisons with the false discovery rate controlled at <5% was performed (Benjamini and Yekutieli, 2005). All statistical analyses were performed using Rstudio version 4.0.2 (Rstudio Team, 2020).

3. Results

3.1. Study population characteristics

The main characteristics of the participants are given in Table 1. The participants included in the study were 26.4 years old on average, with professional firefighters being the oldest and new firefighters the youngest, which in the case of professionals corresponds with the length of the firefighting career. Professional firefighters and new firefighters had higher BMI compared to the control group. The highest rate of former smoking was reported among professional firefighters (Table 1).

Regarding liver function and serum lipids biomarkers, the correlation matrix is presented in supplementary material (Fig. S2). The strongest positive correlation observed was between CHOL and LDL. Serum lipids (CHOL, LDL, TG) were correlated with each other and with BMI. Moreover, CHOL and LDL were correlated with age and length of FF career. Length of FF career was also significantly positively correlated with age and BMI. There was no clear correlation pattern among the liver enzymes. Statistically significant differences between the sub-cohorts were observed in ALT, GGT, CHOL, LDL (PROF had higher levels compared to CTRL and NEW FF) and ALP (PROF had lower levels compared to NEW FF) (Table 1) (Fig. S3). PROF had also significantly higher proportion of participants with levels above the physiological limits in case of CHOL (compared to NEW FF) and LDL (compared to NEW FF and CTRL) (Table 1).

1-OH-NAP, 2-OH-NAP, 2-OH-FLU, and 2/3-OH-PHEN were detected in all samples, with 2-OH-NAP having the highest median concentration. For PFAS, the highest detection frequency was observed for PFNA and PFOA, and PFOS had the highest median concentration (Table 2, Table S2). There were no or only weak inter-compound correlations between PFAS and OH-PAHs; however, intra-class correlations were stronger suggesting similar sources of exposure (Fig. S4). Firefighters (PROF and NEW FF) had higher total PFAS concentrations compared to controls. ΣOH-PAHs levels were not different among PROF, NEW FF (phases 1 and 3), and CTRL. ΣOH-PAHs in NEW FF from phase 2 was significantly higher compared to the median concentrations in other sub-cohorts (Table 2).

3.2. Cross-sectional associations

The results from MLR applied cross-sectionally to the overall study population ($n = 164$) suggest a negative association between the levels of PFOS and TAG ($\beta = -11.6\%$, $p = 0.0225$), and positive associations between all OH-PAHs and BIL ($p < 0.05$). However, after FDR correction for multiple testing, no associations remained statistically significant (Fig. 2, Table S3).

The BWQS regression model for the mixture indicates similar results – the only statistically significant association of the full mixture was with bilirubin ($\beta = 28.6\%$, CrI = 14.6–45.7%), with PFHxS, 1-OH-PYR, and 2/3-OH-PHEN as the most active compounds in the mixture (Fig. 3). Nearly significant positive tendencies were observed between the mixture and CHOL and LDL. When the PFAS mixture was considered separately (without OH-PAHs), a negative association between the mixture and TG became significant (Table 3).

Table 1
Characteristics of the study population. ×- statistically different from new firefighters in training; *- statistically different from new firefighters in training and controls.

Characteristics		Overall study population	New firefighters in training	Professional firefighters	Controls
		n = 164	n = 58	n = 52	n = 54
		Mean ± SD			
Age (years)		26.4 ± 4.3	25.0 ± 3.6	28.4 ± 3.6	26.0 ± 4.9
BMI		25.82 ± 2.70	26.31 ± 2.83	26.14 ± 2.35	24.99 ± 2.72
Former smoking					
yes		21 (13%)	7 (12%)	10 (19%)	4 (7.4%)
no		143 (87%)	51 (88%)	42 (81%)	50 (92.6%)
Length of FF career (years)		1.76 ± 2.77	0.88 ± 0.67	4.58 ± 3.43	0.00 ± 0.00
Biomarkers	**Normal range**	**Median ± IQR**			
ALP (μkat/L)	≤2.15	1.17 (1.0–1.3)	1.21 (1.1–1.4)	1.10 × (0.98–1.2)	1.19 (1.0–1.4)
	out of the range:	0.6%	0.0%	0.0%	1.9%
ALT (μkat/L)	≤0.68	0.42 (0.33–0.55)	0.40 (0.31–0.54)	0.48 * (0.38–0.64)	0.41 (0.32–0.49)
	out of the range:	13.4%	13.8%	21.2%	5.6%
AST (μkat/L)	≤0.62	0.47 (0.39–0.59)	0.46 (0.40–0.57)	0.45 (0.38–0.60)	0.50 (0.4–0.62)
	out of the range:	22.6%	20.7%	23.1%	24.1%
GGT (μkat/L)	≤1.00	0.34 (0.26–0.43)	0.33 (0.25–0.40)	0.39 * (0.29–0.53)	0.31 (0.25–0.36)
	out of the range:	1.2%	0.0%	3.8%	0.0%
BIL (μmol/L)	≤18.70	13 (9.0–17)	14 (9.0–17)	13 (9.0–17)	12 (10–16)
	out of the range:	17.7%	19.0%	15.4%	18.5%
CHOL (mmol/L)	≤5.00	4.50 (4.1–5.2)	4.20 (4.0–4.7)	4.90 * (4.4–5.4)	4.45 (3.7–5.2)
	out of the range:	29.3%	12.1%	48.1% ×	29.6%
LDL (mmol/L)	≤3.00	2.8 (2.4–3.3)	2.65 (2.4–3.1)	3.15 * (2.7–3.7)	2.75 (2.3–3.3)
	out of the range:	38.4%	29.3%	55.8% *	31.5%
TAG (mmol/L)	≤1.70	1.06 (0.83–1.4)	1.02 (0.83–1.3)	1.15 (0.87–1.6)	1.02 (0.79–1.4)
	out of the range:	18.3%	15.5%	23.1%	16.7%

