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Association of perfluoroalkyl substances (PFAS) with liver function biomarkers in the highly exposed population of the veneto region

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ABSTRACT

Introduction: Epidemiological studies highlight the presence of associations between per- and polyfluoroalkyl substances (PFAS) exposure with liver damage. In 2013, PFAS contamination was discovered in Veneto (Italy), leading to the implementation of a Surveillance Program (SP). Our objective is to investigate the association between PFAS exposure and biomarkers of liver function using single-pollutant and mixture approaches, while exploring the sex-specific differences and the mediating role of obesity in the association.

Methods: The study included 42,094 subjects aged ≥20 years participating in the SP. We used generalized additive models to investigate the association between several PFAS and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, adjusting for possible confounders and stratifying by sex. Results were back-transformed to show predicted percentage changes in outcomes per ln-unit increase in PFAS levels; furthermore, we explored the role of BMI in the abovementioned causal pathway, considering it as a potential confounder or mediator PFAS joint effect was investigated using Quantile G-computation.

Results: One ln-unit increase in PFHxS concentrations was associated with a 1.49% (95%CI: 0.87, 2.12) and a 0.84% (95% CI: 0.27, 1.40) increase in ALT levels, in males and females respectively; one ln-unit increase in PFOA concentrations was associated with a 1.03% (95%CI: 0.50, 1.55) increase in ALT levels in males, and a 0.52% (95% CI: 0.22, 0.82) and a 0.60% (95% CI: 0.25, 0.96) increase in AST levels in females and males. PFOS showed no association with ALT and AST levels. Quantile G-computation revealed that an interquartile increase in the PFAS mixture was associated with a 3.02% increase (95% CI: 1.65, 4.43) and a 1.65% (95% CI: 0.77, 2.5) increase in ALT levels, in females and males. Mediation analysis suggested that BMI suppressed the association between PFAS and ALT levels, with positive direct effects higher than the total effects.

Conclusion: Our findings suggest sex-specific associations between PFAS exposure and liver function biomarkers and underscore the need for additional studies on potential mediators.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) constitute a vast group of man-made chemicals with fluorinated carbon chains of varying lengths, used in various industrial processes and consumer applications (Glüge et al., 2020). PFAS are characterized by their substantial resistance properties, which are responsible for their widespread use but also for their accumulation in the environment, wildlife and humans (Bell et al., 2021; Su and Rajan, 2021; Sunderland et al., 2019). Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most known and studied long-chain PFAS (Socianu et al., 2022).

Humans are primarily exposed to PFAS through drinking water, food and air (Domingo and Nadal, 2019). Populations residing in severely contaminated sites are exposed to high PFAS concentrations over time (US EPA, 2021). In these settings, human biomonitoring studies are being conducted to investigate the association between PFAS exposure

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and its effects on health. Various studies have been performed in the last decades in the USA, China, Australia and in Europe, mainly in Sweden, Italy, France, Germany, Norway and Belgium (Domingo and Nadal, 2019). PFAS have been proven to be associated with several adverse health effects, depending on exposure characteristics and individual factors. They have the capability to disrupt immune, endocrine and cardiometabolic systems, resulting in alterations in thyroid function, liver and kidney disease, as well as lipid and insulin dysregulation (Rappazzo et al., 2017; Sunderland et al., 2019; Fenton et al., 2021); they can also affect the reproductive system, being associated with adverse reproductive outcomes and developmental effects (Rickard et al., 2022). In Europe, PFOS, PFOA and chemicals that can degrade to them have been identified as substances of very high concern under the European Chemicals Regulation (Brendel et al., 2018) and starting from 2009 they have been listed under the Stockholm Convention on Persistent Organic Pollutants (van der Veen et al., 2023). Recently, PFOA was classified as carcinogenic to humans by the International Agency for Research on Cancer (Zahm et al., 2024).

Several animal studies have investigated the association between PFAS exposure and liver function, suggesting that the liver is one of the main targeted organs for PFAS accumulation and toxicity (Roth et al., 2021). In murine models, PFAS have shown the potential to disrupt liver homeostasis with an effect potentially mediated by multiple nuclear receptors (Attema et al., 2022), inducing hepatocellular steatosis (Das et al., 2017; Costello et al., 2022), hepatic inflammation and increased plasma alanine aminotransferase levels (Roth et al., 2021). Little is known regarding the molecular mechanisms involved in PFAS hepatotoxic effects in humans, although there is increasing evidence regarding the activation of peroxisome proliferator-activated receptor alpha (Borghese et al., 2022; Ducatman and Fenton, 2022; Wang et al., 2022).

