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Exposure to perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in adolescents. The Fit Futures study

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ABSTRACT

Background: Prevalence of obesity, hypertension and dyslipidemia has been increasing in children and adolescents worldwide. Exposure to environmental pollutants may contribute to this development. Our aim was to study associations between perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in a population-based sample of adolescents.

Methods: Serum PFAS concentrations were measured in 940 adolescents, mean age 16.4 (SD 1.3) years, from the cross-sectional Fit Futures study by the UHPLC-MS/MS method. The following endpoints were used: hypertension (systolic blood pressure over 130 mmHg and/or diastolic blood pressure over 80 mmHg); obesity (body mass index over 2 z-score, WHO charts for adolescents); dyslipidemia (total cholesterol $\geq$ 5.17 mmol/L, and/or LDL-cholesterol $\geq$ 3.36 mmol/l, and/or apolipoprotein B $\geq$ 1.10 g/L).

Results: Perfluorooctane sulfonate (PFOS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA) and perfluoroundecanoate (PFUnDA) serum concentrations were positively associated with apolipoprotein B, total- and LDL cholesterol. The highest vs. lowest quartiles of total PFAS ($\sum$PFAS), PFNA and PFDA concentrations were positively associated with the risk of dyslipidemia: OR 2.24 (95% CI 1.10–4.54), OR 2.30 (95% CI 1.16–4.57) and 2.36 (95% CI 1.08–5.16), respectively. The highest vs. lowest quartiles of $\sum$PFAS, perfluorohexane sulfonate (PFHxS), PFOS, perfluorooctanoate (PFOA) concentrations were positively associated with the risk of hypertension: OR 1.91 (95% CI 1.12–3.26), OR 2.06 (95% CI 1.16–3.65), 1.86 (95% CI 1.08–3.19) and 2.08 (95% CI 1.17–3.69) respectively. PFHxS and perfluoroheptane sulfonate (PFHpS) concentrations were positively associated with obesity.

Conclusions: This cross-sectional study showed a possible link between several PFAS and dyslipidemia, hypertension and obesity in Norwegian adolescents.

1. Introduction

The process of atherosclerosis leading to cardiovascular disease starts in childhood and progresses further throughout the life-course. Cardiovascular risk factors in children and adolescents such as overweight and obesity, hypertension and dyslipidemia are associated with markers of atherosclerosis in aorta, carotid and coronary arteries (Berenson et al., 1998; Juhola et al., 2013; (Expert panel, 2011)). Prevalence of overweight and obesity in children and adolescents is increasing worldwide; the obesity epidemic is a major healthcare challenge and a matter of great concern (Skinner et al., 2016; Garnett et al., 2016; Brettschneider et al., 2017). According to the last report from the World Health Organization, 18% of adolescents and children worldwide are overweight or obese, with the prevalence rising up to 33% in some countries (WHO). In Norway, overweight was found in 20% of girls and 22% of boys of school age, and the prevalence of overweight and obesity has been increasing over the past three decades (Kolle et al., 2009; Juliusson et al., 2007; Krokstad, 2011; Evensen et al., 2016). Overweight and obesity are associated with hypertension and dyslipidemia in children and adolescents (Sorof and Daniels, 2002). Hypertension is a major cardiovascular risk factor which contributes to development of stroke, myocardial infarction, heart failure, chronic kidney disease and peripheral artery disease later in life (Daniels, 2019). A recent meta-analysis of 47 studies from different populations showed a pooled

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prevalence of hypertension of 4% in children and youth 19 years and younger (Song et al., 2019). There has been an increase in the prevalence of hypertension in children and adolescents probably due to the overweight epidemic, and the prevalence is consistently higher in boys compared to girls (Flynn et al., 2017). The prevalence of dyslipidemia has also been increasing in adolescent populations, and this disorder of lipid metabolism remains one of the major cardiovascular risk factors (Expert panel, 2011). One of the theories about the causes of the worldwide epidemic of overweight, obesity, hypertension and dyslipidemia is a combination of unfavorable diet choices, inadequate physical activity, psychosocial stress, as well as possible effects of environmental pollutant exposures, especially endocrine-disrupting chemicals (EDCs) (Schell et al., 2012).

Several reports suggest that prenatal exposure to EDCs may increase risk of overweight and obesity in children (Valvi et al., 2012; Karlsen et al., 2017). There is emerging evidence that the newer group of POPs, perfluoroalkyl substances (PFAS), have endocrine disrupting properties and may be involved in the pathogenesis of dyslipidemia and weight gain (Pedersen et al., 2016; Geiger et al., 2014; Maisonet et al., 2015; Zeng et al., 2015). Exposure to several PFAS had a positive association with total cholesterol and LDL-cholesterol (Geiger et al., 2014; Maisonet et al., 2015; Zeng et al., 2015; ; Christensen et al., 2016; Koshy et al., 2017; Seo et al., 2018; He et al., 2018; Mora et al., 2018; Li et al., 2020; Steenland et al., 2009). PFAS are environmentally persistent chemicals that are widely used in many consumer products since 1940s. PFAS are used in firefighting foams, paper-, textile-, and leather impregnation, cosmetics, in ski waxes and are by-products in polytetrafluoroethylene (PTFE) products as for example Teflon. Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) were relatively newly defined in the Stockholm convention as POPs (Stockholm Convention). Few studies have examined the possible unfavorable effects of PFAS on cardiovascular risk factors in adolescents (Geiger et al., 2014; Maisonet et al., 2015; Lin et al., 2009). The aim of our study was to investigate associations between serum concentrations of several PFAS and serum lipids concentrations, dyslipidemia, hypertension, overweight and obesity in a population-based cohort of adolescents in Northern Norway.