Abbreviations: IQR – interquartile range; BMI – body-mass index; FF – firefighting; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase, BIL – total bilirubin, CHOL – total cholesterol, LDL – low-density lipoprotein cholesterol, TG – triglycerides.

3.3. Prospective associations

To assess the prospective effects of internal exposure on liver and serum lipids in NEW FF, associations between the exposure from phases 1 and 2 and liver and serum lipids biomarkers from phase 3 were determined. Phase 1 levels of serum PFUnDA and PFDA showed a negative association with ALT. Phase 1 2-OH-FLU and 3-OH-FLU were positively associated with BIL from phase 3. After FDR correction, no associations remained statistically significant (Table S4). Internal exposure from phase 2 was measured 4 h after indoor training with fire; hence, increased concentrations of OH-PAHs in urine were observed in this phase (Table 2). Phase 2 2-OH-NAP and PFHxS showed significant positive and negative associations with ALP from phase 3, respectively. Negative associations were observed between phase 2 2-OH-FLU and 3-OH-FLU and phase 3 CHOL; and between 2/3-OH-PHEN, 2-OH-FLU and 3-OH-FLU and phase 3 LDL. Phase 2 PFNA showed a positive association with BIL. After FDR correction for multiple testing, all associations became insignificant (Table S4).

3.4. Sensitivity analyses

When we stratified our study population into sub-cohorts (NEW, PROF, and CTRL), the cross-sectional associations from MLR indicated similar trends (Table S5). In the NEW FF sub-cohort, we detected positive associations between PFNA, 2/3-OH-PHEN, 2-OH-FLU and 3-OH-FLU and bilirubin, negative associations between 2/3-OH-PHEN and CHOL and LDL, and negative associations between 1-OH-NAP, 2-OH-NAP, 2-OH-FLU, and 3-OH-FLU, and TG. In the case of PROF, positive associations between 1-OH-NAP and ALP, PFDA and LDL, and between 1-OH-NAP, 2/3-OH-PHEN, and 2-OH-FLU and TG were detected. A negative association between PFUnDA and TG was also observed. In the case of CTRL, positive associations between 4 OH-PAHs (1-OH-NAP, 2-OH-NAP, 1-OH-PYR, 2/3-OH-PHEN, 2-OH-FLU) and BIL and between 1-OH-PYR and CHOL were detected. However, after correction for multiplicity, all associations became insignificant (Table S5).

BWQS regressions were run for 3 population subsets, each time excluding one sub-cohort and including the other two (*A* – PROF and NEW FF; *B* –NEW FF and CTRL; *C* – PROF and CTRL). The results from the stratified BWQS models are summarized in Table 4. Regarding subsets A and B, the results are very similar to the main analysis – significant positive association with BIL (β_{A_BIL} = 23.4%, β_{B_BIL} = 38.8%). However, when excluding NEW FF (subset *C* – PROF and CTRL), besides the association with BIL (β_{C_BIL} = 25.8%), the mixture also showed significant positive associations with CHOL and LDL (β_{C_CHOL} = 29.5%, β_{C_LDL} = 26.7%).

4. Discussion

In this original cohort study including occupationally exposed firefighters at different professional stages, we found that exposure to the mixture of PFAS and PAHs is associated with an increase in bilirubin and changes in the lipid serum profile.

In general, lower bilirubin levels and dyslipidaemia are considered indicators of the risk of developing CVDs (Choi et al., 2018; Ekstedt

Table 2
Levels of PFAS in serum and SG-adjusted levels of OH-PAHs in the urine of participants. "*" – significantly different from other sub-cohorts; "(1)" – significantly different from new firefighters in training in phase 1; "(2)"– significantly different from new firefighters in training in phase 3.

