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Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region

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ABSTRACT

Background: Residents of a large area of the Veneto Region (North-Eastern Italy) were exposed for decades to drinking water contaminated by perfluoroalkyl substances (PFAS). PFAS have been consistently associated with raised serum lipids, mainly in cross-sectional studies and in background exposure contexts, but the shape of the dose-response relationships has been poorly investigated. The objectives of our study were to evaluate the association between serum PFAS and serum lipids and their dose-response patterns in a large exposed population.
Methods: A cross-sectional study was conducted in 16,224 individuals aged 20–39 years recruited in the regional health surveillance program. 15,720 subjects were analysed after excluding pregnant women (n = 327), participants reporting use of cholesterol lowering medications (n = 67) or with missing information on the selected covariates (n = 110). Twelve PFAS were measured by HPLC-MS in serum; three (PFOA, PFOS and PFHxS) were quantifiable in at least 50% of samples. Non-fasting serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured by enzymatic assays in automated analysers and low-density lipoprotein cholesterol (LDL-C), non-HDL cholesterol and total/HDL cholesterol ratio were calculated. The associations between natural log (ln) transformed PFAS and lipids were assessed through generalized additive models using linear regression and smoothing thin plate splines, adjusted for potential confounders.
Results: There were strong positive associations between the ln-transformed PFOA, PFOS, and PFHxS and TC, HDL-C, and LDL-C, and between ln PFOA and PFHxS and triglycerides. Each ln-increase in PFOA was associated with an increase of 1.94 mg/dL (95% CI 1.48–2.41) in TC, with 4.99 mg/dL (CI 4.12–5.86) for PFOS and 2.02 mg/dL (CI 1.45–2.58) for PFHxS.
Conclusions: Investigation of the shape of exposure-response associations using splines showed a positive association with the largest increases per unit of PFAS in cholesterol levels occurring at the lower range of PFAS concentrations for each compound.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) comprise a diverse group of anthropogenic organic chemicals that have been manufactured since the late 1940s to be used in various industrial and commercial applications as surfactants and surface coatings owing to their grease- and water-repellent properties and low surface tension (Fujii et al., 2015; Harada et al., 2007). PFAS are resistant to biodegradation, photoxidation, direct photolysis, and hydrolysis (OECD, 2018) and have been detected in all environmental media including air, surface water, groundwater, soil, and food (ATSDR, 2018). Human exposure to perfluoroalkyl compounds in particular within PFAS is widespread and mainly occurs through ingestion of contaminated food and dust (Fraser et al., 2013; Lorber and Egeghy, 2011). Drinking water

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has been described as a major source of exposure in some cases of local contamination. One of the largest known incidents involved more than 70,000 people in the mid-Ohio Valley (USA) where PFAS released from a fluoropolymer-producing plant contaminated surface water and groundwater (Frisbee et al., 2010). PFAS are not metabolized in humans or laboratory animals and undergo urinary and biliary excretion (Fujii et al., 2015; Harada et al., 2007). Some PFAS, such as perfluorohexanesulfonic acid (PFHxS), perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are slowly eliminated by the human body, with estimated half-lives ranging between 2.5 and 6 years (Li et al., 2018). Many studies conducted worldwide have reported measurable serum levels of PFHxS, PFOA, and PFOS in most of the general population (Calafat et al., 2007; Ingelido et al., 2010; Zhang et al., 2013).

PFAS have been associated with several health conditions (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2018), including dyslipidemia, one of the major risk factors for cardiovascular disease. Associations between serum PFAS and serum lipids have been evaluated among employees with occupational exposure (Costa et al., 2009; Olsen et al., 2007; Sakr et al., 2007; Wang et al., 2012), communities living near a manufacturing facility with high levels of PFAS in the drinking water (Fitz-Simon et al., 2013; Frisbee et al., 2010; Li et al., 2020; K. Steenland et al., 2009; Winquist and Steenland, 2014), and the general population with background PFAS exposure (Eriksen et al., 2013; Fisher et al., 2013; Fu et al., 2014; Geiger et al., 2014; Lin et al., 2019; Maisonet et al., 2015; Nelson et al., 2009; Skuladottir et al., 2015; Starling et al., 2014; Zeng et al., 2015). Most of these epidemiological studies showed positive associations between PFAS (mainly PFOA and PFOS) and total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), whereas associations with high-density lipoprotein cholesterol (HDL-C) and triglycerides were to a higher extent inconsistent. Only few studies had a longitudinal design (Fitz-Simon et al., 2013; Lin et al., 2019; Maisonet et al., 2015; Winquist and Steenland, 2014), while all the other were cross-sectional.

Almost all the published studies involved north-American or north-European populations, therefore results may not be generalizable to other contexts with different lifestyle habits. Moreover, a few studies considered the possible confounding effect of dietary factors (Donat-Vargas et al., 2019; Jain and Ducatman, 2019; Lin et al., 2019). Also, the dose-response relationships have been poorly investigated so far, since only a few studies have employed adequate statistical methods to explore the presence of non-linear relationships (Li et al., 2020; Maisonet et al., 2015).

In spring 2013, groundwater of a vast area of the Veneto Region (North-Eastern Italy) was found to be contaminated by PFAS. Immediately after, an environmental monitoring program was established to determine the extent and level of groundwater and drinking water contamination with twelve PFAS and to identify its source. A manufacturing plant located in the town of Trissino that produced PFAS since the late 1960s was identified as the only likely source of water contamination. The groundwater contamination plume extends over an area of 190 km^2 and reached public waterworks serving municipalities across the provinces of Vicenza, Verona, and Padua. Measurements of drinking water samples indicated that PFOA was the main contaminant ("Keeping our water clean: the case of water contamination in the Veneto Region, Italy," n.d.). Water treatment plants were immediately equipped with granular activated carbon filters which led to an abrupt reduction in PFAS concentrations in drinking water distributed by public waterworks (Pitter et al., 2020). Therefore, residents were exposed to high concentrations of PFAS, particularly PFOA, through drinking water until autumn 2013. A publicly funded health surveillance program is under way to aid in the prevention, early diagnosis, and treatment of chronic disorders possibly associated with PFAS exposure (Pitter et al., 2020).

The objectives of our study are to evaluate the relationships between the serum concentrations of several PFAS and serum lipid profiles assessed within the health surveillance program, taking into account possible anthropometric, socio-demographic and lifestyle confounders for such relationships and to evaluate if serum PFAS concentrations are associated with an increased risk of clinically relevant dichotomous outcomes: high TC, high LDL-C, low HDL-C and high triglycerides. Moreover, we systematically evaluate sex-specific associations.