Studies conducted on humans are fewer, but the available literature regarding PFAS exposure and liver injury suggests a positive association between PFOA, PFOS, perfluorononanoic acid (PFNA) and markers of liver damage as highlighted in a recent systematic review (Costello et al., 2022). Coherence in results among human and rodent studies has been observed, providing support for the possibility of a causal relationship. However, it is imperative to further investigate several understudied aspects (Costello et al., 2022). In fact, only a limited number of studies explored the combined effect of multiple PFAS exposures on liver function biomarkers using mixture approaches (Stratakis et al., 2020; Liu et al., 2022; Borghese et al., 2022; Kim et al., 2023), despite the fact that individuals are simultaneously exposed to different PFAS with possible additive, synergistic or antagonistic effects (Domingo and Nadal, 2019; Feng et al., 2022). Moreover, since measured PFAS serum concentrations often differ based on sex and obesity status (Qi et al., 2020; Lin et al., 2021; Jain and Ducatman, 2022), the presence of sex-specific and obesity-related differences in PFAS toxicity has been emphasized (Attanasio, 2019; Jain, 2019; Jain and Ducatman, 2019), but further investigation is still needed.

In 2013, a large area of the Veneto Region, in North-eastern Italy, was found to be contaminated by PFAS, which were detected in surface water, groundwater and drinking samples (Ingelido et al., 2018). A fluorochemical manufacturing plant that had been operating in the area since the 1960s was identified as the main source of PFAS contamination in public water works and private wells. Water treatment plants were promptly equipped with granular activated carbon filters, resulting in a significant reduction in PFAS concentrations in drinking water (Ingelido et al., 2018; Pitter et al., 2020). The Veneto Region implemented a free-of-charge population-based biomonitoring program (the Health Surveillance Program) to investigate the associations between PFAS exposure and several adverse health outcomes.

The objective of this cross-sectional study is to examine the associations between PFAS exposure and biomarkers of liver function in a highly exposed Italian cohort, using both single-pollutant and mixture approaches. Furthermore, we aim at investigating the presence of sex-specific differences and exploring the potential role of body mass index (BMI) in the examined associations.

2. Materials and methods

2.1. Study population

Starting from 2017, participation in the Health Surveillance Program was offered to residents of the contaminated area of Veneto Region, encompassing nearly 140,000 inhabitants across 30 municipalities. Detailed information about the recruitment process has been described elsewhere (Pitter et al., 2020). Participants were invited to complete a software-based questionnaire covering their diet, lifestyle, socio-demographic characteristics, self-reported anthropometric measures, and to provide non-fasting blood and urine samples for PFAS quantification and several biochemical parameters evaluation (Pitter et al., 2020). Written informed consent was obtained from all the participants.

In this study, we included all adult participants in the Surveillance Program aged 20 years and older (n = 43,412). We excluded participants with a self-reported history of liver disease (infectious hepatitis, liver cirrhosis, biliary obstruction, liver carcinoma, autoimmune liver disease, n = 893), pregnant women (n = 390) and subjects with missing information in the covariates of interest (n = 34). The final study population comprised a total of 42,095 subjects. The covariates of interest included age (in years), sex, education level (categorized as elementary/middle school, high school, university/higher), alcohol consumption, smoking status, body mass index (BMI) and medical laboratory in which the biochemical analyses were performed (Arzignano, San Bonifacio, Legnago). Alcohol consumption was categorized as none, 1–2 alcoholic units (AU)/week, 3–6 AU/week, 7+ AU/week. Smoking status was defined as never, current or former smoker. BMI was calculated for all participants after checking the accuracy of data regarding the self-reported height and weight and was categorized as <25 kg/m^2 (normal weight), 25.0–29.9 kg/m^2 (overweight), 30.0 kg/m^2 and above (obesity). The abovementioned covariates were selected among the available ones based on previously published studies on associations between PFAS and liver function biomarkers (Borghese et al., 2022; Liu et al., 2022).

2.2. Quantification of serum PFAS

Blood samples were centrifuged and frozen serum samples were sent to the centralized Agency for Environmental Prevention and Protection of the Veneto Region (ARPAV) laboratory for measurement of 12 different PFAS: PFOA, PFOS, PFNA, perfluorohexanesulfonic acid (PFHxS), perfluorobutanoic acid (PFBA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUnA) and perfluorododecanoid acid (PFDoA). Analysis of PFAS was performed using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) (Shimadzu UFLC XR 20 Prominence coupled to Sciex API 4000) as previously described in detail (Pitter et al., 2020; Batzella et al., 2024). The limit of detection (LOD) and limit of quantification (LOQ) were of 0.1 and 0.5 μg/L, respectively; observations below the LOQ were assigned a value of 0.125 μg/L, equal to LOQ/$\sqrt{2}$. For this analysis, only PFAS detected in more than 50% of the participants' samples were considered: PFOA and PFOS (both detected in 99.8% of participants), PFHxS (detected in 97.0% of participants) and PFNA (detected in 55.5% of participants).

2.3. Liver function biomarkers

As histological confirmation of liver damage is not commonly available in epidemiological studies, the presence of liver toxicity is usually evaluated using serum measurements of liver function biomarkers or imaging assessments of hepatic steatosis (Giannini et al.,

2005). In the present study, we considered serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. We primarily focused our attention on ALT as indicator of liver injury, since alterations in AST levels alone can be due to non-hepatic causes, being associated with damage to other organ systems, such as muscular or cardiovascular (Kwo et al., 2017; Lala et al., 2023). Blood samples were sent to the three Local Health Unit laboratories for analysis of clinical biomarkers. All the laboratories in which the measurements were performed regularly followed an external quality assurance program (Pitter et al., 2020).