	Overall study population	New firefighters in training			Professional firefighters	Controls
	n = 164	n = 58			n = 52	n = 54
	Phase III	Phase I	Phase II	Phase III	Phase III	Phase III
PFAS		**Median (25th – 75th percentile) [ng.mL^{-1}]**				
PFOA	1.03 (0.76–1.3)	1.18 (1.0–1.5)	1.22 (0.88–1.5)	1.12 (0.83–1.3)	1.21 (0.92–1.5)	0.82 * (0.54–1.1)
PFNA	0.36 (0.26–0.45)	0.41 (0.3–0.55)	0.41 (0.32–0.51)	0.39 (0.28–0.46)	0.4 (0.29–0.54)	0.29 * (0.24–0.36)
PFDA	0.16 (0.12–0.22)	0.19 (0.12–0.27)	0.21 (0.15–0.29)	0.18 (0.12–0.26)	0.19 (0.14–0.25)	0.13 * (0.1–0.16)
PFUnDA	0.06 (0.04–0.08)	0.05 (0.02–0.08)	0.07 (0.03–0.09)	0.07 (0.04–0.09)	0.05 (0.04–0.07)	0.06 (0.03–0.08)
PFHxS	0.45 (0.34–0.58)	0.46 (0.35–0.59)	0.45 (0.35–0.55)	0.44 (0.39–0.56)	0.49 (0.38–0.66)	0.43 (0.33–0.54)
PFOS	2.72 (1.9–3.8)	2.82 (2.1–4.0)	3.13 (2.0–4.2)	2.90 (2.0–3.9)	3.22 (2.3–4.8)	2.19 * (1.5–2.7)
∑PFAS	4.78 (3.9–6.4)	5.38 (4.2–6.4)	5.67 (4.1–6.7)	5.00 (4.1–6.5)	5.56 (4.6–7.6)	3.93 * (2.9–4.9)
OH-PAHs		**Median (25th – 75th percentile) [ng.mL^{-1}]**				
1-OH-NAP	2.09 (1.2–3.7)	2.94 (1.6–4.9)	13.3 * (8.6–20)	1.95 (1.2–3.7)	2.43 (1.6–3.9)	1.7 (1) (1.2–3.3)
2–OH–NAP	5.11 (3.3–8.3)	5.48 (3.6–10)	18.1 * (11–27)	5.37 (3.6–8.6)	6.37 (3.8–8.8)	4.81 (3.0–6.6)
2–OH–FLU	0.37 (0.26–0.52)	0.36 (0.30–0.52)	0.99 * (0.69–1.6)	0.38 (0.27–0.54)	0.39 (0.28–0.49)	0.36 (0.22–0.51)
3–OH–FLU	0.08 (0.05–0.15)	0.11 (0.07–0.16)	0.2 * (0.13–0.3)	0.07 (0.05–0.15)	0.11 (0.06–0.16)	0.08 (0.05–0.12)
1–OH–PYR	0.13 (0.08–0.22)	0.15* (0.1–0.24)	0.46 * (0.31–0.63)	0.22 * (0.13–0.33)	0.11 (0.07–0.15)	0.10 (0.06–0.14)
2,3–OH–PHEN	0.24 (0.16–0.36)	0.25 (0.16–0.37)	0.62 * (0.49–0.86)	0.26 (0.18–0.29)	0.20 (2) (0.15–0.3)	0.26 (0.18–0.4)
∑OH–PAHs	8.69 (5.4–13)	10.16 (6.2–18)	37.81 * (21–53)	9.07 (5.5–15)	9.70 (5.6–13)	6.57 (4.8–12)

Abbreviations: IQR – interquartile range; PFAS – perfluoroalkyl substances; PFOA – perfluorooctanoate; PFNA – perfluorononanoate; PFDA – perfluorodecanoate; PFUnDA – perfluoroundecanoate; PFHxS – perfluorohexane sulfonate; PFOS – perfluorooctane sulfonate; OH-PAHs – hydroxylated polycyclic aromatic hydrocarbons; 1-OH-NAP – 1-hydroxynaphtalene; 2-OH-NAP – 2-hydroxynaphtalene; 2-OH-FLU – 2-hydroxyfluorene; 3-OH-FLU – 3-hydroxyfluorene; 1-OH-PYR – 1-hydroxyprene; 2/3-OH-PHEN – 2/3-hydroxyphenanthrene.