2. Materials and methods

2.1. The study population

All first level high school students in the municipalities of Tromsø and Balsfjord in Northern Norway (n = 1117) were invited to participate in the Tromsø study Fit Futures 1 (TFF1) in 2010–2011, and 1038 participated (93%), the majority aged 15–19 years (97%). The participants came to the Clinical Research Unit at the University Hospital of North Norway (UNN, Tromsø) for a half-day visit during school-hours. The participants filled in an electronic questionnaire including 143 questions on family background, lifestyle (physical activity, use of chewed tobacco, smoking), dietary habits, and general health. The food frequency questionnaire (FFQ) used in the previous Tromsø study of adults was adapted for adolescents and its validity regarding information on dietary habits for adolescents was previously assessed (Torris et al., 2017). The questionnaire had 29 dietary questions related to possible sources of environmental pollutants and information characterizing healthy/unhealthy diet (e.g. intake of fruits and vegetables, fat fish and lean fish, dairy products, junk food and snacks). The example of the diet question: “How often do you usually eat vegetables?” (rarely/never; 1–3 times per month; 1–3 times per week; 4–6 times per week; 1–2 times per day; 3–4 times per day; 5 times or more per day).

Trained research nurses performed standardized height, weight and blood pressure measurements, blood sampling, and structured interviews (medication and disease, in addition to menstruation and pregnancy in girls). Medication use was self-reported and later coded by a medical professional using the WHO Anatomical Therapeutic Chemical Classification System (ATC) codes. Altogether 940 participants provided blood samples for environmental pollutants analyses (84% of all first level high school students in the area who were invited to participate in the study). None of the girls were pregnant at the time of the TFF1 study.

2.2. Ethics

Written informed consent was obtained from all participants and those aged less than 16 years brought written informed consent from parents. The study was approved by the Regional Committee for Medical and Health Research Ethics, North Norway (2015/1384/REK nord).

2.3. Physical examination

Systolic and diastolic blood pressure (SBP, DBP) were measured 3 times after 2-min seated rest on the participant’s right upper arm with an oscillometric digital automatic device Dinamap ProCare 300 monitor (GE Healthcare, Oslo, Norway), measurements being separated by a 1-min interval. The average of the second and the third SBP and DBP measurements was further used. Body height and weight were measured to the nearest 0.1 cm and 0.1 kg on a Jenix DS 102 Stadiometer (Dong Sahn Jenix, Seoul, Korea). Body mass index (BMI) was calculated as weight (kg)/height (m^2).

2.4. Laboratory analyses

Blood samples for PFAS analyses were obtained from the antecubital vein in BD vacutainer® tubes with no additive (Becton, Dickinson and Company, New Jersey, US), and serum was transferred to Supelco glass vials (Sigma-Aldrich Norway AS, Oslo, Norway) with Pasteur glass pipettes. Samples were stored at −40 °C prior to PFAS analysis. All the equipment was tested for possible PFAS contamination and no substantial background PFAS contamination was detected. All laboratory analyses were performed at the Department of Laboratory Medicine, UNN. Sample preparation, instrumental analysis, quantification, accuracy and precision of the method for PFAS assessment, as well as quality controls have been described in detail in earlier publication (Huber and Brox, 2015). Concisely, samples were extracted automatically on the liquid handler Tecan Freedom Evo 200 (Männedorf, Switzerland). The ultrahigh pressure liquid chromatography triple-quadrupole mass-spectrometry (UHPLC-MS/MS, Waters Acquity UPLC system, Xevo TQ-S mass spectrometer, Waters, Milford, MA, USA) was applied for analysis of perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS), perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorododecane sulfonate (PFDoDS), perfluorooctane sulfonamide (PFOSA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA) and perfluorotetradecanoate (PFTeDA) concentrations in serum. Quantification was conducted applying the Masslynx and Targetlynx software (TargetLynx Application manager, Version 4.1, Waters, Milford, MA, USA (Waters)) and achieved by the internal-standard method with isotope-labelled PFAS. For quality control, four blank samples, four standard reference material (SRM) 1958 samples (NIST, Gaithersburg, MD, USA) and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of 96 samples. Differences from the assigned mean reference concentrations were between 5 and 11%. Analytical coefficient of variation (CV) was below 10% for all PFAS except for PFUnDA with CV 12%. Limits of detection (LOD), detection rates and ranges of PFAS concentrations in this population have been previously published (Averina et al., 2018).