2. Materials and methods

2.1. Participants and study design

This cross-sectional study was performed within the health surveillance program offered to the community of Veneto region that was exposed for several decades to PFAS via contaminated drinking water. The health surveillance program is a free population-based screening program offered by the regional Health Service to residents in the municipalities that were identified as the area served with a PFAS-contaminated public water supply, the so called "Red Area". The program, which has been described in more detail elsewhere (Pitter et al., 2020), started in January 2017 and is still ongoing. At the beginning, the Red Area comprised 21 municipalities but at a later stage nine more municipalities were included. The initial target population comprised 84,795 people born between 1951 and 2002 but later eligibility has been extended to include younger population (born between 2003 and 2014). The eligible population was identified through the Regional Health Registry and the invitation letters were sent by mail with the free offer of health examinations collecting the following information and samples: i) a structured interview on socio-demographic characteristics, personal health history and lifestyle habits, self-reported height and weight; ii) measurement of blood pressure; and iii) non-fasting blood and urine samples. Program visits are performed at public health facilities that are located throughout the contaminated area to ensure easy accessibility.

The population investigated in the present study is a subgroup of the surveillance program target population born between 1978 and 1999 and aged 20–39 years at recruitment, for whom enrolment has been completed. A total of 16,224 eligible young adult residents living in the investigated municipalities were enrolled in the surveillance plan from January 2017 to July 2019, and participation rate was 61%. Workers at the manufacturing plant with occupational exposure to PFAS were not included in the studied population. In addition, pregnant women at the time of participation in the study ($n = 327$) were excluded from the analysis because the levels of serum lipids may be altered in pregnancy and in addition pregnancy can have an impact on the exposure, distribution or excretion of PFAS. Participants who had reported using cholesterol lowering medications such as Statins, Fibrates and red rice (used as a natural statin) were also excluded ($n = 67$). Finally, 110 participants (less than 1%) with missing information on the selected covariates were excluded, leaving a total of 15,720 subjects included in the analyses.

2.2. PFAS exposure

Serum concentrations of twelve PFAS were measured by high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) [Prominence UFLC XR 20 (Shimadzu) coupled to API 4000TM LC-MS/MS System (Sciex)]: perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorodecanoic acid (PFDeA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA). Details of the analytical method have been described elsewhere (Pitter et al., 2020).

Method performances

allow analytes to be detected as low as 0.1 ng/mL (limit of detection LOD) and to be quantified above 0.5 ng/mL (limit of quantification

LOQ). Only three PFAS quantifiable in at least 50% of samples were considered for the analyses: PFOA (detected in 99.87% of people), PFOS (detected in 99.71% of people) and PFHxS (detected in 96.74% of people). Values below the LOQ were assigned a value equal to $LOQ/\sqrt{2}$.

The most extreme outliers in PFAS concentrations were excluded from analysis: (PFOA > 700 mg/L (n = 6), PFOS > 50 mg/L (n = 15), PFHxS > 100 mg/L (n = 3)).

2.3. Outcome assessment

Three laboratories within the Red Area (Arzignano, San Bonifacio, Legnago) have carried out the analyses of clinical biomarkers, including serum lipids, on subjects participating in the Health Surveillance Program.

Several plasma lipid parameters were considered: TC; HDL-C; LDL-C; non-HDL cholesterol (non-HDL-C); total/HDL cholesterol ratio (TC/HDL-C); triglycerides. TC and HDL-C were measured by a direct enzymatic colorimetric assay using cholesterol esterase and cholesterol oxidase. Triglycerides were measured using an assay based on glycerolphosphate oxidase-peroxidase aminophenazone. The measurement of serum lipids was performed in a Cobas automated clinical chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany) in two laboratories and in an AU automated clinical chemistry analyser (Beckman-Coulter, CA, USA) in the third laboratory. The three laboratories regularly follow an external quality assurance program. LDL-C was calculated by the Friedewald equation when triglycerides were less than 400 mg/dL (for 112 subjects it was not possible to calculate it). The non-HDL-C fraction, which is used as an estimation of the total number of atherogenic particles in plasma [VLDL + intermediate-density lipoprotein (IDL) + LDL], has been shown to be a better predictor of risk of cardiovascular risk than LDL-C alone (Liu et al., 2006; J. Perk et al., 2012; Ridker et al., 2005). Non-HDL-C was estimated by subtracting HDL-C from TC. In addition, TC/HDL-C ratio was calculated as another predictor of ischemic heart disease risk (Lemieux et al., 2001).

To facilitate the recruitment process, blood samples were not required to be fasting and were obtained throughout the course of the day.

Furthermore, lipid parameters have been considered as dichotomous outcomes in the analyses, using the following desirable values recommended by available guidelines for subjects at lower to moderate cardiovascular risk as cut offs: TC ≤ 190 mg/dL, HDL-C ≥ 40 mg/dL, LDL-C ≤ 115 mg/dL, non-HDL-C ≤ 145, and triglycerides ≤ 175 mg/dL (Gulizia et al., 2017; Nordestgaard et al., 2016; Joep Perk et al., 2012).

2.4. Data cleaning and covariates

Socio-demographic and medical history data were collected directly from participants via structured computer-assisted questionnaire using in-person interviews at study enrolment. Interviews were administered by trained public health nurses and lasted roughly 20 min. Standard data checks and cleaning procedures (e.g. range and consistency checks) were used to minimize errors and missing values and to maximize data quality. All original variables were examined, and decision rules were created for outliers and missing values.

We obtained information on age, sex, education level, smoking - habits, body mass index (BMI, calculated using self-reported weight and height), physical activity, personal history of disease, medication, alcohol consumption, and food intakes. Data on food consumption (meat, fish/seafood, milk/yogurt, cheese, eggs, bread/pasta/cereals, sweets/snacks/sweet beverages, fruits/vegetables) were transformed from number of servings per day/week/month to number of servings per week for all the food categories to allow harmonized diet pattern classification. BMI was classified as underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9), obese (≥30). Alcohol consumption was categorized in 0, 1–2, 3–6, 7 + alcohol units per week. Smoking status was subdivided into current smokers, previous smokers and non-smokers. Degree of physical activity (Light, Moderate, or Heavy) was defined based on an algorithm that combined information reported by the subject on intensity, duration, and frequency of all types of physical activity practiced during the week.

Countries of birth were classified into two categories based on geographical areas including: Italy plus other Highly Developed Countries (HDC) defined as Western Europe, North America, Oceania, Israel and Japan, and High Migratory Pressure Countries (HMPC), including Central-Eastern Europe, North Africa, Sub-Saharan Africa, Asia and Central and South America. The time-lag between the beginning of the study (1st January 2017) and the date of enrolment was calculated for each participant and included as a possible covariate (number of months). Information on the laboratory in charge of the analyses of serum lipids was considered as a possible confounder in statistical analyses.

Covariates to be included as potential confounders of the lipid/PFAS association were selected from available variables, based on the literature and through the construction of a directed acyclic graph (DAG) (Greenland S 2011). The minimally sufficient adjustment set was identified using DAGitty v1.0 (www.dagitty.net) implemented in R (R Development Core Team 2010, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/).

2.5. Statistical analysis

The serum concentrations of PFAS overall and stratified by sex, were summarised with the geometric mean (GM), arithmetic mean, standard deviation (SD) and percentiles. Since data on PFAS were markedly skewed to the right, concentrations were natural log (ln) transformed in order to improve the model fit. To improve normality and homogeneity of variance of the residuals in the tested associations, triglycerides values were ln-transformed as well. Log 10 transformation was also assessed, although the result was more and less the same. Normality was checked using the Shapiro-Wilk test. Spearman's rank correlation (ρ) was used to describe pair-wise relations between the PFAS.