2.4. Statistical analysis

Sociodemographic characteristics, PFAS concentrations and liver enzyme levels were presented using frequencies and proportions for categorical variables and mean, standard deviation, median and 1st-3rd quartiles for continuous variables. PFAS concentrations, ALT and AST levels were natural log-transformed to ensure that the residuals of the models met the normality assumption.

To explore potential sex-specific variations in PFAS-induced hepatotoxicity, we decided a priori to conduct all analyses separately for males and females. Sex differences among covariates, PFAS concentrations and outcomes were assessed using t-tests or chi-square tests. Spearman's correlation was used to describe pairwise relations among the different measured PFAS.

Generalized additive models (GAM) were used to explore the dose-response relationship between PFAS exposures and the outcome variables. Initially, single PFAS concentrations were graphically plotted against predicted outcomes using thin-plate smooth splines. Estimated degrees of freedom (EDFs) were reported to explore the adequacy of polynomial functions compared to the use of splines (with 1 suggesting a linear relationship). In addition, each PFAS was included in the models as a linear term (after log-transformation), to capture potential linear associations, and categorized into the distribution quartiles for the whole population. This approach was chosen to allow a comprehensive examination of the relationships between exposures and outcomes. All models investigating the association between PFAS and ALT/AST levels were adjusted for age (as a continuous variable, modelled using splines), education level, alcohol consumption, smoking status, and medical laboratory. To assess the role of BMI on PFAS/liver enzymes associations, models in the main analysis were also adjusted for BMI as a possible confounder. Estimated coefficients and their respective 95% confidence intervals were back-transformed to be interpreted as predicted percentage changes in ALT and AST levels for a 1 natural logarithm (ln) increase in each PFAS concentration, using $\left[e^{\beta} - 1\right] \times 100$, with β representing the estimated coefficient in its original scale (Borghese et al., 2022; Liu et al., 2022; Kim et al., 2023).

Furthermore, predicted percentage changes in ALT levels for a ln-unit increase in PFAS concentrations were stratified according to alcohol consumption, smoking status, education level, and categorical BMI, all considered as dichotomous variables. In addition, each fully adjusted model was conducted including an interaction term between these covariates and PFAS concentrations, considered one at a time. This was done to assess potential effect modification by these selected covariates, employing the likelihood ratio test to ascertain whether the differences were statistically significant.

2.4.1. Mixture analysis

To account for the strong correlation between PFAS, we then applied a Quantile G-computation regression model (R package: *gcomp*) to assess the joint effect of PFAS exposure on ALT and AST levels. This method is able to quantify the expected change in the outcome given a quantile change in all exposures simultaneously (Keil et al., 2020). The Quantile G-computation allows the chemicals included in the mixture to predict the outcome of interest, potentially exerting either positive or negative effects (Keil et al., 2020). PFAS were categorized in quartiles and combined to calculate the index and its weights, indicating the partial effect due to a specific PFAS in increasing or decreasing ALT and AST levels. The estimated coefficients and their respective 95% confidence intervals were back-transformed to be interpreted as predicted percentage changes in ALT and AST levels for each quartile increase in the PFAS mixture composed of PFOA, PFOS, PFHxS and PFNA. While for other PFAS, the detection rates allowed for an even distribution of approximately 25% of subjects in each category, this was not feasible for PFNA, where the first quartile comprised 45% of the participants. Nevertheless, we believe this did not compromise the interpretability of the quartile-based estimates for PFNA, though this skewness should be taken into account when interpreting the results.

2.4.2. Mediation analysis

To fully explore BMI's role in the association, we considered also that, if PFAS levels influence BMI, which in turn affects liver enzymes levels, BMI should be treated as a mediator in the causal pathway. Available literature suggests that obesity and related measures should be carefully considered in PFAS exposure studies, as they may act as mediators in the association with outcomes of interest (Gilliland and Mandel, 1996; Jain and Ducatman, 2019). In an additional analysis, we explored the potential mediating role of BMI, as depicted in our path diagram of mediation/confounding effects (Fig. 1). We conducted a formal mediation analysis (R package: *mediation*) to investigate whether BMI (as an indicator of obesity) mediated the association between PFAS (independent variables) and ALT levels (outcome variable) following the VanderWeele framework (VanderWeele, 2016a,b). We first assessed whether BMI satisfied all conditions for being considered a mediator, checking a) whether PFAS exposure was significantly associated with the mediator, and b) whether the mediator was significantly associated with ALT levels, after adjustment for PFAS exposure (VanderWeele, 2016a,b). We used multinomial logistic regressions to test the association between BMI categories and PFAS measured levels, and GAM models to investigate the association between BMI categories and ALT levels, in males and females separately. BMI was considered as a categorical variable (as <25 kg/m^2 (normal weight), 25.0–29.9 kg/m^2 (overweight), 30.0 kg/m^2 and above (obese)). In addition, mediation analysis was conducted considering BMI as a dichotomous variable (categorized as <25 kg/m^2 (normal weight) or $\geq$ 25.0 kg/m^2 (overweight/obese)). All mediation models were adjusted for the covariates previously listed. The total effect of PFAS exposure on liver enzymes was divided into direct and indirect effects. The total effect represents the overall effect of PFAS concentration on ALT/AST levels; the direct effect is the portion of the effect not explained by the mediating factor (BMI), while the indirect effect indicates the part of the effect explained by BMI. The mediation effect of BMI in the association, quantified as the proportion of the total effect that is mediated, was calculated as the ratio between the indirect effect and the sum of indirect and mediated effects, all considered as absolute numbers (Lee et al., 2019).