Total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol were measured in fresh serum

samples by enzymatic colorimetric methods by the Cobas 8000 instrument (Roche Diagnostics, F. Hoffmann-La Roche Ltd, Basel, Switzerland). CV was <2% for all the lipids analyses. Apolipoproteins A1 and B (apo A1, apo B) were measured in fresh serum samples by Cobas 8000 instrument by immunoturbidimetric methods standardized according to the IFCC SP1-01 and IFCC SP3-07 reference standards, respectively. CV was <3.5% for apo A and apo B. The Department of Laboratory Medicine, UNN, participates successfully in the external quality control programmes for biochemistry tests for serum lipids (Labquality, Helsinki, Finland; NOKLUS, Bergen, Norway) and in the international quality control programme for environmental pollutants (the Arctic Monitoring and Assessment, AMAP) Ring Test for Persistent Organic Pollutants in Human Serum, organized by the Laboratoire de toxicologie, Institut National de Santé Publique du Quebec, Canada). All the quality controls during the time of the study were within the acceptable limits.

2.5. Definitions of clinical outcomes

There were no records of medicines with the ATC codes C01-C10, so there were no participants using antihypertensive treatment or treatment for dyslipidemia. Therefore, the definitions of hypertension and dyslipidemia were based only on the cut-off levels for blood pressure and serum lipids from the international guidelines for adolescents.

Hypertension in adolescents was defined according to the American Academy of Pediatrics (AAP) 2017 guidelines for screening and managing high blood pressure in children and adolescents (Flynn et al., 2017): **Hypertension:** SBP ≥130 mmHg and/or DBP ≥80 mmHg.

Dyslipidemia was defined according to the Integrated guidelines for cardiovascular health and risk reduction in children and adolescents, the National Heart, Lung and Blood Institute (Expert panel, 2011). One or several parameters define dyslipidemia:

High total cholesterol ≥5.17 mmol/L (≥200 mg/dL).
High LDL cholesterol ≥3.36 mmol/L (≥130 mg/dL).
High apo B ≥ 1.10 g/L (≥110 mg/dL).
Low HDL cholesterol <1.03 mmol/L (<40 mg/dL).
Low apo A1 < 1.15 g/L (<115 mg/dL).

Overweight was defined as BMI over 1 z-score (1 SD) and obesity as BMI over 2 z-score in the WHO BMI charts for children 5–19 years old (WHO).

2.6. Statistical analyses

Statistical analyses were performed by the SPSS program (IBM Corp. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Chi-Square test and Student's t-test were used to evaluate sex differences in lifestyle and cardiovascular risk factors. PFAS concentrations were not normally distributed, sex differences in PFAS concentrations were therefore evaluated by the non-parametric Mann-Whitney *U* test.

Association between serum lipids concentrations, blood pressure, BMI and PFAS serum concentrations were assessed by multiple linear regression analyses. All the PFAS concentrations were $\log_{10}$-transformed before linear regression analyses because of the skewed distribution. The Targetlynx-software was used to calculate Limits of detection (LODs) for each individual sample (LOD*i*) and each individual PFAS analyte with a signal to noise ratio of 3 divided by the related sample amount. Limit of quantification (LOQ) was defined as three times the LOD. To reduce possible bias of left censored data analyses the actual values between LOQ and LOD were used in the regression models. Individual PFAS concentrations below the LOD were replaced by LOD*i* divided by 2. Multiple linear regression analyses of associations between PFAS and serum lipids were performed only for PFAS with detection rate ≥70%: PFHxS, PFHpS, PFHpA, PFOS, PFOA, PFNA, PFDA, PFUnDA. PFAS with detection rate <70% were included in the total PFAS concentration (ΣPFAS) defined as the sum of serum concentrations in ng/mL of all 18 measured PFAS. Additionally, the Spearman correlation and the principle component analysis (PCA) was performed for PFAS serum concentrations.

The selection of the possible confounders for the regression models was based on the previously published results from the Fit Futures study that showed significant associations of diet and some lifestyle variables with PFAS in this population (Averina et al., 2018). Alcohol intake was not substantial in this adolescent population and was not associated with PFAS, therefore it was not considered as a possible confounder. Directed acyclic graphs (DAGs) were used to present a sufficient set of covariates in the primary regression models (Suppl. Figs. 1-2). The following variables were included in the primary regression models for serum lipids: age, sex, BMI, physical activity, use of chewed tobacco (snuff), and dietary variables such as intake of cheese, full fat and semi-skimmed dairy products, fat and lean fish, fish liver, junk food (sausages, pizza, hamburger), fruits and vegetables, snacks (chips, biscuits cakes and buns, candy and chocolate). Each dietary variable was included separately in the regression models. Smoking was not associated with serum lipids, therefore it was not included in the regression models. For the regression analyses with serum triglycerides as dependent variables the covariate "time since last meal" was included in the models. Further, we used backward elimination procedure, which involves starting with all chosen covariates and stepwise deleting the variables whose loss is not important for the model fit. The results presented in the article are the final regression models after the Wald backward elimination and assessment of the goodness-of-fit of the regression models by the Akaike Information criterion (AIC).