We used generalized additive models (GAMs) to analyse the relation between each ln–transformed PFAS and each outcome, with three different approaches, each adjusted for the potential set of confounders. In order to explore the shape of associations between PFAS exposures and lipid levels, the models employed thin plate spline smooth terms (Duchon, 1977) for the exposures and continuous covariates, plotting the predicted values, estimated for a female subject with otherwise typical or average characteristics in the covariates set (Table S5). The degree of smoothing was selected by generalized cross validation as implemented in the R package mgcv (Wood, 2012). The Estimated Degrees of Freedom (EDF) of the GAM models can be interpreted as the degree to which a polynomial function (of the specific variable) should have to fit the data instead of using splines (if it's equal to 1 the relationship is close to linear and the spline model has collapsed to simple linear term). The p-value refers to the significance of the effect of the exposure variable. These models were fitted to the splines derived from the ln PFAS, and graphs show both the plot of predicted outcome against ln PFAS and the same fit back calculated to PFAS on a linear scale.

Secondly, to assess the linear relationship on the ln-transformed PFAS, linear regression coefficient (β) and 95% confidence intervals (CI) were reported. Thirdly, PFOA and PFOS levels were also categorized into deciles to assess the approximate exposure-response pattern while limiting the influence of extreme values. Owing to the PFHxS distribution, PFHxS values were only categorized into quartiles.

Lipid profiles have also been considered as dichotomous outcomes in logistic regression analyses, identifying the subjects with values above the normal range, and below the cut-off for HDL-C. For these analyses by PFAS quartile, a binomial link function has been used in the models and Odds Ratio (OR) were calculated, together with their 95% confidence intervals (95%CI).

All analyses were fully adjusted for the established set of covariates: age, BMI, time-lag between the enrolment and the beginning of the study (each continuous variable modelled using thin plate splines) and categorical covariates including sex, physical activity, smoking habits, country of birth, alcohol consumption, education level, laboratory in charge of the analyses of serum lipids (Arzignano, Legnago, San Bonifacio) and reported food consumption (in tertiles or quartiles of fruit/vegetables, milk/yogurt, cheese, meat, sweet/snacks/sweet beverage, eggs, fish, bread/pasta/cereals per week).

For supplementary, stratification by sex and an interaction term analyses between sex and ln-PFAS linear coefficient were also added to the models.

The level of statistical significance was set at 0.05. The statistical software STATA/SE version 13.0 (Stata Corp LP, College Station, TX, USA) and R (R Development Core Team 2010, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/) were used for statistical analyses.

2.5.1. *Sensitivity analysis*

Several sensitivity analyses were carried out for TC, as follows. To assess the robustness of the results, we have performed the analysis by using different types of smoothing splines, including cubic, penalized and adaptive splines. Regression splines (natural splines and B-splines) were also used to evaluate changes in the shape of the dose-response curve.

To reduce the impact of extreme values, we excluded from the population the extreme 5% of the subjects (TC < p2.5 and TC > p97.5) in each five-year age class.

A linear parametric effect for the variable age and the categorical versions of BMI (Underweight, Normal weight, Overweight and Obese) and time-lag between the enrolment and the beginning of the study (five months categories) were also used in the GAMs instead of the spline effects.

The presence of biases due to the laboratory in charge of the blood sampling have also been assessed through mixed effect models, adopting a multilevel structure where observations referring to the same lab are correlated with each other through a random lab-specific intercept.

We have finally performed models without including the time-lag between the interview and the beginning of the study, diet variables and BMI. Models were also adjusted for season instead of time-lag.

3. Results

The study population included 7620 (48.5%) females and 8100 (51.5%) males, with a mean age of 30 years (SD 5.9). General characteristics of participants according to sex are reported in Table S1.

Table 1 provides descriptive statistics on PFOA, PFOS and PFHxS. The highest serum levels of PFOA, PFOS, and PFHxS were found in males, and the lowest levels were found in females. Among the PFAS, PFOA was detected at the highest level (GM 31.3 ng/mL), followed by PFOS and PFHxS (GMs 3.7 and 3.2 ng/mL, respectively).

Strong correlations were observed between the measured PFAS. PFOA and PFHxS were most strongly correlated ($\rho = 0.91$), the correlation of PFOS with the other two compounds was slightly weaker ($\rho = 0.63$ with PFOA and $\rho = 0.65$ with PFHxS).

Descriptive statistics on lipid serum variables are shown in Table 2. The distribution of all outcomes was approximately normal except for triglycerides that were positively skewed and therefore ln-transformed in subsequent analyses. The median plasma concentration for TC, HDL-C, LDL-C, non-HDL-C, TC/HDL ratio and triglycerides were 177, 59, 98, 119, 3, 105 mg/dL respectively. Serum lipids were generally higher among males than females, except for HDL-C that was higher in females.

The adjusted concentration-response curves between TC and PFAS are shown in Fig. 1, and Figure S2-S6 (EDF and p-values: Table S6) for the other serum lipid concentrations through the plot of predicted values

Table 1
Distributions of serum PFASs concentrations (ng/mL) in the study population (n = 15,720), stratified by sex.

	Total								Male							Female						
	Mean (SD)	GM	Min	p25	p50	P75	Max	<LOQ	Mean (SD)	GM	Min	p25	p50	P75	Max	Mean (SD)	GM	Min	p25	p50	P75	Max
PFOA	59.62 (72.19)	31.27	0.35	13.6	35.8	78.8	1400	0.13%	83.77 (87.46)	49.85	0.35	25.15	58.3	114.7	1400	36.89 (43.03)	20.16	0.35	8.8	22.65	49.4	671
PFOS	4.62 (4.02)	3.72	0.35	2.5	3.7	5.6	142	0.29%	5.72 (4.42)	4.76	0.35	3.3	4.8	6.9	142	3.59 (3.27)	2.95	0.35	2	3	4.4	124
PFHxS	5.95 (6.81)	3.5	0.35	1.6	3.6	7.8	127	3.26%	8.85 (8.28)	5.85	0.35	3	6.5	12	127	3.23 (3.19)	2.16	0.35	1.1	2.2	4.3	41.3

SD: standard deviation.
GM: geometric mean.

Table 2
Distributions of Serum Lipids concentrations (mg/dL) and values out of range in the study population (n = 15,720), stratified by sex.