All statistical analyses were conducted using the statistical software R (R Core Team, 2022); the statistical significance was set at 0.05.

3. Results

A total of 42,095 subjects (22,160 females, 19,935 males) were included in the present study. Table 1 displays the participants' PFAS measured concentrations and liver function biomarkers levels. Table S1 shows the population's sociodemographic characteristics. Both females and males had a median age of 44 years (Q1-Q3: 34–54 years for females, Q1-Q3: 33–54 years for males). PFOA showed the highest concentrations among all PFAS, and median concentration in males was more than double the median in females (63.00 ng/mL vs. 26.60 ng/mL).

Spearman's correlation showed that PFOA and PFHxS were strongly correlated ($r = 0.90$), followed by PFOS and PFNA ($r = 0.69$) and PFOS and PFHxS ($r = 0.64$).

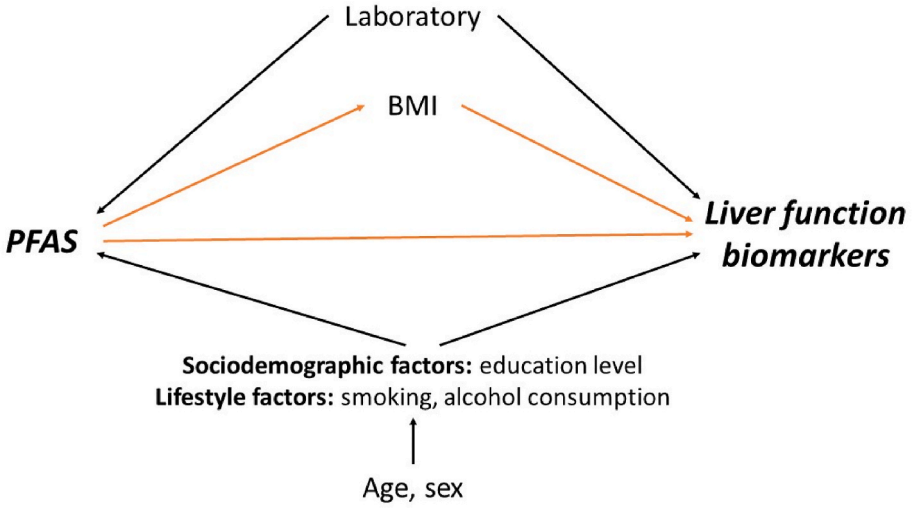

Fig. 1. Path diagram of the association between PFAS exposure and liver function biomarkers.

Table 1
PFAS measured levels (ng/mL) and liver function biomarkers levels (U/L) in the study population (n = 42,095).

	Females (n = 22,160)	Males (n = 19,935)
ALT (U/L)		
Mean (SD)	18.78 (11.01)	28.07 (15.96)
Geometric Mean	16.95	25.08
Median (Q1-Q3)	16.00 (13.00–22.00)	24.00 (18.00–33.00)
AST (U/L)		
Mean (SD)	21.09 (8.17)	25.61 (10.87)
Geometric Mean	20.19	24.36
Median (Q1-Q3)	20.00 (17.00–24.00)	24.00 (20.00–28.00)
PFOA (ng/mL)		
Mean (SD)	44.53 (56.17)	94.92 (106.46)
Geometric Mean	23.54	54.11
Median (Q1-Q3)	26.60 (10.50–57.40)	63.00 (26.55–126.00)
PFHxS (ng/mL)		
Mean (SD)	4.36 (4.94)	10.62 (10.26)
Geometric Mean	2.67	6.83
Median (Q1-Q3)	2.80 (1.30–5.50)	7.70 (3.40–14.45)
PFOS (ng/mL)		
Mean (SD)	4.28 (3.84)	6.28 (4.96)
Geometric Mean	3.42	5.14
Median (Q1-Q3)	3.40 (2.30–5.20)	5.20 (3.50–7.70)
PFNA (ng/mL)		
Mean (SD)	0.52 (0.34)	0.63 (0.59)
Geometric Mean	0.47	0.57
Median (Q1-Q3)	0.35 (0.35–0.60)	0.60 (0.35–0.80)

Figure S1 shows the adjusted concentration-response curves between single ln-transformed PFAS, and liver function biomarkers stratified according to sex (obtained from fully adjusted models). EDFs and p-values for the significance of the effects of PFAS on ALT and AST levels are presented in Table S2. We identified significant associations with liver enzymes levels for most PFAS, except for PFOA in both sexes or PFOS with ALT in males, PFNA with AST in both sexes and PFHxS with AST in males (Table S2). We observed approximately linear relationships between ALT and both PFHxS and PFNA in both sexes, ALT and PFOA in males, and between AST and PFOA, PFHxS and PFNA in both sexes (with EDFs ranging from 1.00 to 1.87). Deviations from the linearity were observed in the relationship between PFOA/ALT, PFOS/ALT and PFOS/AST in females (EDFs ranging from 3.56 to 4.24).