Associations between PFAS and categorical variables such as overweight, obesity, dyslipidemia and hypertension (yes vs. no) were assessed by the binary logistic regression analyses where PFAS concentrations were used as quartile categories. The following covariates were included in the primary logistic regression models for hypertension: age, sex, BMI, physical activity, smoking. The logistic regression models with obesity as dependent variable included age, sex, physical activity, smoking and diet. Smoking was removed from the final regression models for hypertension and obesity, diet was removed from the final regression models for obesity after goodness of fit estimation. Further, we present the results of the final regression models. No mathematical correction was made for multiple comparisons as only planned comparisons were performed. Statistical significance was concluded at a 2-tailed p-value <0.05.

3. Results

General characteristics of the study population are presented in Table 1. Altogether 445 girls and 495 boys, mean age 16.5 (SD 1.4) and 16.3 (SD 1.1) years, respectively, were included in the study. Boys had higher prevalence of low HDL cholesterol than girls, while girls had higher prevalence of high total cholesterol and high LDL cholesterol. About 1/5 of the entire study population was overweight (21.4%), there was no gender difference in prevalence of overweight and obesity. Boys had higher mean systolic blood pressure and remarkably higher prevalence of hypertension than girls. Smoking was not different between the genders, however boys used more snuff than girls. Prevalence of sedentary lifestyle was also higher in boys.

Table 2 presents serum PFAS and serum lipids concentrations. Girls had higher total cholesterol, higher HDL and LDL cholesterol, higher apo A1 and apo B than boys. Total sum PFAS (∑PFAS) concentrations were not different between boys and girls, however girls had higher PFOA, PFNA, PFDA and PFUnDA concentrations, while boys had higher PFHxS and PFOS concentrations. The entire study population had measurable concentrations of PFHxS (100% detection rate), PFOS (100% detection rate), PFOA (99.9% detection rate), PFNA (100% detection rate) and PFDA (99.7% detection rate). Detection rates for PFHpA and PFHpS were 74.8% and 97.7%, respectively. For other PFAS the detection rates were below 70%, and their concentrations were included in ∑PFAS (Suppl. Table 1).

Table 1
Characteristics of the study population. The Tromsø study Fit Futures 1.

Parameters	Girls (n = 445)	Boys (n = 495)	p-value[a] two-tailed
Age (years), mean (SD)	16.5 (1.4)	16.3 (1.1)	0.014
BMI (kg/m^2), mean (SD)	22.6 (4.3)	22.4 (4.1)	0.615
Overweight (BMI >1 z-score)	19.3%	23.2%	0.152
Obesity (BMI >2 z-score)	5.4%	8.0%	0.120
High total cholesterol ≥ 5.17 mmol/L	12.3%	5.1%	<0.0001
High LDL cholesterol ≥ 3.36 mmol/L	9.8%	5.7%	0.025
High Apo B ≥ 1.10 g/L	0.9%	1.1%	0.998
Low HDL <1.03 mmol/L	12.1%	25.3%	<0.0001
Low Apo A1 < 1.15 g/L	12.8%	32.0%	<0.0001
Systolic blood pressure, mean (SD)[b]	111.6 (9.6)	122.7 (12.5)	<0.0001
Diastolic blood pressure, mean (SD)[b]	62.8 (7.1)	63.7 (7.7)	0.050
Hypertension[c]	4.0%	28.9%	<0.0001
Dyslipidemia[d]	13.2%	6.5%	0.001
Smoking	20.9%	23.0%	0.478
Chewed tobacco use (snuff)	30.6%	39.6%	0.004
Physical activity hard training/ sport[e]	16.7%	22.7%	0.021
Physical activity high[e]	28.3%	23.4%	0.098
Physical activity moderate[e]	41.3%	24.9%	<0.0001
Sedentary lifestyle	13.7%	29.0%	<0.0001

[a] Pearson Chi Square test or *t*-test.
[b] mmHg.
[c] SBP≥130/80 mmHg.
[d] total cholesterol ≥5.17 mmol/L (≥200 mg/dL) and/or LDL cholesterol ≥ 3.36 mmol/L (≥130 mg/dL) and/or apo B ≥ 1.10 g/L (≥110 mg/dL).
[e] Physical activity: hard training = regular hard training or sports competitions several times per week, high = recreational sports and heavy outdoor activities, moderate = walking, cycling or other moderate exercise at least 4 h per week.