Outcome	Normal range	Male					Female					Total				
		Mean (SD)	Min	Median	Max	Out of range n (%)	Mean (SD)	Min	Median	Max	Out of range n (%)	Mean (SD)	Min	Median	Max	Out of range n (%)
Total C	≤190	179.5 (36.5)	70	176	487	2736 (35.9)	175.7 (32.4)	83	173	394	2477 (30.6)	177.5 (34.5)	70	174	487	5213 (33.2)
HDL-C	≤40	52.9 (12.0)	23	52	121	1016 (13.3)	64.1 (14.2)	25	63	144	287 (3.5)	58.7 (14.3)	23	57	144	1303 (8.3)
LDL-C	≥115	102.3 (32.9)	0	100	420	2517 (33.0)	94.2 (27.4)	0	92	285	1654 (20.4)	98.1 (30.4)	0	95	420	4171 (26.5)
Non-HDL-C	≥145	126.6 (36.8)	11	123	452	2181 (28.6)	111.6 (30.5)	25	108	309	1075 (13.3)	118.9 (34.5)	11	115	452	3256 (20.7)
Triglycerides	≥175	123.4 (88.6)	26	100	2728	149 (2728)	87.0 (49.0)	20	74	649	435 (5.4)	104.7 (73.3)	20	84.5	2728	1789 (11.4)
TC/HDL ratio	/	3.6 (1.0)	1.12	3.36	13.91	/	2.8 (0.7)	1.23	2.7	8.92	/	3.2 (1.0)	1.12	2.97	13.91	/

back transformed from logs to the original PFAS scale. These analyses revealed positive associations between TC, HDL-C, LDL-C and all PFAS with approximately linear relationships (EDF close to one and p-values < 0.05) between the natural logarithm of the three PFAS and these outcomes. No associations emerged with the TC/HDL-C ratio except for PFHxS ($p = 0.02$). Significant associations between PFOA and PFHxS and triglycerides were seen, with a clear departure from linearity in the relationship for PFOA (EDF = 5.58).

These trends were confirmed when exposures were considered as ln-linear predictors or categorical (based on percentiles of exposure) (Table 3). A full model for the association between PFOA and TC is presented in Table S7. Each ln-increase in PFOA was associated with an increase of 1.94 mg/dL (CI 1.48–2.41) in TC, 4.99 mg/dL (CI 4.12–5.86) for PFOS and 2.02 mg/dL (CI 1.45–2.58) for PFHxS. Overall the magnitude of the effect of the three PFAS was lower for HDL-C and essentially null for TC/HDL ratio. Subjects in the highest PFOA decile had respectively 9.10, 2.07, 5.30 and 7.02 mg/dL higher TC, HDL-C, LDL-C, and non-HDL-C than those in the lowest decile. Slightly higher increments per decile were seen for PFOS. Small increments were seen for PFOA and PFHxS concerning TC/HDL-C ratio. Increasing PFOA and PFHxS percentiles was also associated with increased TG.

For TC, the concentration-response curve when PFAS were categorized in deciles (Figure S7) showed a pattern consistent with the smoothed models, with a higher slope at lower PFOA concentrations, which tends to flatten above around 20/30 ng/mL. Likewise, there was a suggestion of a steeper slope across the first two deciles of PFOS but not so pronounced.

Table 4 shows the results of the models relating to the risk of abnormal values vs normal values with the exception of HDL-C where the risk of normal vs non-standard values is reported. Consistent with results that considered continuous outcomes, increasing PFAS quartiles were positively associated with an increased risk of abnormal TC: PFOA OR 1.46 (1.31–1.64), PFOS OR 1.58 (1.41–1.76), PFHxS OR 1.41 (1.25–1.58). All three PFAS were also associated with increased risk of abnormal LDL-C and non-HDL-C. PFOS also showed association with decreased HDL-C (Table 4). The odds of abnormal levels of triglycerides increased around 20% from the lowest to the highest quartile of PFOA and PFHxS serum levels.

When we examined associations according to sex (Tables S2-S3, Figures S8), results concerning TC were similar to the combined analyses. Sex significantly modified the association between all three PFAS and HDL-C, with significant associations seen only among women; conversely, men showed stronger associations with LDL-C and non-HDL-C than females (although with significant p-value for interaction only for PFOS).

Sensitivity analysis were conducted to assess the stability of the association between the three PFAS and TC (data not shown). Changing the type of smoothing splines to fit the dose-response curve did not affect the results, while the use of regression splines showed an extreme sensitivity to number and location of knots. The analysis on the reduced subset (excluding the 5% of subjects having extreme values of TC) gave similar results to the main analyses, although with a lower magnitude of the effects, especially with respect to PFOA and PFHxS. Results were not substantially altered when excluding the variable time-lag between the enrolment and the beginning of the study, nor when excluding the diet variables and BMI as possible confounders. Furthermore, estimates were similar when models were adjusted for season instead of the time-lag variable. Lipids concentrations significantly vary between the three different labs (Table S8). Using a mixed effect model with a random intercept for the laboratory in charge of blood sampling did not also altered the association between PFOA and TC ($\beta = 1.93$ mg/dL (95% CI 1.47–2.40)) with an Intraclass Correlation Coefficient of 0.10.

