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Article

PFAS concentrations and cardiometabolic traits in highly exposed children and adolescents

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Abstract: Background: Residents of a large area of North-Eastern Italy were exposed for decades to high concentrations of perfluoroalkyl and polyfluoroalkyl substances (PFAS) via drinking water. Despite the large amount of evidence in adults of a positive association between serum PFAS and metabolic outcomes, studies focusing on children and adolescents are limited. We evaluated the associations between serum PFAS concentrations and lipid profile, blood pressure and body mass index (BMI) in highly exposed adolescents and children. Methods: A cross-sectional analysis was conducted in 6669 adolescents (14-19 years) and 2693 children (8-11 years) enrolled in the health surveillance program of the Veneto Region. Non-fasting blood samples were obtained and analyzed for perfluorooctanoic acid (PFOA) perfluorooctane sulfonate (PFOS), perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), total cholesterol (TC) high-density lipoprotein cholesterol (HDL-C) and triglycerides. Low-density lipoprotein cholesterol (LDL-C) was calculated. Systolic and diastolic blood pressure (BP) were measured and BMI z-score accounting for age and sex was estimated. The associations between ln-transformed PFAS (and categorized into quartiles) and continuous outcomes were assessed using generalized additive models. Analyses were stratified by gender and adjusted for potential confounders. Results: Among adolescents, significant associations were detected between all investigated PFAS and TC, LDL-C, and to a fewer extent HDL-C. Among children, PFOS and PFNA had significant associations with TC, LDL-C and HDL-C, while PFOA and PFHxS had significant associations with HDL-C only. Increased serum concentrations of PFAS, particularly PFOS, were associated with decreased BMI z-score. No statistically significant associations were observed between PFAS concentrations and BP. Conclusions: Our study supports a consistent association between PFAS concentration and serum lipids, stronger for PFOS and PFNA and with a greater magnitude among children compared to adolescents, and a negative association of PFAS with BMI.

Keywords: perfluoroalkyl substances (PFAS); children; adolescents; lipid profile; cholesterol; generalized additive model

1. Introduction

Over the past few decades, per- and polyfluoroalkyl substances (PFAS) contamination has grown into a serious global health threat. Because of its favorable

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properties, PFAS are used in numerous consumer products and industrial applications to confer waterproof, greaseproof, stain-proof and low-friction properties [1]. PFAS are among the most ubiquitous synthetic chemicals in the world and environment and human exposure to PFAS can occur throughout the life cycles of these chemicals and products containing them [2]. Human are exposed to PFAS via ingestion of contaminated food and drinking water [3], inhalation of indoor air and indoor dust [4] and use of consumer products. In humans, PFAS half-lives in serum may vary greatly with expected variation in children (small blood volumes and large fraction of exposures coming from drinking) compared to adults [5]. Among the numerous PFAS congeners, only few PFAS have been thoroughly studied from an epidemiological perspective, especially perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Adverse impacts have been consistently reported for PFOA and PFOS on the endocrine, immune, and metabolic outcomes (lipid profile, blood pressure, obesity and metabolic syndrome) in occupationally exposed, highly exposed, and general populations [6]. Despite the large amount of evidence in adults, studies focusing on the association between serum PFAS and metabolic outcomes in children and adolescents are limited, and the potential associations for some outcomes have been scarcely studied in European children and adolescent's population [7–9].

Given the emerging consensus that the development of cardiovascular disease in adulthood is preceded by metabolic changes occurring in childhood, and considering interspecies and interindividual differences in dose/concentration-response assessment and different exposure conditions, it is important to identify the risk associated to exposure to PFAS in this population. Because both childhood and adolescence are distinguished by marked changes in growth, sexual maturity and hormonal secretion, the risk of endocrine disruption by extrinsic agents may be altered during these life phases [10]. Furthermore, the number of underlying factors (e.g. prevalent chronic or acute disease or medication use) confounding the associations between environmental exposure and potential health consequences in children and adolescents is likely to be smaller in this population and is worthy of further insight.

Residents in a large area of the Veneto Region (North-Eastern Italy) were exposed to high concentrations of PFAS, particularly PFOA, via contaminated drinking water from a manufacturing plant active since the late 1960s, until autumn 2013 when water treatment plants were equipped with granular activated carbon filters [3]. In this context, the objective of this study was to evaluate the associations between serum PFAS concentrations and various cardiometabolic traits (lipid profile, blood pressure, and body mass index) in a large group of highly exposed adolescents aged 14-19 years and children aged 8-11 years recruited as part of a community-based health surveillance in Veneto Region, Italy [3].

2. Materials and Methods

2.1. Study design and recruitment

The health surveillance program in Veneto Region is a cross-sectional study realized in 2017 to document exposure to PFAS, and to evaluate associated adverse health outcomes in a highly exposed community residents of 30 municipalities in the Vento Region, Italy. The Project has been more completely described elsewhere [3]. The participants in this study were 9,475 adolescents aged 14 to 19 years and children aged 8 to 11 years at their enrollment in the health surveillance program, representing one of the largest community-based study to date investigating potential associations between PFAS exposure and human health effects in these age groups. Potential participants were sent an invitation letter that explained the health surveillance program and fixed an appointment. Participants <19 years old had to be accompanied by a parent or legal guardian. To facilitate recruitment at different times of the day while minimizing the research impact on school attendance, participants were not required to be in fasting

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conditions. Informed consent from the participants or their parents/legal guardians was obtained orally and recorded in the individual's clinical charts.