3.1. PFAS and serum lipids

Associations of serum lipids with serum PFAS concentrations are presented in Table 3. The results are from the final multiple linear regression models adjusted for age, sex, BMI, lifestyle and diet variables. The final regression models for total and LDL cholesterol with all covariates are presented in Suppl. Tables 4 and 5. Serum total cholesterol, LDL cholesterol and apo B concentrations were positively associated with PFOS, PFNA, PFDA and PFUnDA. Serum HDL cholesterol and apo A1 were positively associated only with PFUnDA concentrations. ΣPFAS concentrations were significantly positively associated ($p < 0.05$) with apo B and LDL-cholesterol with β-coefficients 0.06 (95% CI 0.002–0.13) and 0.27 (95% CI 0.02–0.52), respectively (data not shown in Table 3). There was no association between serum lipids and PFHxS, PFHpS, PFHpA and PFOA (data not shown).

3.2. PFAS and dyslipidemia

Logistic regression analyses with adjustment for age, sex, BMI and diet variables showed significant positive associations of dyslipidemia with ∑PFAS, PFOS, PFNA, PFDA and PFUnDA (Table 4). The associations of PFOS and PFUnDA with dyslipidemia were significant only for 3rd quartile vs. 1st quartile, but not for the concentrations over the 4th quartile. Female gender and BMI, were positively associated with dyslipidemia. Low HDL and low apo A1 were not associated with individual PFAS concentrations; therefore, these parameters were not included in the dyslipidemia definition used in Table 4.

3.3. PFAS and hypertension

There was no significant association between blood pressure as a continuous variable and different serum PFAS concentrations tested in the linear regression analysis with adjustment for possible confounders. Age, male gender and BMI were positively associated with blood pressure. The combined variable hypertension (SBP >130 and/or DBP>80 mm Hg) in this adolescent population was positively associated with ΣPFAS, PFHxS, PFOA and PFOS concentrations over 4th quartile vs. 1st quartile after adjustment for possible confounders (Table 5). There was no association between hypertension and PFHpA, PFNA, PFDA, PFUnDA (data not shown). PFHpS concentrations over 4th quartile had a tendency to a positive association with hypertension with OR 1.74 (95% CI 0.97–3.11), p = 0.063 (compared with the 1st quartile).

∑PFAS had a positive association with hypertension and dyslipidemia, however we did not observe a stronger compound effect of 18 PFAS compared with the effects of single PFAS (Tables 4 and 5). Several PFAS serum concentrations included in ∑PFAS were weakly positively correlated with each other (Suppl. Table 2). PFOS was moderately correlated with PFOA and PFHxS with Spearman correlation coefficients (ρ) 0.40 and 0.44, respectively. Only PFHpS and PFOS, PFNA and PFDA were strongly positively correlated (ρ > 0.7). The PCA analysis of PFAS serum concentrations was performed. After the Scree plot evaluation and elimination of all components with eigenvalues <1.0, three principle components (PC) were retained (Suppl. Table 3). PC1 was defined by PFHpA, PFOA, PFNA, PFDA; PC2 was defined by PFHxS, PFHpS, PFOS; PC3 was defined by PFHxA, PFUnDA, PFDoDA, PFTrDA. There was no strong correlation between the principle components (Suppl. Table 3). PC scores were generated from the PCA and included as covariates in the regression analyses instead of the ∑PFAS variable. PC2 and PC3 scores were not statistically significantly associated with serum lipids and blood pressure when included in the regression models together with PC1. PC1 score had a significant positive association with systolic blood pressure, LDL-cholesterol and apolipoprotein B in the multiple linear regression analysis. PC1 score was significantly positively associated with hypertension in the logistic regression analysis (Suppl. Table 3). The results of the PCA compared with the regression analysis for single PFAS indicate that the positive association of PC1 with hypertension was mostly due to PFOA effect, as other single PFAS that defined PC1 were not significantly associated with hypertension. The positive association of PC1 with dyslipidemia (high LDL-cholesterol and apo B) was mostly due to PFNA and PFDA effects. PFNA and PFDA were strongly positively correlated, their effects on dyslipidemia are difficult to separate from each other.

Table 2
Serum lipids and perfluoroalkyl substances (PFAS) concentrations. The Tromsø study Fit Futures 1.

Serum concentrations	LOD[a]	Girls (n = 445)[b]	Boys (n = 495)[b]	p-value[c] two-tailed
∑PFAS	–	10.6 (4.77)	11.0 (4.76)	0.236
PFHxS	0.02 ng/mL	0.80 (0.53)	0.95 (0.64)	<0.0001
PFHpS	0.01 ng/mL	0.14 (0.06)	0.16 (0.07)	<0.0001
PFOS	0.04 ng/mL	5.71 (2.64)	6.52 (3.09)	<0.0001
PFOA	0.30 ng/mL	2.14 (1.26)	1.86 (0.67)	<0.0001
PFNA	0.03 ng/mL	0.61 (0.40)	0.48 (0.20)	<0.0001
PFDA	0.03 ng/mL	0.27 (0.20)	0.19 (0.09)	<0.0001
PFUnDA	0.03 ng/mL	0.17 (0.13)	0.14 (0.10)	<0.0001
Total cholesterol	1.3 mmol/L	4.25 (0.72)	3.91 (0.77)	<0.0001
HDL cholesterol	0.1 mmol/L	1.45 (0.33)	1.24 (0.28)	<0.0001
LDL cholesterol	0.2 mmol/l	2.46 (0.65)	2.30 (0.70)	<0.0001
Triglycerides	0.1 mmol/L	1.05 (0.48)	1.13 (0.55)	0.023
Apolipoprotein A1	0.25 g/L	1.37 (0.21)	1.22 (0.18)	<0.0001
Apolipoprotein B	0.20 g/L	0.66 (0.16)	0.61 (0.17)	<0.0001