4. Discussion

This cross-sectional study demonstrates that in a population of over

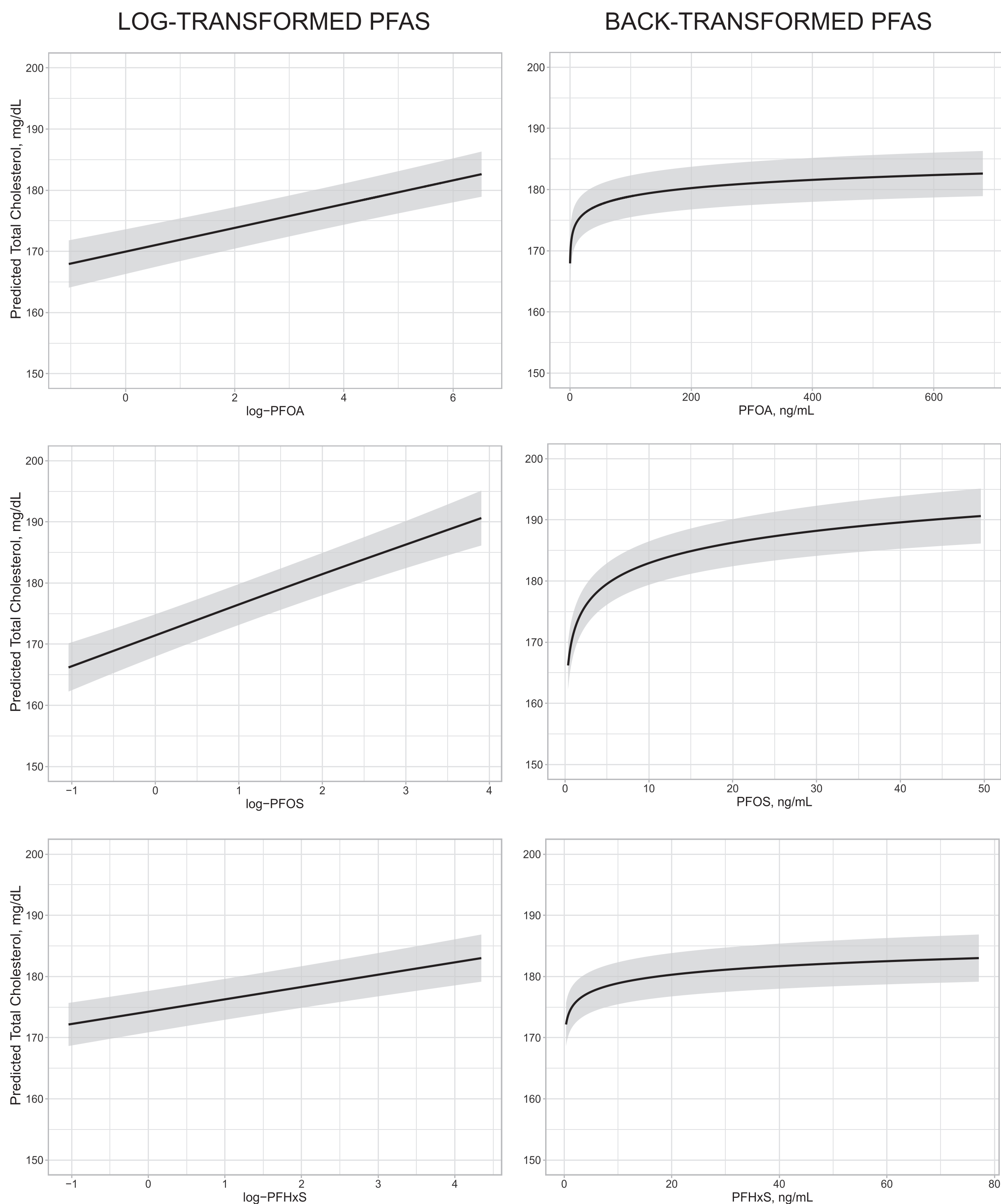

Fig. 1. Exposure–response curves for PFAS exposure and total cholesterol concentrations from GAM models using thin plate spline, with 95% confidence intervals. The predicted levels are based on average characteristics (presented in Table S5) used as covariates in the models.

15,000 young adults exposed to PFAS, mainly PFOA, through drinking water, serum concentrations of PFOA, PFOS, and PFHxS were associated with serum concentrations of TC, non-HDL-C, LDL-C, and HDL-C. The shape of exposure-response associations was investigated using smoothing splines showing an approximately linear relationship between the In-transformed PFAS and above mentioned outcomes. The largest increases in cholesterol levels per unit of PFAS were seen at the lowest range of PFAS concentrations, with shallower slopes per unit of PFAS at higher PFAS concentrations. Moreover, our modelling took into consideration the possible confounding effect of dietary habits. The latter aspect is very important since some food categories, like eggs, milk, fish, and meat, can both have an impact on the serum lipid profile and can contribute to the dietary intake of PFOA and PFOS (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2018; Lorber and Egeghy, 2011). However, results did not differ with or without including all the diet variables as the possible confounders in the models. Consistent with this association with measured lipids, PFAS were associated with an increased probability of having abnormal levels of TC,

Table 3
Association between PFAS (ln ng/mL) and Serum Lipids (mg/dL) from GAM models: adjusted β* coefficients and 95% Confidence Intervals (CI).

PFAS	TC		HDL-C		LDL-C		Non-HDL-C		TC/HDL		ln-Tryglicerides	
	β*	IC 95%	β*	IC 95%	β*	IC 95%	β*	IC 95%	β*	IC 95%	β*	IC 95%
PFOA												
D1 (0.35–5.5)	*171.75*¶		*60.54*¶		*95.58*¶		*2.94*¶		*111.23*¶		*4.30*¶	
D2 (5.6–10.3)	2.83	(0.61–5.05)	0.59	(−0.26–1.44)	1.43	(−0.52–3.38)	0.01	(−0.05–0.07)	2.23	(0.1–4.37)	0.04	(0.01–0.08)
D3 (10.4–16.8)	3.51	(1.27–5.76)	0.45	(−0.41–1.31)	1.4	(−0.56–3.37)	0.04	(−0.01–0.1)	3.03	(0.87–5.18)	0.07	(0.04–0.1)
D4 (16.9–24.9)	4.89	(2.63–7.15)	1	(0.13–1.86)	2.89	(0.91–4.86)	0.03	(−0.02–0.09)	3.84	(1.67–6.01)	0.05	(0.02–0.09)
D5 (25–35.3)	4.15	(1.88–6.37)	0.91	(0.04–1.78)	2.2	(0.22–4.18)	0.03	(−0.02–0.09)	3.2	(1.03–5.38)	0.04	(0.01–0.08)
D6 (35.4–48.9)	5.39	(3.11–7.64)	1.32	(0.44–2.2)	2.76	(0.76–4.76)	0.04	(−0.02–0.1)	4.01	(1.82–6.21)	0.07	(0.04–0.1)
D7 (49–66.4)	4.80	(2.48–7.06)	1.43	(0.54–2.32)	2.29	(0.26–4.32)	0.01	(−0.05–0.07)	3.32	(1.1–5.55)	0.05	(0.02–0.09)
D8 (66.5–93.3)	6.72	(4.38–8.95)	1.81	(0.91–2.71)	3.04	(0.99–5.09)	0.04	(−0.02–0.1)	4.87	(2.62–7.12)	0.09	(0.06–0.13)
D9 (93.4–140.7)	6.91	(4.53–9.15)	1.87	(0.95–2.78)	3.87	(1.79–5.95)	0.05	(−0.01–0.11)	4.99	(2.71–7.27)	0.08	(0.04–0.11)
D10 (141–1400)	9.10	(6.68–11.36)	2.07	(1.14–3)	5.3	(3.18–7.43)	0.07	(0.01–0.13)	7.02	(4.69–9.35)	0.09	(0.06–0.13)
per ln-ng/mL	1.94	(1.48–2.41)	0.49	(0.32–0.67)	1.12	(0.71–1.52)	1.44	(0.99–1.88)	0.01	(0–0.03)	0.02	(0.01–0.03)
PFOS												
D1 (0.35–1.7)	*171.34*		*60.02*¶		*94.40*¶		*2.96*¶		*111.34*¶		*4.35*¶	
D2 (1.8–2.2)	4.33	(2.14–6.51)	1.13	(0.29–1.97)	2.94	(1.03–4.85)	0	(−0.05–0.06)	3.19	(1.09–5.29)	0.02	(−0.02–0.05)
D3 (2.3–2.7)	5.22	(3.09–7.35)	1.84	(1.02–2.66)	3.5	(1.64–5.37)	−0.01	(−0.06–0.05)	3.38	(1.33–5.43)	0	(−0.03–0.03)
D4 (2.8–3.2)	5.29	(3.11–7.46)	1.8	(0.96–2.63)	3.45	(1.55–5.35)	0.01	(−0.04–0.07)	3.52	(1.43–5.61)	0.01	(−0.02–0.05)
D5 (3.3–3.7)	5.94	(3.71–8.13)	2.12	(1.26–2.98)	4.52	(2.57–6.48)	0	(−0.05–0.06)	3.82	(1.68–5.97)	0	(−0.03–0.03)
D6 (3.8–4.3)	6.57	(4.36–8.71)	2.42	(1.56–3.27)	4.26	(2.32–6.2)	0.01	(−0.05–0.06)	4.14	(2.01–6.27)	0.01	(−0.02–0.04)
D7 (4.4–5.1)	8.07	(5.87–10.25)	2.24	(1.4–3.09)	5.72	(3.79–7.65)	0.03	(−0.03–0.08)	5.86	(3.74–7.98)	0.02	(−0.02–0.05)
D8 (5.2–6.2)	8.68	(6.44–10.87)	2.94	(2.08–3.81)	6.19	(4.22–8.15)	−0.01	(−0.07–0.05)	5.73	(3.57–7.89)	0	(−0.04–0.03)
D9 (6.3–8.2)	10.20	(7.93–12.33)	3.43	(2.55–4.3)	7.22	(5.23–9.2)	0	(−0.05–0.06)	6.78	(4.6–8.96)	0.02	(−0.01–0.05)
D10 (8.3–142)	11.77	(9.45–13.94)	2.95	(2.06–3.84)	9.67	(7.64–11.7)	0.05	(−0.01–0.11)	8.86	(6.63–11.09)	0.01	(−0.02–0.05)
per ln-ng/mL	4.99	(4.12–5.86)	1.43	(1.1–1.76)	3.97	(3.21–4.73)	3.56	(2.73–4.4)	0.01	(−0.01–0.04)	0	(−0.01–0.01)
PFHxS												
Q1 (0.35–1.6)	*173.58*¶		*60.94*¶		*96.26*¶		*112.69*¶		*2.95*¶		*4.34*¶	
Q2 (1.7–3.6)	3.19	(1.76–4.62)	0.73	(0.18–1.27)	2.27	(1.02–3.52)	2.47	(1.1–3.84)	0.02	(−0.01–0.06)	0.02	(0–0.04)
Q3 (3.7–7.8)	3.3	(1.81–4.78)	1.13	(0.56–1.7)	1.63	(0.33–2.93)	2.15	(0.73–3.58)	0.01	(−0.03–0.05)	0.03	(0.01–0.05)
Q4 (7.9–77.1)	5.57	(3.91–7.23)	1.16	(0.53–1.8)	3.82	(2.37–5.27)	4.4	(2.81–5.99)	0.06	(0.01–0.1)	0.04	(0.01–0.06)
per ln-ng/mL	2.02	(1.45–2.58)	0.46	(0.24–0.67)	1.31	(0.81–1.8)	1.56	(1.02–2.09)	0.02	(0–0.03)	0.02	(0.01–0.02)