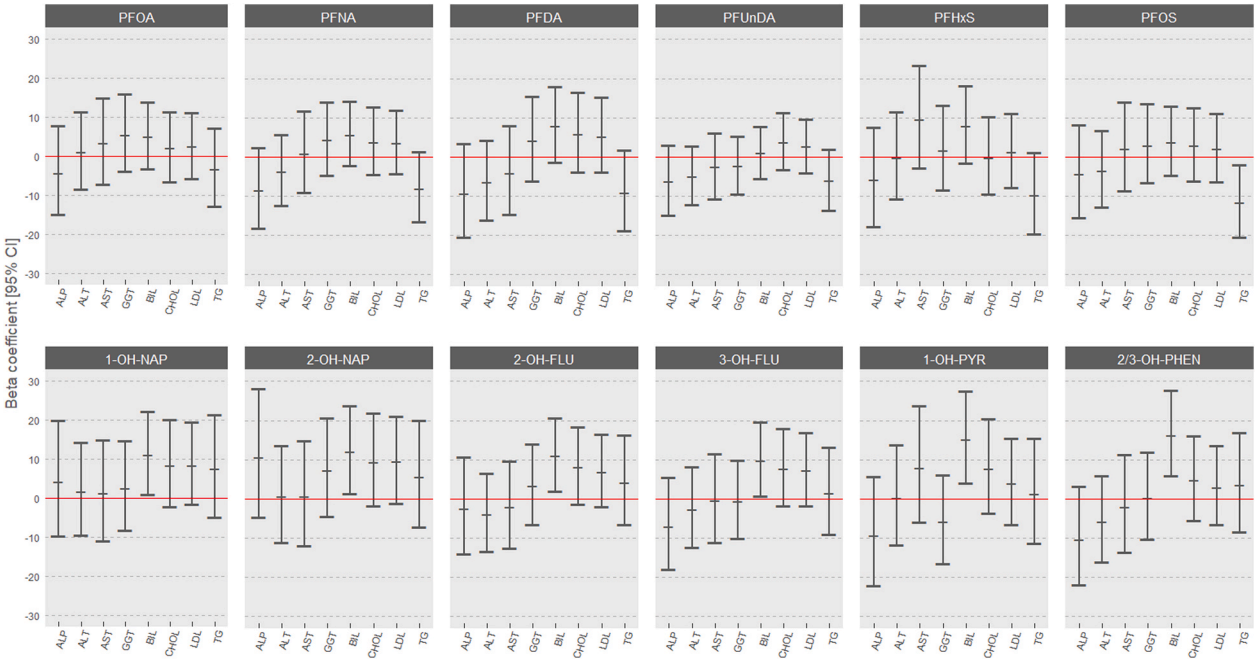

Fig. 2. Adjusted β-coefficients and 95% confidence intervals (CI) between the internal exposure (SG-adjusted urinary levels of OH-PAHs metabolites and serum PFAS levels) and biomarkers of liver function and serum lipids from cross-sectional multiple linear regression models (n = 164). Estimates are expressed as percent change in the median of biomarker upon doubling exposure levels. All models adjusted for age, BMI, former smoking, length of FF career, and study sub-cohort.

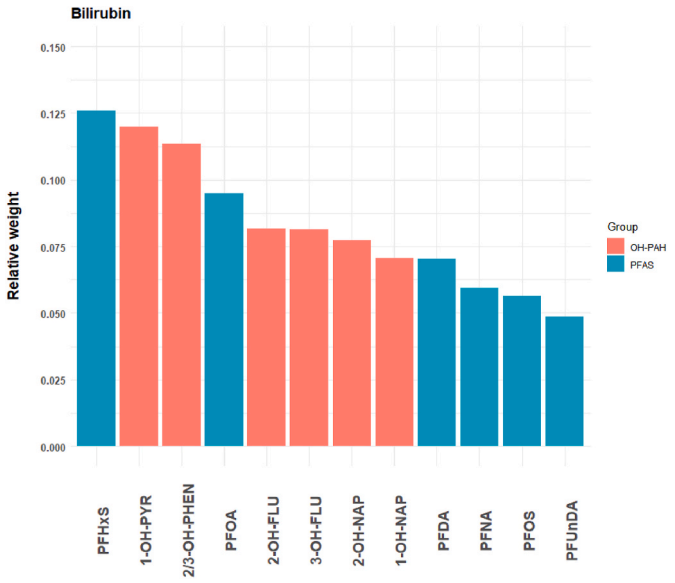

Fig. 3. Bayesian weighted quantile sum regression mixture composition estimates for total bilirubin (weights between 0 and 1).

et al., 2015; Ismaiel and Dumitraşcu, 2019; Méndez-Sánchez et al., 2017; Soderberg et al., 2010). Exposure to PFAS and PAHs, both common environmental pollutants, has been previously associated with the alteration of these biomarkers; however, the associations are not consistent. Firefighters are of particular interest due to their relevant occupational exposure and observed increased incidence of CVD (Soteriades et al., 2011, 2019). In spite of this, relatively few studies have focused on the associations between the exposure of firefighters and CVD biomarkers and risk factors; however, most of them suggest significant associations (Andersen et al., 2018; Oliveira et al., 2020; Semmens et al., 2016). The most robust association observed within this study was the positive association between exposure to the mixture of PFAS and PAHs and total serum bilirubin, tested by BWQS regression and supported by the results from MLR. Biliverdin, a precursor of bilirubin, is a product of the degradation of haemoproteins (e.g., haemoglobin, cytochrome P450) by haem oxygenase (HO), which is subsequently transformed into highly lipophilic bilirubin by biliverdin reductase. In blood, it is bound to the plasma protein albumin and then transported to the liver, where conjugates are formed, mostly bilirubin glucuronide by the action of UDP-glucuronosyltransferase 1, and then excreted via bile (Tomaro et al., 2002). The most active components of the mixture were PFHxS, 1-PYR, and 2/3-OH-PHEN (Fig. 3), which is partially in line with the results from MLR (Table S3). The association was significant also when PFAS and PAH mixtures were assessed individually (Table 3).