[a] Level of detection (LOD).
[b] For PFAS serum concentrations: geometric mean (interquartile range, IQR), ng/mL), geometric mean is 10^{β} where β is a mean log 10 concentration; for serum lipids concentrations: mean (SD), for total cholesterol, HDL and LDL cholesterol in mmol/l, for apolipoproteins AI and B in g/L.
[c] The Mann-Whitney *U* test for PFAS, *t*-test for lipids.

Table 3
Associations of serum lipids with serum log 10-tranformed-PFAS serum concentrations after adjustment for possible confounders (multiple linear regression analyses). The Tromsø study Fit Futures 1.

Parameters	PFOS		PFNA		PFDA		PFUnDA	
	β-coef.[a]	p-value	β-coef.[a]	p-value	β-coef.[a]	p-value	β-coef.[a]	p-value
Total cholesterol	0.38 (0.10; 0.66)	0.008	0.15 (0.04; 0.26)	0.008	0.35 (0.12; 0.57)	0.003	0.35 (0.17; 0.53)	<0.0001
LDL-cholesterol	0.30 (0.05; 0.55)	0.021	0.14 (0.05; 0.24)	0.004	0.34 (0.14; 0.54)	0.001	0.24 (0.07; 0.40)	0.004
HDL-cholesterol	0.08 (−0.03; 0.20)	0.152	−0.001(−0.05; 0.04)	0.980	−0.01 (−0.10; 0.09)	0.923	0.14 (0.06; 0.21)	<0.0001
Triglycerides	0.006 (−0.18; 0.20)	0.947	−0.01 (−0.08; 0.07)	0.902	0.01 (−0.15; 0.17)	0.901	−0.05 (−0.18; 0.08)	0.435
Apolipoprotein A1	0.03 (−0.05; 0.11)	0.502	0.02 (−0.03; 0.08)	0.440	0.03 (−0.03; 0.09)	0.283	0.10 (0.05; 0.15)	<0.0001
Apolipoprotein B	0.07 (0.004; 0.13)	0.036	0.04 (0.02; 0.07)	0.001	0.09 (0.04; 0.14)	<0.0001	0.05 (0.01; 0.09)	0.009

[a] Final regression models adjusted for age, sex, BMI and for lifestyle and diet variables: total cholesterol for intake of junk food (sausages, pizza, hamburger), snacks (chips, biscuits cakes and buns), full fat dairy products, fat and lean fish; LDL-cholesterol and apolipoprotein B for intake of junk food, full fat dairy products, fat and lean fish; HDL-cholesterol for chewed tobacco use (snuff), vegetables intake, fish liver intake; apolipoprotein A1 for vegetables and fruits intake, fish liver, snacks and candy intake; triglycerides for physical activity, intake of cheese, fish liver and for time since the last meal.

Table 4
Association between PFAS serum concentrations and dyslipidemia in Norwegian adolescents. The Tromsø study Fit Futures 1.

PFAS	Concentration ng/mL	Dyslipidemia/Non-dyslipidemia[b]	OR (95% CI)[c] for dyslipidemia[d]	p-value
∑PFAS				
Quartile 1[a]	2.59–8.60	15/214	1.0	
Quartile 2	8.61–10.72	22/206	1.86 (0.90–3.86)	0.096
Quartile 3	10.73–13.37	25/201	2.18 (1.06–4.47)	0.034
Quartile 4	13.38–200.8	27/203	2.24 (1.10–4.54)	0.026
PFOS				
Quartile 1[a]	1.28–4.86	17/214	1.0	
Quartile 2	4.87–6.21	22/207	1.66 (0.82–3.37)	0.161
Quartile 3	6.22–7.80	31/192	2.43 (1.23–4.77)	0.010
Quartile 4	7.81–99.2	19/211	1.36 (0.65–2.83)	0.411
PFNA				
Quartile 1[a]	0.120–0.400	16/213	1.0	
Quartile 2	0.401–0.496	22/204	1.74 (0.85–3.55)	0.130
Quartile 3	0.497–0.676	19/209	1.24 (0.59–2.63)	0.571
Quartile 4	0.677–5.35	32/198	2.30 (1.16–4.57)	0.018
PFDA				
Quartile 1[a]	0.016–0.157	11/213	1.0	
Quartile 2	0.158–0.207	26/201	2.34 (1.08–5.05)	0.031
Quartile 3	0.208–0.288	25/206	2.19 (1.01–4.74)	0.048
Quartile 4	0.289–1.89	27/204	2.36 (1.08–5.16)	0.031
PFUnDA				
Quartile 1[a]	0.005–0.104	16/213	1.0	
Quartile 2	0.105–0.151	19/209	1.44 (0.68–3.06)	0.347
Quartile 3	0.152–0.217	30/200	2.30 (1.14–4.63)	0.020
Quartile 4	0.218–0.852	24/202	2.00 (0.95–4.20)	0.066