Tables 2 and 3 show the coefficients between PFAS concentrations and ALT/AST levels (both ln-transformed) after adjusting for different sets of covariates. In models conducted not considering BMI for adjustment, in both males and females, an increase in serum concentrations of PFHxS was positively associated with increased ALT; the association was significant also for PFHxS/AST in females. A positive association was also found between PFOA and AST, while a negative association was found between PFOS levels and ALT/AST in males. In the fully adjusted models, which considered BMI as a potential confounder in the association, the estimates differed substantially from those obtained without BMI adjustment. We found positive associations between PFOA levels and ALT levels, especially in males, as well as with PFHxS and PFNA levels in both sexes. For a one ln-unit increase in PFOA concentrations, ALT showed an increase of 1.03% (95%CI: 0.50, 1.55) in males; for a one ln-unit increase in PFHxS concentrations, ALT showed an increase of 1.49% (95%CI: 0.97, 2.12) in males and 0.84% (95%CI: 0.27, 1.40) in females. We also observed a null association between PFOS levels and ALT levels in both males and females. In addition, we observed positive associations between PFOA and AST levels in both sexes and PFHxS and AST levels in females. Results were confirmed when observing the predicted percentage changes in ALT/AST levels for changes in PFAS concentrations by quartiles.

We found that alcohol consumption significantly modified the association of PFOA and PFHxS with ALT levels, only in males: higher PFAS effects were observed in drinkers compared to non-drinkers, while the other characteristics did not significantly modify the PFAS/ALT associations (Table S3).

Results from the mixture analysis confirm the presence of an overall positive association between the PFAS mixture and ALT levels, with a 3.02% (95%CI: 1.65, 4.43) and a 1.65% (95%CI: 0.77, 2.55) increase in ALT in females and males, respectively, for each quartile increase in the mixture of PFAS. Table S4 shows the weights of each compound in the PFAS mixture, representing their individual contribution in the overall association with ALT and AST levels. When considering the association between PFAS and liver enzymes using Quantile G-computation, PFHxS showed the higher positive contribution to the mixture effect on ALT levels, followed by PFNA; whereas PFOS and PFOA showed negative contributions. In addition, PFOA and PFHxS showed the higher positive contributions to the PFAS mixture effect on AST levels in females, whereas PFOS showed negative contributions.

When evaluating the assumptions for mediation analysis, multinomial logistic regressions, conducted separately for females and males, showed a significant association between all four PFAS and BMI, with increases in PFAS levels associated with a reduced probability of being overweight or obese (Table S5). Moreover, GAM models showed increases in ALT levels across BMI categories, with higher predicted ALT levels for overweight and obese subjects compared to normal weight ones. Estimates from mediation analysis are presented in Table 4. The mediation models showed direct effects of PFAS on ALT greater than the

Table 2
GAM models conducted to investigate the association between PFAS and ALT.

	Predicted percentage changes in ALT levels			
	Not adjusting for BMI		Adjusting for BMI	
	Females	**Males**	**Females**	**Males**
	Coef (95% CI)	***Coef (95% CI)***	***Coef (95% CI)***	***Coef (95% CI)***
PFOA				
ln(PFOA)	−0.08 (−0.55, 0.38)	0.52 (−0.03, 1.07)	0.14 (−0.31, 0.60)	**1.03 (0.50, 1.55)**
Q2	0.91 (−0.43, 2.27)	1.96 (−0.16, 4.12)	**1.38 (0.06, 2.72)**	**2.47 (0.45, 4.54)**
Q3	−0.58 (−2.03, 0.88)	**2.24 (0.18, 4.33)**	−0.03 (−1.46, 1.42)	**3.29 (1.31, 5.30)**
Q4	−0.50 (−2.29, 1.31)	1.87 (−0.11, 3.89)	0.3 (−1.46, 2.09)	**3.66 (1.73, 5.62)**
PFHxS				
ln(PFHxS)	**0.70 (0.12, 1.27)**	**0.87 (0.22, 1.53)**	**0.84 (0.27, 1.40)**	**1.49 (0.87, 2.12)**
Q2	1.27 (−0.04, 2.60)	1.49 (−0.76, 3.79)	**1.51 (0.22, 2.82)**	1.92 (−0.23, 4.12)
Q3	1.22 (−0.24, 2.70)	1.91 (−0.26, 4.12)	**1.48 (0.04, 2.93)**	**3.06 (0.98, 5.19)**
Q4	1.19 (−0.81, 3.23)	**2.41 (0.29, 4.58)**	1.68 (−0.29, 3.68)	**4.28 (2.22, 6.38)**
PFOS				
ln(PFOS)	−0.48 (−1.32, 0.37)	**−1.97 (-2.96, -0.98)**	0.39 (−0.44, 1.23)	−0.12 (−1.07, 0.85)
Q2	−1.11 (−2.42, 0.22)	−0.93 (−2.95, 1.13)	−0.71 (−1.99, 0.60)	0.53 (−1.43, 2.52)
Q3	−0.27 (−1.72, 1.21)	**−2.10 (-4.03, -0.13)**	1.05 (−0.39, 2.52)	0.15 (−1.74, 2.07)
Q4	−0.60 (−2.26, 1.09)	**−3.34 (-5.21, -1.43)**	0.97 (−0.69, 2.65)	0.09 (−1.76, 1.98)
PFNA				
ln(PFNA)	1.09 (−0.28, 2.48)	1.46 (−0.04, 2.97)	**1.54 (0.19, 2.91)**	**1.91 (0.48, 3.36)**
Q2	−1.14 (−2.67, 0.41)	0.74 (−1.10, 2.61)	−1.03 (−2.53, 0.49)	1.03 (−0.73, 2.81)
Q3	1.03 (−0.47, 2.55)	**2.12 (0.46, 3.80)**	1.21 (−0.26, 2.71)	**2.20 (0.63, 3.80)**
Q4	1.21 (−0.44, 2.88)	**1.70 (0.02, 3.41)**	**1.67 (0.05, 3.31)**	**2.24 (0.64, 3.88)**
Mixture	**2.96 (1.55, 4.39)**	0.92 (−0.01, 1.85)	**3.02 (1.65, 4.43)**	**1.65 (0.77, 2.55)**