2.2. Data collection

At four different centers (Lonigo, Legnago, San Bonifacio, and Noventa Vicentina), trained nurses collected personal and medical data, anthropometric measures (height, weight), blood pressure and blood samples from the participants. Analyses of clinical biomarkers, including serum lipids, were carried out from three different laboratories (Arzignano, San Bonifacio, Legnago). Medical history, medications, socio-demographic information, and lifestyle habits were collected using interviewer-administered questionnaires.

2.3. Outcomes of Interest

2.3.1. Lipid profile

Several plasma lipid parameters including total cholesterol (TC); high-density lipoprotein cholesterol (HDL-C); low-density lipoprotein cholesterol (LDL-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 16 subjects it was not possible to calculate it).

2.3.2. Overweight and Obesity

BMI was recalculated after checking the accuracy of data regarding the self-reported height and weight. Since the relationship between weight and height changes considerably during the childhood, we converted BMI to a BMI z-score accounting for age and sex using the recommended WHO Growth References for school-aged children and adolescents [11]. The Z-score system expresses the anthropometric value as a number of standard deviations or Z-scores below or above the reference mean or median value [12]. A BMI z-score of ≥2 indicates obesity.

This measure was obtained using the R package z-scorer, calculated as number of standard deviations above or below the reference median value, based on the WHO Growth References for school-aged children and adolescents (https://www.who.int/nutgrowthdb/about/introduction/en/index4.html).

2.3.3. Blood pressure

Blood pressure (BP) was measured by trained nurses with participants first sitting at rest for at least five minutes, according to the European Society of Hypertension recommendations. A validated semi-automatic sphygmomanometer with an appropriate cuff size for the arm circumference was used. If the first measure was above normal values for age, gender and height, a second measurement was taken at least two minutes apart.

2.4. Serum PFAS measurement

Serum concentrations of twelve PFAS, consisting of PFOS, 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), were measured by high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (HPLC MS/MS,

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Shimadzu UFLC XR 20 Prominence coupled to Sciex API 4000). Details of the analytical procedure for measuring the twelve PFAS in serum samples have been described previously [3]. Method performances allow analytes to be detected as low as 0.1 ng/mL (LOD) and to be quantified above 0.5 ng/mL (LOQ). Following published practices, levels less than the LOD were imputed to be LOD/√2, and we limited our statistical analyses to PFAS detected in ≥ 40% of the samples. Three of the twelve PFAS were detected in more than 98% of the serum specimens (PFOA (detected in 99.96% of people), PFOS (detected in 99.75% of people), PFHxS (detected in 97.59% of people)), the one exception being PFNA (43.81%).

2.5. Covariates

We obtained information on age, gender, country of birth, education level, smoking habits, self-reported height and weight, physical activity, history of certain diseases, medication and food intakes, including salt intake. 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. Data on food consumption were transformed from number of serving per day/week/month to number of serving per week for all the food categories to create harmonized diet pattern classification. 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 in two categories based on geographical areas including: Italy plus other Highly Developed Countries, and High Migratory Pressure Countries. The time-lag between the beginning of the study (1st January 2017) and the date of enrollment was calculated for each subject and included as possible covariate (number of months).

Information on the center in charge of the blood pressure and anthropometric measurements, was considered as possible confounder in statistical analyses on Blood Pressure and BMI. Lipids models instead were adjusted by laboratory in charge of the analyses of clinical biomarkers.

Covariates potentially confounders of the cardiometabolic health and PFAS associations were selected through the construction of a directed acyclic graph (DAG) representing the existing literature, and the identification of a minimally sufficient set of variables to control confounding. 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/).

2.6. Statistical analysis

After excluding pregnant teenagers and subjects with incomplete exposure or outcome data (supplementary material, Figure S1), 6,669 adolescents and 2,693 children were included in the statistical analyses. The serum concentrations of PFAS were expressed as arithmetic mean, standard deviation (SD) and percentiles. Spearman's correlation (ϱ) was used to describe pair-wise relations between the PFAS. A Mann-Whitney test was used to test for gender differences in the distribution of outcomes and exposure variables.

All continuous outcome (Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Systolic and Diastolic Blood Pressure), except BMI z-score, were analyzed considering a different subset of observations, excluding those with specific self-reported diseases and medications. PFOA, PFOS, PFHxS and PFNA are treated as both continuous, to determine the association between the outcome and the PFAS concentration by linear regression analysis, and categorical-quartiles of increasing exposure, with the lowest PFAS quartile as a reference group in order to examine any dose-response trends and limit the influence of extreme values. For PFNA, due to the high proportion of the measures below the LOQ, PFNA was classified in three categories: low (below the Limit of Quantification), medium

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and high. The latter two were defined using a cut-off based on the median of the distribution of PFNA above the limit of classification. For analysis as a continuous variable, PFAS values were natural log (ln)-transformed to correct skewed distribution and improve normality of the data.

The relation between each ln-transformed PFAS and continuous outcomes was analyzed using Generalized Additive Models (GAMs). Thin plate spline smooth terms were used for the exposure and continuous covariates, in order to explore non-linear shapes of possible association between PFAS and outcomes. Degree of smoothing was selected by generalized cross validation as implemented in the R package mgcv. The interpretation of spline analyses doesn't produce interpretable coefficients besides of EDF, which represents the degree that a Polynomial function (of a specific variable) should have to fit the data instead of using splines. This implies that a graphical interpretation of predicted values is the only option. Therefore, since the spline analysis showed some associations compatible with a linear relationship on the ln-transformed PFAS, linear regression coefficient (β) and 95% confidence intervals (CI) were reported.

All analyses were fully adjusted for the established set of covariates.

All the analyses have been also stratified according to gender and an interaction term between gender and ln-PFAS was also added to the main models.

A p-value of < 0.05 was considered statistically significant. 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.