[a] Reference group.
[b] Number.
[c] Adjusted for sex, age and diet (junk food, snacks, full fat dairy and fat fish intake).
[d] Total cholesterol ≥5.17 mmol/L and/or LDL cholesterol ≥3.36 mmol/L and/or high apo B ≥ 1.10 g/L.

3.4. PFAS and overweight/obesity

Logistic regression analyses with adjustment for age, sex and physical activity outside school showed no association of overweight and obesity with total ΣPFAS, PFOS, PFOA, PFNA, PFDA and PFUnDA concentrations. After adjustment for sex, age and physical activity outside school, PFHxS and PFHpS concentrations were positively associated with obesity (Table 6). There was no significant association between BMI as a continuous variable and different serum PFAS concentrations in the linear regression analysis.

4. Discussion

PFAS are abundant in the environment, dietary intake is the main source of PFAS and the entire general population is exposed to these substances (Domingo and Nadal, 2017). PFOS and PFOA are defined as POPs in the Stockholm convention (Stockholm Convention) and their use is therefore restricted and controlled, while other PFAS are still not regulated.

In the present population-based cross-sectional study of Norwegian adolescents, several PFAS (∑PFAS, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnDA) were positively associated with cardiovascular risk factors such as unfavorable lipid profile and hypertension. These associations remained positive after adjustment for possible confounders. The EFSA 2020 report has concluded that there was clear evidence for an association between exposure to PFOS, PFOA and PFNA and increase of serum total cholesterol (Schrenk et al., 2020). Meanwhile, the evidence for other PFAS was not sufficient to conclude about the positive association with total and LDL cholesterol according to the EFSA 2020 report (Schrenk et al., 2020) The results of the present study in adolescents showed a positive association of exposure to PFOS, PFNA, PFDA and PFUnDA with total cholesterol and LDL-cholesterol. These results are consistent with several previously published studies in adults (Geiger et al., 2014; Maisonet et al., 2015; Zeng et al., 2015; Bao et al., 2017; Christensen et al., 2016; Koshy et al., 2017; Seo et al., 2018; He et al., 2018; Mora et al., 2018; Li et al., 2020; Steenland et al., 2009). There

Table 5
Association between PFAS serum concentrations and hypertension in Norwegian adolescents. The Tromsø study Fit Futures 1.

PFAS	Concentration ng/mL	Hypertension/non-hypertension[b]	OR (95% CI)[c] for hypertension[d]	p-value
∑PFAS				
Quartile 1[a]	2.59–8.60	35/200	1.0	
Quartile 2	8.61–10.72	36/199	1.13 (0.64–1.99)	0.679
Quartile 3	10.73–13.37	37/198	1.28 (0.72–2.27)	0.394
Quartile 4	13.38–200.8	53/182	1.91 (1.12–3.26)	0.018
PFHxS				
Quartile 1[a]	0.18–0.55	25/210	1.0	
Quartile 2	0.56–0.71	46/191	1.63 (0.90–2.94)	0.103
Quartile 3	0.72–1.12	37/196	1.25 (0.69–2.28)	0.461
Quartile 4	1.13–84.72	53/182	2.06 (1.16–3.65)	0.013
PFOA				
Quartile 1[a]	0.28–1.56	35/202	1.0	
Quartile 2	1.57–1.92	42/192	1.28 (0.74–2.22)	0.370
Quartile 3	1.93–2.44	47/187	1.45 (0.85–2.49)	0.175
Quartile 4	2.45–13.97	37/198	2.08 (1.17–3.69)	0.013
PFOS				
Quartile 1[a]	1.28–4.86	33/202	1.0	
Quartile 2	4.87–6.21	35/200	1.40 (0.78–2.51)	0.261
Quartile 3	6.22–7.80	36/199	1.01 (0.56–1.80)	0.980
Quartile 4	7.81–99.2	57/178	1.86 (1.08–3.19)	0.025

[a] Reference group.
[b] Number.
[c] Adjusted for sex, age, BMI and physical activity outside school.
[d] Systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg.

Table 6
Association between obesity and PFHpS, PFHxS serum concentrations in Norwegian adolescents. The Tromsø study Fit Futures 1.