Studies focusing on the associations between liver functions and levels of urinary OH-PAHs are available (Alhamdow et al., 2017; Min et al., 2015; Xu et al., 2021); however, none of them focuses on bilirubin as a biomarker. Fortunately, similar trends have been observed in studies with rodents and hence support causality – after the administration of phenanthrene, pyrene, or their ozonized products, levels of bilirubin were increased compared to control treatments (Yoshikawa et al., 1985). Zhu et al. observed increased bilirubin levels after pyrene exposure in adult male rats compared to the control treatment (Zhu et al., 2018). Both studies used pyrene because, in general, pyrene is present in all PAH mixtures at relatively high concentrations, and, since its metabolite 1-OH-pyrene is stable and easy to measure, it is frequently used as a biomarker of exposure to PAHs (Kim et al., 2013).

Regarding PFAS, previous epidemiological studies focusing on PFAS exposure provide evidence suggestive of liver damage due to the alteration of serum enzymes and bilirubin levels associated with PFAS

Table 3
β-coefficients from BWQS mixture models with 95% credibility interval (CrI) for the full mixture (PFAS and OH-PAHs), PFAS-mixture, and OH-PAHs-mixture. All models were adjusted for age, BMI, former smoking, length of FF career, and study sub-cohort (n = 164). β represents a relative change in the median of the biomarker upon a doubling concentration of the mixture. **Bold** and "*" refer to statistically significant results.

	Full mixture				PFAS mixture				OH-PAH mixture			
	β	95% CrI			β	95% CrI			β	95% CrI		
ALP	−10.2	−24.4	7.5		−10.1	−21.0	3.2		−0.9	−12.1	12.0	
ALT	−7.5	−20.2	7.2		−6.5	−16.3	3.6		−1.3	−10.8	8.5	
AST	−3.2	−17.7	14.3		−1.4	−13.1	11.3		−1.8	−11.6	9.4	
GGT	6.4	−7.2	22.4		4.0	−6.9	16.5		3.2	−6.6	13.0	
BIL	**28.6**	**14.6**	**45.7**	*	**11.7**	**1.3**	**23.1**	*	**16.0**	**6.9**	**26.5**	*
CHOL	10.8	−3.0	27.8		2.6	−8.0	14.0		7.5	−1.7	17.0	
LDL	9.9	−4.0	24.2		1.2	−8.2	11.3		7.7	−0.6	16.7	
TG	−11.2	−24.9	5.8		**−14.3**	**−24.4**	**−3.3**	*	3.5	−7.1	15.7	

Abbreviations: PFAS – perfluoroalkyl substances, OH-PAH – hydroxylated polycyclic aromatic hydrocarbons, ALT – alanine aminotransferase, GGT – gamma-glutamyl transferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, BIL – total bilirubin, CHOL – total cholesterol, LDL – low-density lipoprotein, TG – triglycerides.

Table 4
β-values from BWQSR mixture models with 95% credibility interval (CrI) for the full mixture (PFAS and OH-PAHs) for specific subsets. **A** – new firefighting trainees (NEW FF) and firefighters professionals (PROF), n = 110; **B** – NEW FF and control sub-cohort (CTRL), n = 112; and **C** – PROF and CTRL, n = 106. All models were adjusted for age, BMI, former smoking, length of FF career, and study sub-cohort.

	A				B				C			
	β	95% CrI			β	95% CrI			β	95% CrI		
ALP	−0.5	−18.3	21.1		−18.9	−35.0	1.3		−10.8	−32.4	17.1	
ALT	−11.0	−25.9	6.9		−10.1	−24.4	7.7		−0.5	−18.0	19.7	
AST	−13.4	−29.8	6.9		−5.1	−20.8	13.9		4.7	−15.2	27.3	
GGT	4.1	−13.5	25.8		4.6	−10.0	22.3		15.5	−5.0	40.5	
BIL	**23.4**	**7.5**	**42.2**	*	**38.8**	**21.9**	**57.9**	*	**25.8**	**7.8**	**48.2**	*
CHOL	−1.1	−15.0	14.5		4.1	−11.1	22.0		**29.5**	**10.3**	**53.6**	*
LDL	0.2	−13.3	14.6		3.1	−11.5	20.1		**26.7**	**8.3**	**48.5**	*
TG	−12.4	−27.0	5.5		−13.3	−26.5	3.0		−1.8	−27.9	30.8	

exposure (Costello et al., 2022; Darrow et al., 2016; Gallo et al., 2012a; Gleason et al., 2015a; Lin et al., 2010; Omoike et al., 2021; Salihovic et al., 2018). Our results are in line with observations from the NHANES study, which suggested positive associations of total serum bilirubin with PFOS and PFOA, and an inconsistent positive association with PFHxS (Gleason et al., 2015a). In another study, BIL was positively associated with internal exposure to PFNA and PFHxS (Lin et al., 2010). Gallo et al. observed a clear positive association between bilirubin and serum PFOS levels (Gallo et al., 2012a). Positive associations between BIL and PFHxS, PFNA, PFOA, and PFOS were also observed in the recent NHANES study (Omoike et al., 2021). In contrast, many available studies report inverse associations of bilirubin with serum PFAS levels (Costa et al., 2009; Darrow et al., 2016; Olsen and Zobel, 2007; Sakr et al., 2007b) which is in line with the fact that decreased levels of BIL are associated with an increased risk of CVDs (Méndez-Sánchez et al., 2017).