Note: all models were adjusted for age (spline), education level, alcohol consumption, smoking status and medical laboratory.

Table 3
GAM models conducted to investigate the association between PFAS and AST.

	Predicted percentage changes in AST levels			
	Not adjusting for BMI		Adjusting for BMI	
	Females	**Males**	**Females**	**Males**
	Coef (95% CI)	***Coef (95% CI)***	***Coef (95% CI)***	***Coef (95% CI)***
PFOA				
ln(PFOA)	**0.51 (0.21, 0.81)**	**0.48 (0.12, 0.83)**	**0.52 (0.22, 0.82)**	**0.60 (0.25, 0.96)**
Q2	**0.92 (0.06, 1.80)**	**2.26 (0.90, 3.65)**	**0.95 (0.08, 1.83)**	**2.40 (1.04, 3.77)**
Q3	0.91 (−0.04, 1.87)	**1.87 (0.55, 3.20)**	0.93 (−0.02, 1.89)	**2.13 (0.82, 3.46)**
Q4	**1.64 (0.46, 2.84)**	**2.14 (0.86, 3.43)**	**1.67 (0.49, 2.86)**	**2.59 (1.31, 3.88)**
PFHxS				
ln(PFHxS)	**0.64 (0.27, 1.01)**	0.22 (−0.19, 0.64)	**0.65 (0.27, 1.02)**	0.37 (−0.04, 0.79)
Q2	0.53 (−0.32, 1.38)	0.53 (−0.90, 1.99)	0.55 (−0.30, 1.40)	0.64 (−0.79, 2.09)
Q3	**1.45 (0.51, 2.41)**	0.97 (−0.41, 2.37)	**1.48 (0.53, 2.43)**	1.25 (−0.12, 2.65)
Q4	**1.51 (0.21, 2.83)**	0.71 (−0.63, 2.08)	**1.53 (0.23, 2.85)**	1.17 (−0.17, 2.53)
PFOS				
ln(PFOS)	−0.37 (−0.92, 0.17)	**−1.19 (-1.83, -0.55)**	−0.31 (−0.86, 0.24)	**−0.72 (-1.36, -0.08)**
Q2	**−0.92 (-1.76, -0.06)**	−0.41 (−1.72, 0.91)	−0.86 (−1.71, −0.01)	−0.04 (−1.35, 1.28)
Q3	0.16 (−0.79, 1.12)	**−1.84 (-3.09, -0.57)**	0.25 (−0.70, 1.21)	**−1.27 (-2.52, -0.01)**
Q4	−0.37 (−1.45, 0.72)	**−1.88 (-3.10, -0.64)**	−0.26 (−1.35, 0.83)	−1.01 (−2.24, 0.23)
PFNA				
ln(PFNA)	−0.01 (−0.88, 0.88)	−0.90 (−1.84, 0.04)	0.03 (−0.85, 0.91)	−0.79 (−1.73, 0.15)
Q2	−0.71 (−1.71, 0.29)	−0.67 (−1.83, 0.52)	−0.71 (−1.70, 0.29)	−0.59 (−1.75, 0.58)
Q3	0.01 (−0.95, 0.98)	−0.13 (−1.17, 0.92)	0.02 (−0.94, 0.99)	−0.11 (−1.14, 0.94)
Q4	0.26 (−0.80, 1.32)	−0.80 (−1.85, 0.27)	0.28 (−0.77, 1.35)	−0.66 (−1.71, 0.40)
Mixture	**1.79 (0.89, 2.71)**	−0.21 (−0.79, 0.38)	**1.78 (0.87, 2.69)**	−0.03 (−0.61, 0.55)

Note: all models were adjusted for age (spline), education level, alcohol consumption, smoking status and medical laboratory.

total effects, due to significant negative effects mediated by BMI for all included PFAS, except for PFNA in male subjects. Total effects were non-significant in almost all cases, except for PFHxS. The results provided by the mediation analysis suggest that a higher BMI partially suppresses the impact of PFAS exposure on ALT levels (Table 4). The suppressive role of BMI is also confirmed by sensitivity analyses, where BMI was considered as a dichotomous variable (Table S6).