	Concentration ng/mL	Obesity/non-obesity[b]	OR (95% CI)[c] for obesity	p-value
PFHxS				
Quartile 1[a]	0.18–0.55	9/226	1.0	
Quartile 2	0.56–0.71	20/217	2.09 (0.89–4.91)	0.090
Quartile 3	0.72–1.12	24/209	2.71 (1.17–6.25)	0.020
Quartile 4	1.13–84.72	11/224	1.18 (0.46–3.04)	0.729
PFHpS				
Quartile 1[a]	0.01–0.12	7/226	1.0	
Quartile 2	0,13–0.15	19/211	2.73 (1.05–7.05)	0.039
Quartile 3	0.16–0.19	20/218	2.84 (1.10–7.37)	0.032
Quartile 4	0.20–7.62	18/221	2.36 (0.89–6.25)	0.084

[a] Reference group.
[b] Number.
[c] Adjusted for sex, age, physical activity outside school.

was no association of PFAS with higher HDL that has protective effect, only PFUnDA was positively associated with higher HDL cholesterol, as well as with higher total- and LDL-cholesterol. The similar association between PFUnDA and HDL-cholesterol was described in another study (Fu et al., 2014).

The EFSA 2020 report on effects of PFAS has concluded that there is still insufficient evidence to suggest that PFAS are associated with hypertension and more studies are needed (Schrenk et al., 2020). The results of this study showed a positive association of exposure to PFHxS, PFOS and PFOA with hypertension that is consistent with other studies (Bao et al., 2017; Huang et al., 2019).

Unfavorable effects of PFAS on blood pressure and serum lipids seem plausible as they were also reported in experimental animal studies and cell cultures. Thus, a study of pregnant rats exposed to PFOS and PFNA found increased blood pressure in their offspring (Rogers et al., 2014). Previous studies in cell cultures demonstrated dose-dependent PFAS effects with increased lipid levels and increased expression of peroxisome proliferator-activated receptors (PPARγ and PPARα), which seems to be the plausible biological mechanism that could explain the effect of PFAS on the lipid metabolism (Ma et al., 2018; Yamamoto et al., 2015; Li et al., 2018).

Results from published literature examining associations between PFAS exposure and overweight/obesity are not consistent. Koshy T et al. found no association with overweight in American adolescents exposed to PFHxS, PFOA, PFOS, PFNA, PFDA (Koshy et al., 2017), while a Swedish prospective birth cohort study showed a positive association of PFOS and PFOA exposure with overweight/obesity (Lauritzen et al., 2018). 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 (Domazet et al., 2016). Several studies of prenatal PFAS exposure in humans demonstrated positive association with higher body weight later in life (Karlsen et al., 2017; Lauritzen et al., 2018; Hartman et al., 2017; Mora et al., 2017; EFSA, 2018). The EFSA 2020 report has concluded that there is insufficient evidence for association between exposure to PFAS and obesity (Schrenk et al., 2020). The present study found a positive association between obesity and PFHxS and PFHpS concentrations, however this association was not linear and there was no positive association with other PFAS. Obesity is a complex disease with multifactorial etiology; differences between the populations may be attributable to different genetic and environmental factors, as well as differences in PFAS exposure and different time of follow-up.

The present cross-sectional study observed that several individual PFAS were associated with cardiovascular risk factors already in adolescence. This is consistent with a recently published study from a national cohort of the US population demonstrating that ∑PFAS, PFOS; PFNA, PFDA and PFUnDA were associated with cardiovascular disease risk in the general population (Huang et al., 2018). ∑PFAS, PFOS, PFOA exposure have also been associated with several metabolomics biomarkers associated with oxidative stress and cardiovascular dysfunction (Wang et al., 2017).

The cross-sectional design is the main limitation of the present study. Therefore, the causality of PFAS associations with obesity, hypertension and dyslipidemia cannot be established. However, PFAS have relatively long half-life, therefore the cross-sectional nature of this study may be of lesser importance. Another limitation of the study is the definition of obesity by high BMI, which is a usual approach for epidemiological studies, but it has its limitations because BMI cannot differentiate between bone density, muscle mass, and body fat. The strengths of the study are that the PFAS exposure was measured by analyzing serum samples at the specialized laboratory for analysis of environmental

pollutants and that the study population was recruited from the general population that provided a representative sample of the adolescent population from Northern Norway. Another strength of the study is the possibility to adjust for possible confounders due to the comprehensive data available on diet and lifestyle factors. Thus, it is likely that the observed associations are applicable to other youth populations.

In conclusion, after adjustment for age, sex and lifestyle factors, several PFAS concentrations remained positively associated with the risk of dyslipidemia, hypertension and obesity in this cross-sectional study of the adolescent population from Northern Norway. These findings indicate possible harmful effects of PFAS on cardiovascular system and lipid metabolism that start in adolescence and may lead to cardiovascular disease later in life. Further prospective studies are required to confirm this hypothesis.

Credit author statement

Maria Averina: conceptualization, methodology, formal analysis, writing and original draft preparation, visualization. Jan Brox: supervision, resources, funding acquisition, writing- reviewing and editing. Sandra Huber: methodology, investigation, data curation, validation, writing- reviewing and editing. Anne-Sofie Furberg: conceptualization, project administration, resources, funding acquisition, writing- reviewing and editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

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