Such inconsistency among available studies might be explained by the U-shaped relationship between bilirubin and serum PFAS levels, which was already proposed for PFOA (Gallo et al., 2012a). The authors suggest increasing levels of BIL per increasing levels of PFOA at low PFOA levels and decreasing BIL levels for concentrations of PFOA above about 40 ng/mL (Gallo et al., 2012a). This trend can be observed throughout the available studies – results from strongly/occupationally exposed populations with medians in the range of μg/mL show inverse associations (Costa et al., 2009; Darrow et al., 2016; Olsen and Zobel, 2007; Sakr et al., 2007b), while studies focusing on cohorts with milder exposure (in the range of ng/mL) report positive associations (Gallo et al., 2012a; Gleason et al., 2015a; Lin et al., 2010; Omoike et al., 2021).

Besides bilirubin being the end product of haem metabolism, it is also an endogenous antioxidant protectant. It is capable of scavenging hydroxyl (·OH), hydroperoxyl (HO_2·), and superoxide anion (O_2^-·) radicals while oxidising itself to biliverdin, which is thanks to a large excess of bilirubin reductase subsequently regenerated back into bilirubin. This cycle allows nanomolar concentrations of bilirubin (20–50 nM) (Sedlak et al., 2009) to effectively neutralize millimolar concentrations of toxic oxidant agents (Méndez-Sánchez et al., 2017). Bilirubin metabolism depends on several enzymes, such as haem-oxygenase (catalysing the cleavage of the tetrapyrrole ring of haem (Sedlak et al., 2009)), glutathione-S-transferase (binding lipophilic bilirubin in its non-substrate sites and providing the storage of bilirubin within cells (Fukai et al., 1989)) and UDP-glucuronosyltransferase 1 (catalysing the conjugation of bilirubin with glucuronic acid upon excretion via bile (Kapitulnik, 2004)). All three above-mentioned enzymes are of toxicological significance due to their irreplaceable functions in mitigating oxidative stress (defined as the increased production of reactive oxygen species (ROS)), and/or detoxifying xenobiotics in the human body (Dasari et al., 2018; Doré et al., 1999; Guillemette, 2003; Llesuy and Tomaro, 1994). Hence, increased oxidative stress can potentially affect the metabolism of bilirubin on several levels. Both PAHs and PFAS are known for their potential to increase levels of pro-oxidant moieties in the human body (Lin et al., 2020; Oliveira et al., 2020; Omoike et al., 2021; Wielsøe et al., 2015; Yang et al., 2015). Such exposure can trigger an increase in blood bilirubin, a potent antioxidant responding to exposure-related oxidative stress, probably via the induction of haem-oxygenase (Kapitulnik, 2004; Llesuy and Tomaro, 1994; Ryter and Tyrrell, 2000; Tomaro et al., 2002). When considering a complex mixture, our data suggest that already low levels of PFAS and OH-PAHs (in the range of ng/mL, Table 2, Říhačková et al., 2023) can probably initiate detoxifying activities in the human body. When the exposure is more severe (in the range of hundreds to thousands of ng/mL), it can be assumed that the capacity of antioxidant systems becomes exhausted (Bélanger et al., 1997; Niki, 2010; Sedlak and Snyder, 2004) which may lead to hepatotoxic effects, as observed in other studies. The lower exposure levels in participants from this study might also be the reason for there being no robust associations with liver enzymes detected by MLR or BWQS.

When the PFAS mixture was considered separately, a significant negative association with TG was observed (Table 2). Similar results were reported for the Swedish cohort (Donat-Vargas et al., 2019) as well as for prenatally exposed children (Papadopoulou et al., 2021). However, these hypolipidemic effects were not observed in the majority of previous studies, which reported either positive or non-significant associations with serum lipids (Canova et al., 2020; Ho et al., 2022; Sakr et al., 2007a, 2007b; Steenland et al., 2009). PFAS can affect the metabolism of lipids via several non-exclusive mechanisms, mainly in hepatocytes, including the activation of nuclear receptors such as peroxisome proliferator-activated receptor alpha (PPARα), PPARγ, constitutive androstane receptor (CAR), and pregnane X receptor (Andersen et al., 2021; Beggs et al., 2016; Behr et al., 2020; Canova et al., 2020; Fragki et al., 2021; Ho et al., 2022). The results from animal models (including primates) are in line with the findings from our study – inverse associations between the levels of PFAS and serum lipids, including TG (Guruge et al., 2006; Haughom and Spydevold, 1992; Martin et al., 2007; Seacat et al., 2002), suggesting PPARα is a key player in PFAS toxicity (Donat-Vargas et al., 2019). However, the relative importance of these mechanisms in humans is still debatable (Knutsen et al., 2018), mostly due to the differences between human and animal models as well as the potential non-causality of observations (Donat-Vargas et al., 2019; Ho et al., 2022). When PFAS and PAHs were considered together in one complex mixture, the association was not significant, suggesting different modes of action of PFAS and PAHs, a notion supported also by the opposite (positive) directionality of β-coefficients associated with OH-PAHs from BWQS as well as from cross-sectional MLR models (Fig. 2, Table 3). Although non-significant, these observations are in line with available epidemiological studies which suggest that exposure to PAHs in humans is positively associated with early markers of CVD and atherosclerosis, including levels of serum lipids (Alhamdow et al., 2017; Holme et al., 2019; Shahsavani et al., 2021).