3. Results

3.1. General characteristics of the study population and PFAS internal dose

The general characteristics of children and adolescents who participated in the study are reported in Table 1a and 1b. The mean age was 16.2 years for adolescents and 9.4 years for children. The study population was weighted at 3411 (51%) males and 3258 (49%) females among adolescents and 1356 (50%) males and 1337 (50%) females among children. Most participants were exposed to PFAS via contaminated drinking water from birth (n= 6539, 98% of adolescents, and n=2669, 99% of children). Serum PFOA levels were an order of magnitude higher than PFOS and PFHxS, with a mean (SD) of 59.65 (52.99) μg/L in male adolescents and a mean of 43.23 (38.61) μg/L in female counterparts ($p<0.001$), and 27.58 (22.66) μg/L and 24.83 (20.21) μg/L in male and female children, respectively ($p<0.001$). Similar to other studies, PFOA, PFOS and PFHxS concentration levels were significantly higher in male adolescents compared to females. The gender difference in serum PFOA, PFOS and PFHxS concentrations was more pronounced in the adolescent age group. PFAS concentrations were positively correlated with one another with the highest correlation observed between PFOA and PFHxS with a Spearman correlation coefficient of 0.93 and 0.89 in adolescents and children, respectively. PFHxS and PFNA were the least correlated at 0.34 among adolescents and at 0.20 among children (data not shown).

3.2. Serum lipids

Among adolescents, significant differences were observed in the lipid profiles between males and females, with the latter showing higher levels of TC, HDL-C, and LDL-C, and lower levels of triglycerides (Table 1a). Among children gender differences of lipid profile were limited to HDL-C (higher in males) and triglycerides (higher in females) (Table 1b).

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Table 1a. Characteristics of the ADOLESCENTS included in the study population (n=6669), stratified by gender.

	TOTAL			MALES (n=3411)			FEMALES (n=3258)			Mann-Whitney p-value
	Mean (SD)	min - max	Median (Q1-Q3)	Mean (SD)	min - max	Median (Q1-Q3)	Mean (SD)	min - max	Median (Q1-Q3)	
PFAS										
PFOA	51.63 (47.24)	0.35-599.3	38.9 (20.1-68.8)	59.65 (52.99)	0.35-599.3	45.2 (24.6-79.3)	43.23 (38.61)	0.35-477.3	33.3 (16.5-58.7)	<0.0001
PFOS	4.1 (3.45)	0.35-86.8	3.3 (2.2-4.9)	4.49 (3.59)	0.35-69.1	3.6 (2.5-5.3)	3.68 (3.24)	0.35-86.8	3 (2-4.4)	<0.0001
PFHxS	3.6 (2.85)	0.35-27.2	2.8 (1.6-4.8)	4.22 (3.19)	0.35-27.2	3.4 (2-5.6)	2.95 (2.26)	0.35-21.6	2.4 (1.3-3.9)	<0.0001
PFNA	0.51 (0.26)	0.35-3.7	0.35 (0.35-0.6)	0.51 (0.24)	0.35-3.7	0.35 (0.35-0.6)	0.5 (0.27)	0.35-3.2	0.35 (0.35-0.6)	<0.0001
OUTCOMES										
TOTAL CHOLESTEROL	150.49 (27.13)	60 -294	148 (132-166)	145.04 (26)	77-290	143 (127-161)	156.2 (27.1)	60-294	153 (138-172)	<0.0001
HDL CHOLESTEROL	54.15 (11.91)	19 -124	53 (46-61)	50.05 (10.24)	19-99	49 (43-56)	58.44 (12.02)	24-124	57 (50-66)	<0.0001
LDL CHOLESTEROL	79.49 (23.04)	0 -243	77 (64-93)	76.71 (22.99)	0-243	75 (61-90)	82.4 (22.74)	6-211	80 (67-95)	<0.0001
TRYGLICERIDES	84.86 (50.28)	17-699	71 (54-100)	92.32 (58.22)	17-699	76 (56-110)	77.03 (38.83)	17-505	67 (52-90)	<0.0001
BMI z-score	0.3 (1.15)	-4.73-4.54	0.26 (-0.46-1.04)	0.34 (1.08)	-4.73-4.26	0.28 (-0.38-1.03)	0.25 (1.22)	-3.73-4.54	0.24 (-0.56-1.05)	0.00452
SYSTOLIC BP	114.5 (13.89)	70-190	115 (105-120)	117.86 (14.07)	70-190	120 (110-129)	110.98 (12.8)	70-180	110 (100-120)	<0.0001
DIASTOLIC BP	66.86 (9.62)	30-124.5	70 (60-71)	67.47 (10.04)	30-105	70 (60-75)	66.23 (9.11)	40-124.5	65 (60-70)	<0.0001

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Table 1b Characteristics of the CHILDREN included in the study population (n=2128), stratified by gender.