Q1-Q4:quartile 1-quartile 4.
D1-D10: decile 1 – decile 4.
* adjusted by age, BMI, time-lag between the enrolment and the beginning of the study, sex, physical activity, smoking habits, country of birth, alcohol consumption, education level, laboratory in charge of the analyses of serum lipids and reported food consumption (in tertiles or quartiles of fruit/vegetables, milk/yogurt, cheese, meat, sweet/snacks/sweet beverage, eggs, fish, bread/pasta/cereals per week)
¶ predicted values of each outcome for the 1st percentile (decile or quartile) of the PFAS distribution

Table 4
Association between PFAS and abnormal Serum Lipids from GAM models adjusted by several covariates, using PFAS quartiles: adjusted odds ratios (OR*) and 95% Confidence Intervals (CI).

PFAS	TC		HDL-C**		LDL-C		Non-HDL-C		Triglycerides	
	OR*	IC 95%	OR*	IC 95%	OR*	IC 95%	OR*	IC 95%	OR*	IC 95%
PFOA										
Q1	1		1		1		1		1	
Q2	**1.20**	(1.08–1.33)	1.14	(0.94–1.38)	**1.14**	(1.02–1.28)	1.10	(0.97–1.25)	1.04	(0.88–1.24)
Q3	**1.25**	(1.12–1.39)	1.13	(0.93–0.90)	1.11	(0.98–1.19)	1.11	(0.97–1.27)	1.13	(0.95–1.34)
Q4	**1.46**	(1.31–1.64)	1.10	(0.90–1.34)	**1.34**	(1.19–1.52)	**1.35**	(1.18–1.55)	**1.24**	(1.05–1.48)
PFOS										
Q1	1		1		1		1		1	
Q2	**1.19**	(1.07–1.32)	1.07	(0.89–1.29)	1.10	(0.98–1.23)	1.02	(1.12–1.36)	**0.83**	(0.71–0.98)
Q3	**1.37**	(1.23–1.53)	**1.25**	(1.03–1.15)	**1.21**	(1.08–1.37)	**1.21**	(1.06–1.37)	0.94	(0.80–1.11)
Q4	**1.58**	(1.41–1.76)	**1.40**	(1.15–1.70)	**1.52**	(1.35–1.72)	**1.37**	1.20–1.57)	0.90	(0.76–1.06)
PFHxS										
Q1	1		1		1		1		1	
Q2	**1.18**	(1.06–1.30)	1.12	(0.92–1.37)	**1.21**	(1.08–1.35)	**1.20**	(1.05–1.36)	1.11	(0.93–1.32)
Q3	**1.19**	(1.07–1.32)	1.18	(0.97–1.45)	**1.15**	(1.02–1.29)	1.12	(0.98–1.28)	1.17	(0.98–1.40)
Q4	**1.41**	(1.25–1.58)	1.15	(0.93–1.41)	**1.37**	(1.20–1.55)	**1.36**	(1.18–1.57)	**1.22**	(1.02–1.46)

*adjusted by age, BMI, time-lag between the enrolment and the beginning of the study, sex, physical activity, smoking habits, country of birth, alcohol consumption, education level, laboratory in charge of the analyses of serum lipids and reported food consumption (in tertiles or quartiles of fruit/vegetables, milk/yogurt, cheese, meat, sweet/snacks/sweet beverage, eggs, fish, bread/pasta/cereals per week)
** risk of HDL ≥ 40 vs below; Q1-Q4:quartile 1-quartile 4

non-HDL-C, and LDL-C and, for PFOS, protective levels of HDL-C. Limited to PFOA and PFHxS, a weak association was also found with triglycerides.

This is the first study investigating the association between PFAS and serum lipids in a Mediterranean country, in which lifestyle and dietary habits are very different from those of North-American, North-European, or Far Eastern countries. We analysed a large group of highly exposed community residents aged 20–39 years with median serum concentrations of 35.8 ng/ml for PFOA, which is about 22 times higher than the PFOA serum concentration in the Veneto citizens with only background exposures (1.64 ng/mL) (Ingelido et al., 2018), and that allowed us to model the outcome variables across a wide range of serum PFOA concentrations. For PFHxS the level of exposure in the exposed population (median: 3.6 ng/mL) was slightly higher (≈1.44 times) than those with background exposure (median: 2.49 ng/mL) (Ingelido et al., 2018). The median PFOS level (5.84 ng/mL) measured in the Veneto residents with background exposure (Ingelido et al., 2018) was 1.6 times higher than the median PFOS level in the serum samples of the highly exposed residents in the Veneto region (3.7 ng/mL).