In the case of CHOL and LDL, no significant associations resulted from MLR models. The results from the assessment of mixture effects (Table 3) are in line with observations from other studies (Alhamdow et al., 2017; Costa et al., 2009; Dong et al., 2019; Emmett et al., 2006; Holme et al., 2019; Olsen and Zobel, 2007; Sakr et al., 2007a, 2007b; Shahsavani et al., 2021) revealing the positive directionality of β-coefficients between exposure to the PFAS/PAHs mixture and serum CHOL (β = 10.8%) and LDL (β = 9.9%), although the associations were not statistically significant (95% CrI_{CHOL} = −3.0 – 27.8% and 95% CrI_{LDL} = −4.0 – 24.2%). When the NEW FF sub-cohort was excluded as part of the sensitivity analysis, the associations with CHOL and LDL became significant (Table 4). Interestingly, in the case of NEW FF, the prospective associations between phase 2 OH-PAHs and phase 3 CHOL and LDL were negative (Table S4), while mostly positive tendencies were observed in cross-sectional sensitivity analyses in the PROF and CTRL sub-cohorts (Table S5). In rodents, oral exposure to PAHs caused the dysregulation of lipid metabolism by altering the expression of genes responsible for *de novo* fatty acid synthesis and the accumulation of lipids in hepatocytes, suggesting the increased uptake of lipids from the blood by the liver (Jin et al., 2014; Li et al., 2019, 2020a). The alteration of serum lipids via this pathway due to the single-point exposure of NEW FF during indoor firefight training in the 6th week of the study (phase 2) might be an explanation for the observed trends that were inconsistent with other sub-cohorts. Hence, it can be hypothesised that results from the prospective analysis of NEW FF reflect a biological response to a short-term single exposure of higher magnitude (phase 2), and that this single exposure also influenced the results from the cross-sectional analysis of NEW FF in phase 3. In contrast, it can be assumed that the results from PROF and CTRL reflect a biological response to rather long-term stable exposure. However, in NEW FF, it cannot be ruled out that the observed results may have been affected by reduced sample size (n = 58) and by differences in the metabolization and excretion rate of PAHs due to the intensive physical training undertaken by participants before and during

the study period, which might have affected basal metabolic rates, since the confounding variable of physical activity was not included in the models (Durand et al., 2011; Speakman and Selman, 2003).

Levels of BIL, CHOL, and LDL are considered risk factors for CVDs; hence, the results from this study suggest that individuals exposed to higher levels of PFAS and PAHs are more prone to develop an unfavourable cardiometabolic profile in terms of the exhaustion of bilirubin antioxidant capacity and the alteration of serum lipid levels, both leading to an increased risk of developing CVDs in the future. Firefighters are among these individuals because firefighting as well as firefighting training increase internal exposure to PFAS and/or PAHs (Barros et al., 2021; Clarity et al., 2021; Durand et al., 2011; Fent et al., 2020; Jin et al., 2011; Laitinen et al., 2012; Laitinen et al., 2014a; Oliveira et al., 2020). There is a need to continuously monitor such exposure, identify the exposure sources (during both on- and off-duty periods), and minimise it. It is essential to communicate this information about potential health risks and how to reduce them through safety training with both firefighters and policymakers. Since PAHs and PFAS are omnipresent environmental pollutants (Giesy and Kannan, 2002; Kim et al., 2013), not only firefighters, but also other occupationally and non-occupationally exposed individuals (e.g., people living in contamination hot-spots (McMahon et al., 2022), production workers (Sakr et al., 2007a), coke-oven workers, and chimney sweeps (Wagner et al., 2015)) might face increased health risks.

The major strengths of this study can be summarized in 3 points:

a) the use of the cohort from central Europe with a special focus on the occupational exposure of firefighters including in total 110 firefighters at various professional stages, which is rare due to challenges arising from collaboration with fire rescue teams;
b) the collection of a rich dataset of both exposure biomarkers (6 PFAS in blood and 6 OH-PAHs in urine) and biomarkers of liver function and serum lipids accompanied with data from questionnaires;
c) the use of a complex statistical approach, including the assessment of associations of liver function and serum lipids biomarkers with both individual compounds by means of linear regression with adjustments for multiple comparisons, and their mixtures by means of Bayesian weighted quantile sum regression.