	TOTAL			MALES (n=3411)			FEMALES (n=3258)			Mann-Whitney p-value
	Mean (SD)	min - max	Median (Q1-Q3)	Mean (SD)	min - max	Median (Q1-Q3)	Mean (SD)	min - max	Median (Q1-Q3)	
PFAS										
PFOA	26.21 (21.52)	0.35-316.3	20.9 (12.9-316.3)	27.58 (22.66)	0.35-316.3	22.5 (13.45-316.3)	24.83 (20.21)	0.35-209.3	19.8 (12.2-209.3)	<0.0001
PFOS	2.56 (2.48)	0.35-96	2.2 (1.6-96)	2.63 (2.97)	0.35-96	2.2 (1.6-96)	2.5 (1.86)	0.35-39	2.1 (1.5-39)	0.037
PFHxS	2.22 (1.48)	0.35-14.6	1.9 (1.2-14.6)	2.34 (1.55)	0.35-14.6	2 (1.3-14.6)	2.1 (1.4)	0.35-13.8	1.8 (1.1-13.8)	<0.0001
PFNA	0.43 (0.16)	0.35-3.1	0.35 (0.35-3.1)	0.43 (0.14)	0.35-1.2	0.35 (0.35-1.2)	0.43 (0.18)	0.35-3.1	0.35 (0.35-3.1)	0.193
OUTCOMES										
TOTAL CHOLESTEROL	160.8 (27.55)	77-389	159 (143-389)	160.99 (28.03)	80-357	159 (142-357)	160.6 (27.08)	77-389	158 (143-389)	0.873
HDL CHOLESTEROL	59.51 (12.08)	21-111	59 (51-111)	60.66 (12.47)	24-107	60 (52-107)	58.34 (11.56)	21-111	58 (50-111)	<0.0001
LDL CHOLESTEROL	88.52 (24.8)	16-323	86 (72-323)	88.17 (25.09)	19-271	86 (71-271)	88.88 (24.5)	16-323	87 (73-323)	0.165
TRYGLICERIDES	63.98 (31.81)	16-383	57 (43-383)	60.9 (31.05)	16-303	54 (40-303)	67.11 (32.27)	22-383	60 (46-383)	<0.0001
BMI z-score	0.71 (1.31)	-5.68-5.61	0.7 (-0.2-1.65)	0.7 (1.21)	-3.87-5.61	0.68 (-0.18-1.57)	0.72 (1.41)	-5.68-4.71	0.73 (-0.24-1.72)	0.401
SYSTOLIC BP	99.95 (11.06)	70-160	100 (90-160)	99.71 (11.06)	70-160	100 (90-160)	100.2 (11.05)	70-142	100 (90-142)	0.388
DIASTOLIC BP	61.99 (8.26)	45-100	60 (57-100)	62 (8.32)	45-100	60 (56-100)	61.97 (8.21)	45-93	60 (57-93)	0.914

Table 2 displays the association between single PFAS and serum lipids. Among adolescents, significant associations were detected between all investigated PFAS and TC, LDL-C, and to a fewer extent HDL-C in the multivariable model adjusting for various confounders and the associations remained significant after categorizing PFAS (Table 2). Among children, PFOS and PFNA had significant associations with TC, LDL-C and HDL-C, while PFOA and PFHxS had significant associations with HDL-C only (Table 2). The magnitude of the associations, measured by the increase in each lipid biomarker for a ln-increase of serum concentration of PFAS, varied according to the PFAS congener and the age group. As regards TC, LDL-C, and HDL-C, the largest effects were observed for PFNA, followed by PFOS; moreover, these effects were larger in children compared to adolescents (Table 2). The associations were all non-linear; in some cases, they approximated log-linearity, while in others they had an irregular shape.

In adolescents, PFAS/lipids associations were not modified by gender except for HDL-C with effect significantly higher in girls (supplementary material, Table S1). In children, the effect of PFOS and PFHxS on TC and LDL-C was significantly higher in girls than in boys (Supplementary material, Table S2).

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Table 2 Association between ln-PFAS (ln ng/mL) and Serum Lipids (mg/dL) from GAM models: adjusted β* coefficients and 95% Confidence Intervals (CI).