4. Discussion

The present study investigated the association between exposure to several PFAS substances and levels of ALT and AST, considered as biomarkers of liver function, in a highly exposed population of adults aged 20 years or older in the Veneto Region, Italy.

We reported positive associations between PFOA levels and ALT levels, particularly in males, as well as with PFHxS and PFNA levels in both sexes. Additionally, we observed a null association between PFOS levels and ALT levels in both males and females.

In the investigated population, PFOA had the highest measured concentrations, especially among males; concentrations of PFHxS, PFOS and PFNA were relatively lower compared to PFOA, that was possibly the primary contaminant produced by the fluorochemical plant situated

Table 4
Mediation analysis with BMI as a categorical mediator for ALT, stratified by sex.

		Total effect (95% CI)	Mediated effect (95% CI)	Direct effect (95% CI)	Proportion mediated (%)
PFOA	Females	−0.157 (−0.563, 0.327)	**−0.295 (-0.442, -0.100)**	0.139 (−0.316, 0.542)	68.1%
	Males	0.557 (−0.076, 0.989)	**−0.460 (-0.881, -0.290)**	**1.022 (0.618, 1.527)**	31.2%
PFOS	Females	−0.525 (−1.441, 0.475)	**−0.912 (-1.112, -0.567)**	0.390 (−0.505, 1.295)	70.2%
	Males	**−1.899 (-2.879, -1.150)**	**−1.785 (-2.319, -1.452)**	−0.116 (−1.155, 0.739)	93.9%
PFHxS	Females	**0.653 (0.213, 1.294)**	**−0.183 (-0.345, -0.031)**	**0.838 (0.343, 1.443)**	18.0%
	Males	**0.977 (0.131, 1.482)**	**−0.514 (-0.904, -0.307)**	**1.499 (0.793, 1.980)**	25.7%
PFNA	Females	1.122 (−0.143, 2.645)	**−0.413 (-0.706, -0.181)**	**1.542 (0.460, 3.035)**	21.3%
	Males	1.696 (−0.004, 2.805)	−0.188 (−0.932, 0.005)	**1.888 (0.723, 3.131)**	9.16%

in Trissino, Veneto Region.

In this study, we considered serum levels of ALT and AST as outcomes of interest. Measurements of these liver enzymes are critical for the diagnosis and assessment of the presence of liver disease (Giannini et al., 2005; Kim et al., 2008) and are widely investigated in epidemiological studies, particularly in cases where liver biopsies or imaging assessments of liver disease are not available for all participants (Costello et al., 2022). Contrary to ALT, AST is a non-specific marker of liver injury, which can be found circulating in the bloodstream due to several causes beyond liver-related problems (Xu et al., 2015), so results from models investigating levels of AST should be interpreted with particular caution, as increasing AST levels can be due to several conditions. However, most of the associations identified between PFAS and ALT were also seen between PFAS and AST.

Our results are partially comparable to previously published studies and contribute to the knowledge provided by a recent review investigating exposure to per- and polyfluoroalkyl substances and markers of liver injury (Costello et al., 2022). In the review, the authors outlined a positive relationship between PFOA/PFOS/PFNA and ALT levels in adults, in both cross-sectional and longitudinal settings, although sensitivity analyses did not confirm the results obtained for PFOS. In addition, they did not report a statistically significant relationship between PFOA/AST levels and between PFHxS/ALT levels (Costello et al., 2022).

In our population, we observed a positive association between PFOA and ALT levels, which aligns with the findings reported in the review (Costello et al., 2022). Additionally, we identified a positive association between PFHxS and ALT levels, as well as between PFOA and AST levels, across both sexes. However, contrary to the review's suggestion of a positive association between PFOS and ALT, our analysis revealed a null association in both sexes when analyses were adjusted for BMI. This finding is not consistent with what has been reported in previously published literature, as several studies underlined that PFOS can damage liver tissue and disrupt its function, although the exact mechanisms remain unclear (Wang et al., 2022). When exploring the association between liver enzymes and PFOS, particularly in light of available evidence, we suggest interpreting the results showing no association with caution. First, PFOS concentrations in our study population were relatively low and comparable to those of populations with background exposure (median of 5.84 ng/mL (Ingelido et al., 2018)), with PFOA being the main and most relevant contaminant in the Veneto Region (median of 1.64 ng/mL (Ingelido et al., 2018)). Additionally, residual bias from uncontrolled confounding factors could significantly influence the estimates of the association, especially at low exposure levels.