In terms of limitations of the study, despite the high participation of firefighters, the sample size was from the statistical point of view relatively small, which limited drawing firm conclusions, particularly for the stratified analyses. Small sample size along with homogeneity of the study population also limited generalization to the whole Czech male population. Although the models presented in this paper were controlled for factors such as age, BMI, former smoking, and others, residual confounding by unmeasured factors, such as physical activity and fitness, cannot be excluded. Despite the proposed molecular mechanisms, the causality of the observed associations cannot be clearly confirmed due to cross-sectional character of analyses or limited sample size in case of prospective analyses. Lastly, until recently women did not participate in firefighting in the Czech Republic, hence, only men were included in this study. However, in August 2022, the first woman qualified as a professional firefighter and began to participate in incident responses, hence, more women are expected to follow. Due to sex-related effects of PFAS (Sen et al., 2022), specific monitoring of female firefighters in Czech Republic will soon be required.

5. Conclusion

There are emerging gaps in the occupational epidemiology of firefighting, one of the most hazardous occupations. This study investigated the effects of PAH and PFAS exposure on liver function and serum lipids, with a special focus on firefighters at different professional stages. The findings from studying both the effects of individual compounds (by means of linear regression) as well as the effects of complex mixtures (by means of Bayesian weighted quantile sum regression) suggest that increased exposure to these compounds, typical in firefighters, is associated with increased levels of bilirubin (a potent antioxidant with a proposed U-shaped dose-response curve) and increased levels of total cholesterol and low-density lipoprotein (risk factors for developing cardiovascular diseases). Due to the low sample size and the cross-sectional design of the study, further research is required to confirm the associations observed.

Ethics approval

The study was approved by the ELSPAC Ethics Committee, ethical approval number No: ELSPAC/EK/1/2019. All participants received an information brochure and participated in personal interviews in order to be fully informed about the study and their participation. In addition, informed consent was obtained from each participant before participation began. All data were pseudonymized to protect the identity of the participants.

CRediT author contributions

The authors made substantial contributions to the acquisition, analysis, and interpretation of the data and the drafting and revision of the manuscript. All authors also approved the final version of the paper and agreed to be accountable for all aspects of the work.

N.P.: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Visualisation; **L.M.**: Methodology, Investigation, Writing – Review & Editing; **N.S.**: Methodology, Investigation, Writing – Review & Editing, **K.Ř.**: Conceptualization, Investigation, Visualisation, Writing – Review & Editing; **A.P.**: Conceptualization, Investigation, Writing – Review & Editing; **J.K.**: Methodology, Validation, Writing – Review & Editing, **P.Š.**: Methodology, Validation, Writing – Review & Editing; **A. B. P.**: Methodology, Investigation, Writing – Review & Editing; **P.G.**: Methodology, Visualisation, Writing – Review & Editing; **M.V.**: Methodology, Resources, Investigation, Writing – Review & Editing, Supervision; **P.Č.**: Methodology, Resources, Investigation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Funding

This work was funded by the European Union's Horizon 2020 research and innovation program under grant agreement No 857487 and supported under grant agreement No 857340, grant agreement No 857560 and grant agreement No 874627. This work was supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) - Funded by the European Union – Next Generation EU. This work was supported by the European Union's Horizon Europe research and innovation programme in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC) under grant agreement No 101057014. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the Health and Digital Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

LM is funded by a Juan de la Cierva-Incorporación fellowship (IJC2018-035394-I) awarded by the Spanish Ministerio de Economía, Industria y Competitividad. ISGlobal acknowledges support from the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019–2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program.

Declaration of competing interest

The authors declare that they have no known competing financial

interests nor personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Research Infrastructure RECETOX RI (No LM2018121 and LM2023069) and the large research infrastructure project BBMRI.cz (No LM2023033) financed by the Ministry of Education, Youth and Sports, and Operational Programme Research, Development, and Education – project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) and CETOCOEN PLUS (CZ.02.1.01/0.0/0.0/15_003/0000469) for supportive background.

The authors thank Ludmila Šebejová and Zuzana Jašková from CELSPAC Biobank for support with serum isolation and the preparation of serum and urine aliquots, and for storing samples in the CELSPAC biobank facility; Lenka Andrýsková for her help with addressing the ethical aspects of the study, Iva Poláková and Janka Vranová from the RECETOX Trace laboratories for sample preparation; Ondřej Mikeš for his help with the preparation of exposure questionnaires; and Tomáš Janoš for the determination of urine specific gravity. We would also like to thank Richard Hůlek, Mazen Ismael, Zuzana Luhová and Jiří Bilík from RECETOX Information systems and data services for the preparation of a data warehouse and infrastructure to store and manage the CELSPAC – FIREexpo cohort data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2023.114215.

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