	ADOLESCENTS				CHILDREN			
PFAS	**TC**	**HDL**	**LDL**	**TRIGLYCERIDES**	**TC**	**HDL**	**LDL**	**TRIGLYCERIDES**
	Coef (95% CI)	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**
log_pfoa	1.05 (0.31, 1.80)	-0.17 (-0.47, 0.14)	1.03 (0.39, 1.66)	0.01 (0.00, 0.03)	0.85 (-0.44, 2.14)	0.64 (0.09, 1.19)	0.17 (-0.98, 1.32)	0 (-0.02, 0.02)
II Q	1.18 (-0.60, 2.96)	-0.02 (-0.74, 0.71)	1.19 (-0.33, 2.71)	0.01 (-0.02, 0.04)	0.45 (-2.43, 3.33)	0.71 (-0.51, 1.94)	-0.23 (-2.8, 2.34)	-0.01 (-0.05, 0.04)
III Q	1.68 (-0.18, 3.55)	0.10 (-0.67, 0.86)	1.55 (-0.05, 3.15)	0.00 (-0.03, 0.04)	0.31 (-2.59, 3.21)	1.35 (0.12, 2.58)	-1.09 (-3.68, 1.5)	-0.01 (-0.05, 0.03)
IV Q	3.20 (1.20, 5.20)	-0.30 (-1.12, 0.52)	2.99 (1.27, 4.70)	0.04 (0.01, 0.07)	2.97 (0.02, 5.93)	1.46 (0.20, 2.71)	1.58 (-1.06, 4.21)	-0.01 (-0.06, 0.03)
log_pfos	3.32 (2.20, 4.45)	1.17 (0.71, 1.63)	2.66 (1.70, 3.62)	-0.02 (-0.04, 0.00)	6.22 (4.32, 8.13)	1.91 (1.1, 2.73)	4.52 (2.8, 6.23)	-0.01 (-0.04, 0.02)
II Q	3.75 (1.98, 5.52)	1.02 (0.29, 1.74)	3.03 (1.51, 4.54)	-0.01 (-0.04, 0.02)	3.80 (1.05, 6.56)	2.32 (1.14, 3.49)	2.08 (-0.39, 4.55)	-0.02 (-0.07, 0.02)
III Q	4.10 (2.26, 5.95)	1.30 (0.54, 2.05)	3.11 (1.53, 4.69)	-0.02 (-0.05, 0.01)	5.82 (2.96, 8.68)	2.35 (1.13, 3.57)	3.52 (0.96, 6.09)	0.01 (-0.04, 0.05)
IV Q	5.84 (3.88, 7.79)	1.83 (1.04, 2.63)	4.63 (2.96, 6.31)	-0.03 (-0.06, 0.01)	8.34 (5.51, 11.17)	2.99 (1.78, 4.20)	5.83 (3.28, 8.39)	-0.04 (-0.08, 0)
log_pfhxs	1.49 (0.60, 2.37)	-0.05 (-0.41, 0.31)	1.44 (0.68, 2.19)	0.01 (-0.01, 0.02)	1.30 (-0.28, 2.88)	0.8 (0.12, 1.47)	0.54 (-0.87, 1.96)	-0.01 (-0.03, 0.01)
II Q	1.96 (0.20, 3.73)	-0.16 (-0.88, 0.56)	2.03 (0.52, 3.55)	0.01 (-0.02, 0.04)	-1.04 (-3.84, 1.76)	0.46 (-0.73, 1.65)	-1.70 (-4.19, 0.8)	0 (-0.04, 0.04)
III Q	1.72 (-0.10, 3.54)	0.14 (-0.60, 0.88)	1.60 (0.05, 3.16)	0.00 (-0.03, 0.03)	0.56 (-2.35, 3.46)	1.68 (0.44, 2.91)	-1.22 (-3.81, 1.38)	0 (-0.04, 0.04)
IV Q	3.80 (1.83, 5.77)	0.07 (-0.74, 0.87)	3.65 (1.97, 5.33)	0.02 (-0.02, 0.05)	1.95 (-0.99, 4.89)	1.32 (0.07, 2.56)	0.76 (-1.86, 3.39)	-0.02 (-0.07, 0.02)
log_pfna	3.79 (2.04, 5.54)	1.24 (0.53, 1.96)	3.35 (1.85, 4.84)	-0.03 (-0.06, 0.00)	8.13 (4.44, 11.83)	2.11 (0.53, 3.69)	6.19 (2.87, 9.51)	-0.02 (-0.07, 0.04)
medium	3.43 (1.91, 4.95)	1.07 (0.45, 1.69)	2.35 (1.05, 3.65)	0.00 (-0.02, 0.03)	2.95 (0.39, 5.50)	1.31 (0.22, 2.40)	1.58 (-0.71, 3.87)	-0.01 (-0.05, 0.03)
high	3.58 (1.83, 5.33)	1.45 (0.73, 2.17)	3.04 (1.54, 4.54)	-0.03 (-0.06, 0.00)	7.53 (3.81, 11.25)	1.8 (0.21, 3.38)	6.06 (2.73, 9.39)	-0.02 (-0.07, 0.04)

3.3. Blood pressure

No statistically significant associations were observed between any of the investigated four PFAS concentrations and blood pressure (Table 3) neither in adolescents nor in children, after adjustment for covariates when these cardiometabolic trait was continuous outcomes in the regression models. Results stratified by gender are showed in supplementary tables S3 (adolescents) and S4 (children).

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Table 3 Association between PFAS (ln ng/mL) and Blood Pressure (mm/Hg) from GAM models: adjusted β* coefficients and 95% Confidence Intervals (CI).

	ADOLESCENTS		CHILDREN	
PFAS	**SYSTOLIC BP**	**DIASTOLIC BP**	**SYSTOLIC BP**	**DIASTOLIC BP**
	Coef (95% CI)	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**
log_pfoa	-0.16 (-0.53, 0.20)	-0.11 (-0.37, 0.15)	-0.51 (-1.02, -0.01)	0.16 (-0.23, 0.54)
II Q	-0.44 (-1.31, 0.43)	-0.23 (-0.84, 0.39)	-0.08 (-1.20, 1.05)	0.58 (-0.28, 1.44)
III Q	-1.01 (-1.92, -0.10)	-0.28 (-0.93, 0.36)	-0.22 (-1.35, 0.91)	0.37 (-0.5, 1.24)
IV Q	-0.44 (-1.42, 0.54)	-0.08 (-0.77, 0.61)	-0.98 (-2.14, 0.18)	0.68 (-0.21, 1.57)
log_pfos	-0.47 (-1.02, 0.08)	-0.44 (-0.82, -0.05)	-0.42 (-1.18, 0.33)	0.03 (-0.54, 0.61)
II Q	-0.67 (-1.54, 0.2)	-0.54 (-1.15, 0.08)	-0.13 (-1.22, 0.95)	0.67 (-0.16, 1.5)
III Q	-0.96 (-1.87, -0.06)	-0.66 (-1.30, -0.02)	0.18 (-0.95, 1.31)	0.91 (0.05, 1.77)
IV Q	-1.34 (-2.3, -0.38)	-0.78 (-1.45, -0.10)	-0.8 (-1.92, 0.33)	-0.1 (-0.95, 0.75)
log_pfhxs	-0.22 (-0.65, 0.21)	-0.15 (-0.45, 0.16)	-0.68 (-1.3, -0.06)	0.15 (-0.33, 0.62)
II Q	-0.34 (-1.21, 0.52)	0.02 (-0.59, 0.63)	-0.53 (-1.62, 0.57)	0.69 (-0.15, 1.54)
III Q	-0.71 (-1.6, 0.18)	-0.17 (-0.79, 0.46)	-1.38 (-2.52, -0.24)	0.19 (-0.68, 1.06)
IV Q	-0.37 (-1.33, 0.59)	-0.29 (-0.97, 0.39)	-1.12 (-2.28, 0.03)	0.3 (-0.58, 1.19)
log_pfna	-0.56 (-1.41, 0.30)	0.08 (-0.52, 0.68)	-0.13 (-1.59, 1.33)	-0.28 (-1.39, 0.83)
medium	-0.58 (-1.32, 0.17)	0.38 (-0.15, 0.90)	-0.45 (-1.45, 0.56)	0.32 (-0.45, 1.08)
High	-0.33 (-1.19, 0.53)	0.07 (-0.54, 0.68)	-0.23 (-1.69, 1.23)	-0.91 (-2.03, 0.2)