We observed that PFOA and PFHxS effects on ALT levels were more pronounced in male drinkers compared to male non-drinkers. These results may be due to the fact that alcohol can impact liver health, contributing to organ damage (Bandiera et al., 2020; Mehlig et al., 2022).

Our analyses were conducted considering exposure to individual PFAS as well as a mixture of different PFAS, considering the fact that humans are exposed to multiple environmental pollutants simultaneously (McCarthy et al., 2021). Few studies have adopted this approach when evaluating the effects of exposures to multiple substances on liver enzymes (Borghese et al., 2022; Liu et al., 2022). Results from mixture analysis, employing Quantile G-Computation, showed the presence of an overall positive association between the PFAS mixture and ALT levels, with a 3.02% (95%CI: 1.65, 4.43) and a 1.65% (95%CI: 0.77, 2.55) increase in ALT levels, in females and males respectively, for each quartile increase in the mixture of PFAS. The same positive association was highlighted between the PFAS mixture and AST levels in females.

Our results show some sex-specific differences in PFAS hepatotoxicity, which were evident for each of the PFAS investigated, prompting the necessity of considering males and females separately when assessing the effect of PFAS exposures on the liver homeostasis. Changes in liver enzymes levels were more pronounced in males, although with small magnitudes observed in both sexes. Evidence of sex differences has already been observed in adolescent populations (Attanasio, 2019) and in animal studies investigating PFAS effects on multiple metabolic pathways (Roth et al., 2021), while other studies conducted on adults did not observe sex-specific differences (Costello et al., 2022).

In the present study, we used cross-sectional collected data, in which all variables are measured at the same time, making it difficult to fully determine whether BMI should be considered as a confounder (affecting both PFAS and ALT levels), as a mediator (with PFAS levels affecting BMI, and BMI influencing ALT levels) or an effect modifier. However, our decision to present results considering all these approaches aims to deepen our understanding of BMI's possible role on the health effects of PFAS exposure. While no significant evidence of effect modification was observed for BMI, in mediation analysis the mediated effect of BMI resulted in a reduction in the observed effect of PFOA/PFHxS concentrations on the outcome, indicating a suppression effect, as direct and mediated effect of PFAS on liver enzymes showed opposite directions (MacKinnon et al., 2000). Estimates provided in the mediation analysis considering the effect of PFOS on ALT should be interpreted with caution, considering the non-linear shape of the dose-response curve.

This study possesses several strengths, primarily in relation to the large sample size involved; in addition, information on the covariates of interest was available for almost all the participants and the number of missing data was extremely limited, resulting in a reduced number of subjects being excluded from the analysis due to this reason. We employed both single-pollutant and mixture techniques, adopting an approach that has been used only in a few studies investigating the effects of PFAS on the liver thus far (Borghese et al., 2022; Liu et al., 2022; Kim et al., 2023). To our knowledge, this study is also the first to consider BMI as a mediator, instead of a confounder, in the association between PFAS exposure and ALT levels.

However, the study also has some limitations. Firstly, due to the structure and organization of the Health Surveillance Plan monitoring, we were unable to include information in the study regarding other enzymes different from ALT and AST useful for investigating liver function, such as gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and total bilirubin. In addition, it is important to note that the information regarding height and weight, which was used to calculate the participants' BMI, was self-reported, potentially limiting

the accuracy of our estimates. Finally, it was not possible to retrieve adequate and comprehensive information regarding dietary habits, and levels of physical activity, so these aspects were not evaluated in this analysis, although we consider them to play a relevant role in the relationship between PFAS and liver function.

5. Conclusions

In this study, we observed the presence of significant associations between exposure to almost all PFAS investigated and levels of liver function biomarkers, resulting in small increases in both ALT and AST levels for PFOA, PFHxS and PFNA exposure and in a null effect following PFOS exposure. We also noted the presence of sex-related differences among female and male subjects. Results from mixture analysis confirm those obtained using single-pollutant approaches, and mediation analysis suggests that BMI may act as a partial suppressor of the effect of exposure on the outcome.

While our findings indicate that the impact of PFAS on liver enzymes appears to be small in terms of magnitude, we advocate for thorough consideration of PFAS exposure when assessing liver health. Even though individual exposures may cause minor harm, the liver is constantly exposed to various harmful substances, such as medications, alcohol and drugs, heavy metals and other pollutants, and their cumulative effect can lead to significant organ dysfunction. Therefore, in the context of liver homeostasis, it is crucial to recognize and further investigate the potential effects of multiple exposures, which could manifest as noticeable organ malfunction.

Additional studies on the same topic, conducted on populations with varying characteristics and different levels of PFAS exposure and stratifying by sex, are needed to enhance the relevance of our findings.

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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. 203638).

CRediT authorship contribution statement

Isabella Rosato: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Erich Batzella:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Gisella Pitter:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Francesca Russo:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Filippo Da Re:** Writing – review & editing, Resources, Investigation, Data curation. **Tony Fletcher:** Writing – review & editing, Methodology, Conceptualization. **Cristina Canova:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.120082.

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