Notably, our results are consistent with those of the C8 Mid Ohio Valley study on 46,294 highly exposed community residents aged 18 years or above, both in terms of shape of the observed relationships and in terms of magnitude of the effects. Median serum PFOA was a little higher in this population (35.8 ng/mL) than in the C8 population (median, 27 ng/mL), although the range of PFOA exposure in C8 study (0.25–17556.6 ng/mL) was wider than the present study (0.35–1400 ng/mL). In the present study, the predicted increase in TC from lowest to highest decile was about 9 mg/dL for PFOA and was around 12 mg/dL for PFOS compared to the results of the C8 study as 11 and 12 mg/dL increase in total cholesterol for PFOA and PFOS, respectively (K. Steenland et al., 2009). Similar to the findings here and in the C8 study, a steeper increase in TC was noted in the low range of serum PFOS, PFHxS and PFOA concentrations in the study conducted by Li et al., on 1945 adults aged 20–60 in the community of Ronneby, Sweden (Li et al., 2020). Similar effect for PFOA were also seen in general populations with lower exposure contrasts (Eriksen et al., 2013; Nelson et al., 2009).

Altogether, the present study represents a strong confirmation of previous results, through their replication in a different socio-cultural context and with more detailed adjustment for potential confounders. This confirmation is important, since recently the European Food Safety Authority (EFSA) proposed new values for Tolerable Weekly Intake (TWI) of PFOA and PFOS using the increase in serum TC as a critical effect, based on human epidemiological studies (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2018).

Some aspects of our results deserve to be underlined. First, we see linear-log associations with no evidence of a threshold in the PFAS serum ranges studied. Second, the magnitude of the effect per unit PFAS is higher at low serum PFAS concentrations and then tends to progressively level off at higher concentrations, without disappearing. Thus, if these associations were causal, it would imply that even a low, background exposure has an effect, which would reach zero only if the exposure was zero.

Another notable finding is that the PFOS effect, as seen in the C8 study (K. Steenland et al., 2009), seems to be stronger than PFOA and PFHxS: for every increase in one logarithmic unit of serum PFOS, the increase of serum TC is 4.99 mg/dL, while for PFOA and PFHxS it is of 1.94 mg/dL and 2.02 mg/dL, respectively. However, these differences in slopes for models with each PFAS separately need to be treated with some caution, since a ln PFOA spans larger absolute contrasts than the lower concentration ranges for the other PFAS. This population has had significant exposure to PFOA and the serum levels were highly correlated (particularly PFOA and PFHxS) so it was not possible to statistically distinguish their biological effects, and the apparent effect of one specific PFAS might thus be expected to reflect to some extent the effect of other correlated PFAS.

The observed sex differences in PFAS concentrations are consistent with findings of other cross-sectional studies (Fromme et al., 2009; Kyle Steenland et al., 2009; Yu et al., 2020). Females have lower serum PFAS concentrations than males during their reproductive years (as our study population is) and inputs to these differences are known to most likely include menstrual excretion, previous childbirth with transplacental transfer of PFAS to the developing foetus and breastfeeding (Cariou et al., 2015; Lorber et al., 2015; Manzano-Salgado et al., 2015; Mondal et al., 2014). Another explanation for these differences may be the higher renal clearance of PFAS in women, and longer half-lives have been observed in males (Fromme et al., 2009; Li et al., 2018). Further, stronger relationships observed between PFOA serum and urine concentrations in male participants compared to female participants support the relative importance that non-renal excretion pathways may play in PFAS clearance in women (Worley et al., 2017).

We found that sex modifies the associations between serum PFAS and lipids. Most of the studies have applied the conventional practice of

integrating males and females and adjusting rather than stratifying for sex, which might overlook important differences (Fu et al., 2014; Geiger et al., 2014; Zeng et al., 2015). In a few cases results of the associations between PFAS and cholesterol have been stratified by sex (Frisbee et al., 2010; He et al., 2018; Jain and Ducatman, 2019; Nelson et al., 2010; Winquist and Steenland, 2014). In our study, the magnitude of the effect on non-HDL-Cand LDL-C was larger in males than in females, while on the contrary we observed an effect on HDL-C only in females. This "healthy"-looking association of PFAS with HDL-C for females has been reported previously in adult females (Jain and Ducatman, 2019) and adolescent girls (Nelson et al., 2010) but has not been consistent and the opposite has also been reported (Frisbee et al., 2010; Wang et al., 2012). Compared with males, female in pre-menopause age, as the group analyzed in the present study, have lower levels of LDL-C and higher HDL-C (Anagnostis et al., 2015) reflecting a role of estrogens in regulating liver lipid metabolism towards a more protective pattern by modifying signaling to estrogen receptors (Stevenson et al., 1993). This metabolic pattern in females may play a role in the differential effect of PFAS on lipids between males and females of reproductive age.

The strengths of the present study are the large sample size, the adjustment for a large set of possible confounders and the robustness of statistical methods. The main limitation is the cross-sectional design which precludes the possibility to infer the causal nature of the observed associations. Another potential limitation is that serum lipids were determined in three different laboratories with different analytical platforms. However these different methods have been shown to be correlated (Kim et al., 2014), and we accounted for any laboratory effects through the adjustment by laboratory. Furthermore, in a sensitivity analysis, modelling laboratory in charge of blood sampling with a random intercept did not affect our findings.

Serum lipids levels may vary in the same subject according to season and fasting status. The seasonal variability was controlled adjusting by time since the beginning of the study. Whereas, no adjustment was possible for fasting status because the information on the time since the last meal was lacking. However, circulating levels of plasma lipids and lipoproteins are only modestly influenced by food consumption. Among all studies comparing non-fasting with fasting lipid profiles, only minor increases in plasma triglycerides and no relevant change in TCl, LDL-C, and HDL-C concentrations were observed (Dipankar and Pawar, 2019; Langsted et al., 2014; Langsted and Nordestgaard, 2019; Mora et al., 2008). Numerous population-based studies (totalling > 300,000 non-fasting individuals) and three major statin trials (totalling 43,000 non-fasting individuals) used random, non-fasting blood sampling, thus providing a substantial body of evidence to support a change in the conventional practice of using fasting samples for such studies (Nordestgaard et al., 2016). These findings have led to the inclusion of non-fasting lipid measurements in several national society guidelines (Anderson et al., 2015; Nordestgaard et al., 2016; Rabar et al., 2014). Therefore, we believe it's unlikely that the variability of fasting time significantly affected serum cholesterol levels, whilst we cannot exclude that it might affect serum triglycerides levels. Overall, to our knowledge, fasting has no effect on serum PFAS concentrations, therefore it should not be considered as a confounder.

At present, the mechanisms underlying the association between PFAS and serum lipids are poorly understood and there are some concerns of a possibility of reverse causality or confounding affecting observed associations in cross sectional studies. For example, PFAS can bind to serum lipoproteins, however, the percentage of PFAS bound to serum lipoproteins is low (Butenhoff et al., 2012). The EFSA Panel concluded therefore that this binding cannot explain the magnitude of the association between PFAS and serum cholesterol level observed in epidemiologic studies, and a reverse causation mechanism is unlikely (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2018).