3.4. BMI z-score

Increased serum concentrations of PFAS, particularly PFOS, were associated with decreased BMI z-score. Among adolescents, these associations were more pronounced in females (p-interaction<0.01 for all PFAS) and the effect was stronger for PFOS, while among children they were mixed (no gender-pfas significant interactions), with greater effect seen for PFOS (Table 4).

Table 4 Association between PFAS (ln ng/mL) and BMI z-scores (mm/Hg) from GAM models: adjusted β* coefficients and 95% Confidence Intervals (CI), stratified by gender.

	BMI z-score - ADOLESCENTS			BMI z-score - CHILDREN		
PFAS	**TOTAL**	**MALES**	**FEMALES**	**TOTAL**	**MALES**	**FEMALES**
	Coef (95% CI)	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**	**Coef (95% CI)**
log_pfoa	-0.03(-0.06, 0.01)	0(-0.04, 0.04)	-0.07(-0.11, -0.02)	-0.08(-0.14, -0.01)	-0.07(-0.15, 0.01)	-0.08(-0.18, 0.02)
II Q	-0.1(-0.18, -0.02)	-0.1(-0.21, 0.01)	-0.09(-0.2, 0.02)	0.03(-0.11, 0.17)	0.2(0.01, 0.39)	-0.1(-0.32, 0.11)
III Q	-0.1(-0.18, -0.02)	-0.06(-0.17, 0.05)	-0.11(-0.23, 0)	-0.11(-0.25, 0.03)	0.01(-0.18, 0.2)	-0.2(-0.41, 0.02)
IV Q	-0.06(-0.14, 0.03)	-0.03(-0.14, 0.09)	-0.16(-0.29, -0.02)	-0.17(-0.32, -0.03)	-0.09(-0.27, 0.1)	-0.25(-0.48, -0.02)
log_pfos	-0.11(-0.16, -0.06)	-0.1(-0.17, -0.04)	-0.16(-0.23, -0.09)	-0.27(-0.36, -0.17)	-0.24(-0.36, -0.11)	-0.31(-0.45, -0.17)
II Q	-0.14(-0.22, -0.07)	-0.13(-0.24, -0.02)	-0.11(-0.22, 0)	-0.15(-0.28, -0.01)	-0.01(-0.19, 0.17)	-0.29(-0.5, -0.09)
III Q	-0.16(-0.24, -0.08)	-0.14(-0.26, -0.03)	-0.2(-0.32, -0.09)	-0.25(-0.39, -0.11)	-0.16(-0.35, 0.03)	-0.33(-0.55, -0.12)
IV Q	-0.19(-0.27, -0.1)	-0.16(-0.28, -0.05)	-0.26(-0.39, -0.13)	-0.35(-0.49, -0.21)	-0.3(-0.48, -0.12)	-0.42(-0.63, -0.2)
log_pfhxs	0.03(-0.01, 0.07)	0.06(0.01, 0.11)	-0.03(-0.09, 0.03)	-0.13(-0.21, -0.05)	-0.11(-0.21, -0.01)	-0.14(-0.26, -0.03)
II Q	-0.08(-0.15, 0)	0(-0.12, 0.11)	-0.11(-0.22, 0)	0.06(-0.08, 0.2)	0.12(-0.06, 0.31)	0(-0.2, 0.21)
III Q	0.01(-0.07, 0.09)	0.09(-0.02, 0.2)	-0.04(-0.15, 0.08)	-0.2(-0.34, -0.06)	-0.11(-0.3, 0.08)	-0.25(-0.47, -0.04)
IV Q	0.03(-0.05, 0.12)	0.12(0, 0.23)	-0.11(-0.25, 0.02)	-0.18(-0.32, -0.03)	-0.12(-0.31, 0.07)	-0.23(-0.46, -0.01)
log_pfna	-0.06(-0.14, 0.02)	-0.03(-0.14, 0.07)	-0.13(-0.24, -0.01)	-0.42(-0.6, -0.24)	-0.5(-0.75, -0.25)	-0.36(-0.62, -0.09)
medium	-0.02(-0.08, 0.05)	0.03(-0.06, 0.11)	-0.1(-0.2, 0)	-0.25(-0.38, -0.13)	-0.28(-0.44, -0.13)	-0.21(-0.41, -0.01)
high	-0.04(-0.11, 0.04)	0(-0.1, 0.1)	-0.11(-0.23, 0)	-0.36(-0.54, -0.18)	-0.37(-0.62, -0.12)	-0.35(-0.62, -0.08)

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4. Discussion

4.1. Serum lipids

In this cross-sectional study on 6,669 adolescents and 2,693 children exposed to high levels of PFAS through contaminated drinking water, we found a significant, non-linear association between serum concentrations of four PFAS congeners and common biomarkers of lipid metabolism. Compared to PFOA and PFHxS, PFOS and PFNA exhibited stronger associations and their effects showed a greater magnitude among children compared to adolescents. The concentration-response curves were mostly irregular in shape, but in some instances showed a clear log-linear shape with steeper slopes at lower concentrations.