Another possible explanation for this association, as argued by Steenland and colleagues (Steenland et al., 2010), is the existence of a confounding mechanism that affects serum levels of both PFAS and lipids. PFAS are subject to entero-hepatic circulation (Harada et al., 2007) mediated by a number of transporters located in the gut mucosa and at the hepato-biliary interface, including organic anion transporting polypeptides (Zhou et al., 2017) and bile acids transporters, such as human apical sodium-dependent bile salt transporter (ASBT) and the Na + -taurocholate co-transporting polypeptide (NTCP) (Zhou et al., 2017). Current evidence suggests there is concomitant entero-hepatic reabsorption of both bile acids and PFAS in the intestine (Salihović et al., 2020). Decreased reabsorption of bile acids through the gut mucosa causes the depletion of the bile acids pool and stimulates the liver to increase the synthesis of bile acids from cholesterol, ultimately causing a reduction of serum cholesterol levels. This is the mechanism at the basis of the hypolipidemic action of cholestyramine, a bile acid sequestrant. Of note, a few animal studies and human case reports suggest that cholestyramine may increase fecal PFAS excretion (Genuis et al., 2013, 2010; Johnson, 1984). Taking into account all these elements, one may hypothesise is that some factor, such as differences in activity of bile acids transporters, may affect entero-hepatic reabsorption of both PFAS and bile acids (with ensuing increase of serum cholesterol), thus introducing a correlation, i.e. confounding, between PFAS and serum cholesterol.

The third possibility is that PFAS really increase serum lipids. Longitudinal study designs and reliance on modelled PFOA intake rather than measured serum levels, are less vulnerable to the mechanisms of confounding or reverse causation discussed above. A follow-up study in the C8 Mid-Ohio Valley population reported an association between increasing doctor diagnosed hypercholesterolemia and modelled PFOA serum levels over the period of increasing PFOA exposure (Winquist and Steenland, 2014). Another study (Fitz-Simon et al., 2013) investigating the changes of serum cholesterol in relation to degree of elimination of PFAS from the body showed that a 50% more decrease of serum PFOA and PFOS levels were associated with a decrease of serum total cholesterol of 1.7% and 3.2%, respectively, and with a decrease of LDL cholesterol of 3.6% and 5.0%, respectively. These longitudinal studies supported a causal role of PFOA and PFOS. The latter study also suggests that any causal mechanism would be transient and reversible. In another study conducted by Lin et al., (2019) the relationship of baseline plasma PFAS concentrations with repeated measures of blood lipids on 888 prediabetic adults were examined. The results of the study showed that baseline PFAS was associated with a higher incidence of hyperlipidemia. Additionally, the study found that participants who received an intensive lifestyle intervention of diet and exercise had lower incidence of hyperlipidemia compared to placebo and protective effect of the lifestyle intervention on hyperlipidemia persisted throughout the 15 years of follow-up (Lin et al., 2019). However, although cholesterol is a well-established major cardiovascular risk factor, it is of note that the longitudinal study in the C8 Mid-Ohio Valley population showed no clear evidence of an association between previous PFOA exposure and incidence of cardiovascular disease (Winquist and Steenland, 2014).

Experimental evidence on causal mechanisms relating PFAS to serum lipids is accruing. PFAS have structural similarities with fatty acids and may interfere with fatty acid metabolism and lipid synthesis in the liver (Frayn et al., 1996; Pilz et al., 2006). Fatty acids are endogenous ligands for peroxisome proliferator activated receptor α (PPARα), a transcription factor that regulates lipid homeostasis. In rodents, the activation of PPARα is a well-documented effect of PFAS (ATSDR, 2018). According to a recent experimental study in mice expressing human PPARα and fed with a high-fat diet, exposure to PFOA at a human-relevant level caused liver steatosis, activation of human PPARα and dysregulation of several other genes involved in lipid homeostasis (Schlezinger et al., 2020). Interestingly, the patterns of dysregulation differed between male and female mice, which suggests that the sex difference observed in the present and other epidemiological studies may reflect some sex-specific mechanism linked to lipid metabolism. However, PPARα activation in rodents has been shown to induce a decrease of serum levels of triglycerides and cholesterol, that is the opposite of what has been

observed in epidemiological studies on PFAS and serum lipids though at much higher body burdens (United States Environmental Protection Agency (US-EPA)., 2016). Humans and non-human primates are deemed less responsive to PPARα agonists than rodents (United States Environmental Protection Agency (US-EPA)., 2016). Additionally, PPARα is the target of fibrates, a class of human drugs used to treat hypertriglyceridemia which also have a modest lowering effect on LDL-cholesterol. Thus, any causal mechanism at the basis of the association between PFAS and serum lipids in humans might be PPARα-independent. PFOA and PFOS have been shown to decrease synthesis of a number of bile acids in human hepatoma cells, indicating that both substances have a cholestatic potential which may play a role in the observed increased serum cholesterol levels in humans in epidemiological studies (Behr et al., 2020). Furthermore, exposure to PFAS has been also associated with oxidative stress (Liu et al., 2007; Yao and Zhong, 2005) and endothelial dysfunction (Hu et al., 2003; Qian et al., 2010), both phenomena related to the development of cardiovascular diseases, as well as with DNA damage as shown in a human liver-derived cell line (Wielsøe et al., 2015). Overall, further studies will have to be conducted to elucidate the molecular initiating events triggered by PFAS via adverse outcome pathways approach in humans and for other receptors in the environment.

5. Conclusion

This study provides evidence of PFAS-specific associations with lipids in a large group of young adults who were exposed to contaminated drinking water for decades. Specifically, serum PFOA and PFOS concentrations were positively associated with TC and LDL-C/non-HDL-C, with strongest association seen for PFOS than PFOA or PFHxS. The magnitude of the effect per unit of serum PFAS was higher at low serum PFAS concentrations with no evidence of a threshold.

Overall, we consider that this study contributes to strengthening the evidence from multiple studies, of PFAS, and PFOA in particular, causing an increase in cholesterol. While there has been more detailed control of potential dietary confounders than in other studies, some residual confounding related to absorption and excretion pathways cannot be ruled out and may contribute to some of the associations observed.

More effort is needed to study mechanisms of action of PFAS in human cells and tissues to understand potential causality, and longitudinal studies of cardiovascular risk in relation to PFAS, particularly on lipid subfractions, are warranted.

CRediT authorship contribution statement

Cristina Canova: Conceptualization, Methodology, Resources, Writing - original draft, Supervision. **Giulia Barbieri:** Data curation, Formal analysis, Software. **Maryam Zare Jeddi:** Conceptualization, Writing - review & editing. **Massimo Gion:** Funding acquisition, Writing - original draft. **Aline Fabricio:** Writing - original draft. **Francesca Daprà:** Funding acquisition, Writing - original draft. **Francesca Russo:** Funding acquisition, Investigation, Writing - review & editing. **Tony Fletcher:** Conceptualization, Methodology, Writing - review & editing. **Gisella Pitter:** Investigation, Conceptualization, Methodology, Writing - original draft.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.106117.

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