These findings are quite similar to those we previously reported in a group of more than 16,000 young adults (age 20-39 years) recruited in the Veneto Region's health surveillance program [13]: also in that sub-population, we found that PFOS had a stronger effect compared to PFOA and PFHxS and that the relationship was log-linear.

A number of studies examined the association between serum PFAS and serum lipids in humans [14] but only twelve involved children or adolescents [7–9,15–23]. In those young age groups it may be easier to observe adverse effects of environmental contaminants on the lipid metabolism since the impact of lifestyle habits has been more limited compared to adults. Most of the abovementioned studies found significant associations between one or more PFAS congeners and lipid biomarkers in children or adolescents, with few exceptions [9,20]. The most consistent effects observed across different studies regarded TC and LDL-C, while fewer studies reported a positive association with HDL-C [15,19,20,23]. Results varied between studies in terms of magnitude and strength of the observed associations and also regarding the involved congeners. Such heterogeneity may be at least in part attributed to the limited sample size of most studies (a few hundreds of individuals in most instances) and also to differences in the population ages and exposure levels. All these differences render the available studies difficult to compare with each other. The study by Frisbee and colleagues [15] resembles more closely the present one as it was based on more than 12,000 individuals aged 1-17 years from a community highly exposed to PFAS through drinking water in the Mid-Ohio valley, USA. With a cross-sectional design the authors showed a significant log-linear association of both serum PFOA and PFOS with TC and LDL-C and of PFOS with HDL-C. As regards TC and LDL-C, the magnitude of the association was greater for PFOS. Altogether these results are similar to what we found in the Veneto Region's exposed population.

Although the literature consistently shows an association between serum PFAS concentrations and serum cholesterol levels, it is not clear whether this association is causal or not. To date, most epidemiological studies, including ours, have been cross-sectional, thus limiting any causal inference. Cross-sectional studies are subject to bias such as reverse causation or confounding, as has been suggested also for the PFAS-lipids associations. In particular, a confounding effect due to individual variations in the enterohepatic cycling of PFOS/PFOA and bile acids has been hypothesised [24], and the European Food Safety Authority has recently reviewed its former decision to consider the increase in serum cholesterol as one of the critical effects of PFAS exposure [14,25]. As regards children and adolescents, only four cohort studies have been conducted so far on the associations between maternal serum PFAS during pregnancy and offspring serum lipids later in life [8,9,22,23]. Results were inconsistent, with some studies reporting some positive associations [8,22,23] and others not [9]. Anyhow, even a well-conducted cohort study may provide limited evidence on the causal link between serum PFAS and cholesterol if the biomarker of exposure (i.e., serum PFAS) is determined only once and the outcome is assessed after a long delay, since cholesterol levels may be sensitive to modifications of exposures and the PFAS effect on cholesterol may be transient and reversible. The latter hypothesis is suggested by a study from the Mid-Ohio valley community, that showed a greater decrease in serum cholesterol in association with the

decrease in serum PFAS [26]. Further studies based on repeated measures of PFAS and lipids levels in the same individuals may contribute to shed light on the intricated issue of the PFAS-lipids association.

We observed some significant gender differences in the associations between PFAS and serum lipids, and those gender differences varied according to age range. The effect of PFOA, PFOS, and PFHxS on HDL-C was higher in female adolescents, while the effect of PFOS and PFHxS on TC and LDL-C was higher in female children. In our previous study on the young adult population, we observed that the associations between PFAS and HDL-C were statistically significant only in females [13]. Among other studies, only few assessed gender effect modification, with variable findings: in the Mid-Ohio valley community, larger effects of PFOA and PFOS on TC and LDL-C were found in boys compared to girls [15], whereas in the study by Mora et al. [23] mid-childhood serum PFOA, PFOS and PFDeA were associated with a bigger increase of TC and LDL-C among girls. Another study [9] did not find any significant gender differences. Two studies recruited only females and provided contradictory findings: in a cross-sectional study of girls aged 6-8 years, Fassler et al. [20] showed a positive association of PFOA with HDL, while the cohort study by Maisonet et al. [8] showed a positive association of prenatal PFOA (limited to the lower tertile of the distribution) with TC and LDL-C in followed-up daughters at the ages of 7 and 15 years.

Altogether our findings and the available literature indicate the existence of a very complex interplay between PFAS exposure and individual characteristics such as gender and phase of the life course, possibly related to the variation in the internal doses across genders [27,28] and ages and to the effect of sex hormones on lipid metabolism [29,30]. On this regard, mechanistic evidence is accruing on a pro-estrogenic and anti-androgenic effect of PFAS [31,32]. Further mechanistic studies are needed to understand whether these hormonal perturbations are linked to alterations of lipid metabolism.

4.2. Blood pressure

To our knowledge, only one study has, by now, found a positive association between hypertension and PFAS serum levels in an adolescent population, while they did not find any association of BP as continuous variable and PFAS concentration [7]. Other studies, with both cross-sectional and longitudinal design, did not find any significant association [9,33].

4.3. BMI

PFAS exposure during critical development periods has been reported to be associated with body weight changes. Prenatal PFOA exposure is associated with decreased birth weight in both mice and humans [34,35]. Few cross-sectional studies have examined the relationship between childhood/adolescence exposure to PFAS and BMI/overweight showing inconsistent findings, although the association between prenatal PFOA and BMI was mostly inverse [35,36] or null [37,38], except for one study [22]. In particular, analysis of the BMI trajectory in infants up to 12 years old showed that BMI zenith was lower in magnitude in the highest tertile of PFOA [36]. In children aged 3 to 11 years old, PFHxS was negatively associated with weight-for-age and BMI z-score, but only in males [39]. Recently, Pinney et al. (2019) investigated the relationship between serum PFOA in girls aged 6–8 years and longitudinal changes in adiposity at age 6–18 years. The authors reported an inverse association of PFOA level with BMI z-score, but declining with age [40], which is not in agreement with our data, although the time window of our study is tighter and the impact of puberty could have unmasked this association in the Pinney study. In another cohort study on girls aged 6 to 8 years, increasing serum PFOA concentrations were associated with decreased BMIz and fat mass percent [20].

These findings, together with the inverse association of maternal PFAS and birth-weight, may support evidence of continued negative effects on weight by prenatal PFAS exposures. A meta-analysis conducted by Johnson et al. (2014) evaluated the association

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of PFOA exposure and growth. The authors found that a 1 ng/mL increase in serum or plasma PFOA was associated with a −18.9 g (95% CI: 29.8,−7.9) change in birth weight and−0.1 cm (95% CI: 0.1, −0.02) change in birth length [41]. A second meta-analysis by Verner et al. (2015) found similar associations of PFOA and PFOS with decreased birth weight [42].

Few studies have investigated the association between PFAS and adiposity during puberty, with conflicting results according to sex, timing of exposure, type of study, and levels of exposure. Koshy et al. found no association with overweight in American adolescents exposed to PFHxS, PFOA, PFOS, PFNA, PFDA [18], whereas a Swedish prospective birth cohort study showed a positive association of PFOS and PFOA exposure with overweight/obesity [43]. A large multicenter prospective cohort study (the European Youth Heart Study) showed that childhood exposure to PFOS and PFOA predicted adiposity at 15 and 21 years of age [10]. A very recent cross-sectional study [7] found a positive association between PFHxS and PFHpS serum levels with obesity in Norwegian adoloscents, however this association was not linear and there was no positive association with other PFAS.

Since obesity is a complex disease with multifactorial etiology, differences between studies and populations may be attributable to different genetic and environmental factors, study designs, concentrations of PFAS, or the timing and method of adiposity measurements. In particular the differences between cross-sectional and longitudinal studies might be explained by reverse causation associated with the expanded distribution volumes in obese compared to lean children [38]. For instance, in the longitudinal study by Liu et al. (2020), prenatal PFAS concentrations were overall weakly correlated with postnatal PFAS concentrations [44]. In addition, some of the point estimates for postnatal PFAS concentration were negative, whereas their prenatal counterparts were positive. In our study, the use of a single serum measure may not fully reflect past exposure, and this exposure measure does not allow for assessment of prenatal exposures. However, these substances have long half-lives in humans, which may be upwards of 20 years [28], so exposure misclassification is less likely. Moreover, it was suggested that PFAS exposure during foetal life has a minor impact on childhood anthropometry and weight than PFAS exposure from the environment where children grow up [45].

In agreement with the literature, we also reported a greater magnitude for the association in girls than boys. Indeed, growing evidence suggests that the association of early-life exposure to certain environmental toxicants with placental functions and risk of disease later in life may vary by child sex [46]. One possible mechanism to explain gender differences in the association of PFAS with childhood or adolescent adiposity could be related to increased cortisol levels associated with PFAS exposure [47] and/or associated to placental epigenetic processes with sex-specific effects, as observed for maternal stress [48]. As serum levels of androgens and gonadotropins differ between sexes during mini puberty in early childhood [49], it cannot be excluded that the observed associations may be influenced by sex differences in hormones. In vitro studies [31,32] demonstrated that PFAS have estrogenic and antiandrogenic activities, but the in vivo consequences to hormonal interference by PFAS might also differ to receptor sensitivity differences between the two sexes, due to the physiological homeostasis of sex steroids [50,51]. In addition, PFAS can inhibit 11-β hydroxysteroid dehydrogenase 2 with subsequent increases in glucocorticoid concentrations [52], leading to alterations in placental development and function, and impairment of fetal growth [53].

Altogether, available experimental evidence suggests that PFAS may interfere with metabolic pathways relevant to childhood adiposity and lipid metabolism by different pathways. In addition to cortisol and sex hormones receptors, other targets have been identified in peroxisome proliferator– activated receptors (PPAR) and thyroid metabolism [54–56]. Since thyroid hormones play a crucial role in normal growth and development, altered thyroid function can affect early-life growth and adiposity during critical periods of development. There is a possibility that the effect of PFAS on early-life growth can be

mediated by thyroid hormone disruption. Finally, the wide variety of pathways altered by PFAS may also reflect the inconsistencies across studies among different PFAS molecules, since each compound may have different affinities and magnitudes of effect depending on the targeted pathway and the relative outcome.

5. Conclusions

Our cross-sectional study in children and adolescents supports a positive association between serum PFAS concentration and serum lipids, stronger for PFOS and PFNA and with a greater magnitude among children compared to adolescents, and a negative association of PFAS with BMI.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Flow-chart of study population, Table S1: Association between ln-PFAS (ln ng/mL) and Serum Lipids (mg/dL) from GAM models, stratified by gender: adjusted β* coefficients and 95% Confidence Intervals (CI). ADOLESCENTS, Table S2: Association between ln-PFAS (ln ng/mL) and Serum Lipids (mg/dL) from GAM models, stratified by gender: adjusted β* coefficients and 95% Confidence Intervals (CI). CHILDREN, Table S3: Association between PFAS (ln ng/mL) and Blood Pressure (mm/Hg) from GAM models, stratified by gender: adjusted β* coefficients and 95% Confidence Intervals (CI). Stratified by gender, ADOLESCENTS Table S4. Association between PFAS (ln ng/mL) and Blood Pressure (mm/Hg) from GAM models, stratified by gender: adjusted β* coefficients and 95% Confidence Intervals (CI). Stratified by gender, CHILDREN.

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