ASSOCIATION AMONG SERUM PER- AND POLYFLUOROALKYL SUBSTANCES, LIPID PROFILE AND METABOLIC SYNDROME IN CZECH ADULTS, HBM-EHES SURVEY 2019

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SUMMARY

Objectives: Per- and polyfluoroalkyl substances (PFASs) are a large group of persistent synthetic chemicals widely used commercially. They accumulate increasingly in all environmental components and enter the organisms, including humans. Some of them are associated with the risk of harm to health, among others with metabolic disorders. To test the associations between blood serum levels of PFASs and blood lipid profile as well as metabolic syndrome, we linked human biomonitoring with the Czech Health Examination Survey (CZ-EHES) conducted in 2019.

Methods: A total of 168 participants of the CZ-EHES survey aged 25–64 years were examined including anthropometrical data and analyses for serum PFAS and blood lipid levels. Extended model approach in multiple linear regression models was used for identification of the associations between serum levels of 11 PFASs and lipid profile components. The relation between PFAS serum levels and metabolic syndrome prevalence was tested using a logistic regression model.

Results: Six PFASs were detected over the limit of quantification in at least 40% cases and were examined in subsequent analyses: perfluorodecanoic acid (PFDA), perfluorohexane sulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorooctane sulfonic acid (PFOS), perfluoroundecanoic acid (PFUdA). The most dominant was PFOS with the mean value amounting to 4.81 ng/ml. After adjusting for potential confounders, we found a significant positive association between serum PFHxS and blood total cholesterol ($p = 0.005$) as well as LDL-cholesterol ($p = 0.008$). Significant positive association was also found between PFDA and HDL-cholesterol levels ($p = 0.010$). No significant associations were detected between PFASs and triglycerides, and between PFASs and metabolic syndrome.

Conclusions: We found some evidence of a significant association between blood serum PFAS levels and blood cholesterol levels. Our results did not confirm an association between serum PFASs and the metabolic syndrome prevalence.

Key words: human biomonitoring, serum PFASs, lipid profile, metabolic syndrome

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INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a group of substances extensively used in a wide range of industrial and consumer-production sectors for their stability and beneficial surface-active effects. However, they are hardly decomposed in the environment therefore they have the ability of global contamination, and they accumulate in living organisms (1). PFASs are ubiquitous. The main routes are consumption of primarily contaminated food or food from packaging containing PFASs, drinking water, inhalation, and ingestion of indoor air dust, they can be dermally absorbed or swallowed due to the hand-to-mouth transfer from treated material surfaces (2). The original long-chain PFASs, which have been proven toxic by epidemiological studies, are regulated and gradually replaced by short-chain fluorinated or by non-fluorinated alternatives in consumer products. However, the potential health risks are thereby not reduced. First, some long-chain PFASs are still used under exceptions and at the same time previously used PFASs do not disappear from the environment due to accumulation in marine food chains, groundwater, etc. This results in their constant and widespread levels in the body fluids of populations (3). Second, although short-chain fluorinated substitutes often also have high stability and environmental mobility, they are increasingly used without sufficient knowledge on their potential hazards.

With varying weight of evidence numerous studies identify association between perfluoroalkyl exposure and immune, endocrine, reproductive, developmental, and metabolic outcomes (4–8). As regards the effects on metabolism, PFASs have ability to disrupt lipid homeostasis and can cause liver damage. As it follows from the review of Sunderland et al. (3), dyslipidaemia is the metabolic disorder most associated with PFASs exposure manifesting by increased levels of total cholesterol, LDL-cholesterol and triglycerides; these effects were observed by number

of studies (9–14). Although there is an apparent trend towards positive associations between PFASs and increased serum lipid levels, the findings are not consistent across studies and the causality is still not clear. Toxicological data show that PFAS-induced disruption of lipid metabolism is associated with the accumulation of triglycerides in hepatocytes resulting in the onset of the fatty liver disease. These processes are accompanied by changes in genes regulating lipid homeostasis in particular affecting the transcriptional activity of peroxisome proliferator-activated receptor PPARα (15), but also other nuclear receptors (16).

Obesity has also been found to be associated with PFAS exposure in general populations. As stated by Qi et al. in the review (17), cohort as well as cross-sectional studies reported positive associations between the exposure to PFASs and increased body mass index (BMI) or waist circumference. It is long well known that obesity is an independent risk factor for non-alcoholic fatty liver disease and steatosis (18). The finding of the associations between LDL-cholesterol/triglyceride serum levels and PFAS serum levels being more evident in obese persons led to the assumption that this is probably just due to the accumulation of fat in hepatocytes, and that the combination of increased PFAS exposure and obesity increases the risk of lipid metabolism alterations (19). According to the recent review of Liu et al. (20), obesity seems to be a key factor in the PFAS-induced glycolipid metabolic disease (GLMD), and vice versa exposure to PFASs promotes the obesity-associated GLMD, such as diabetes, cardiovascular and liver diseases.

This work follows on from our previous activities in the Czech Human Biomonitoring (HBM-CZ), where PFASs have been regularly monitored in serum of blood donors (21) and in breast milk of primiparas (22). To explore the associations among serum PFAS levels and lipid profile indicators and metabolic syndrome we linked human biomonitoring with the Czech Health Examination Survey (CZ-EHES 2019), which took place in connection with the compulsory European Health Interview Survey (EHIS) in 2019.

MATERIALS AND METHODS

Study Subjects and Data Collection

In 2010–2011, the EHES survey was piloted in 12 European countries (23) including the Czech Republic. We linked the 2nd round of the national Health Examination Survey (CZ-EHES) realized in 2019 with the human biomonitoring (HBM). The participants were recruited by the professional interviewers of the Czech Statistical Office, who invited all respondents of the simultaneously ongoing European Health Interview Survey (EHIS) aged 25–64 to participate in the CZ-EHES examination survey. The CZ-EHES study and HBM were organized by the National Institute of Public Health in Prague (NIPH). Linkage of CZ-EHES to HBM was implemented in four Czech regions: the capital city Prague, industrial agglomeration Ostrava, medium-sized city of Liberec, and smaller highland town Žďár nad Sázavou. The participants signed an informed consent to take part in the examination, including HBM. The study was approved by the ethics committee of the NIPH.

The health examination was carried out by trained medical professionals according to the uniform EHES manual (24). The examination included measurement of anthropometric parameters (height, weight, and waist circumference), three repeated blood pressure measurements and venous blood sampling. The fasting state was not a mandatory condition in order not to reduce the chance of participation in the survey. Blood samples for this CZ-EHES/HBM study were taken from 168 persons. Laboratory analysis of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) with low-density lipoprotein cholesterol (LDL-C) being calculated, triglycerides (TG) and glycated haemoglobin (HbA1c) was carried out in contracted certified laboratories. The remaining blood serum was pipetted into coded test tubes, frozen at a temperature of −18 °C to −20 °C and shipped to the NIPH laboratories, where chemical analyses for PFASs were carried out.

Analytical Methods

The determination of blood lipids and glycated haemoglobin took place in the network of the biochemical laboratories accredited according to the ČSN EN 15189:2013 standard. TC, HDL-C and TG were determined photometrically. LDL-C was obtained by calculation based on total cholesterol, HDL-C and TG. HbA1c was determined by high-performance liquid chromatography (HPLC) on cation exchanger.

In blood serum samples, 11 PFASs were analysed: perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), perfluoroheptanoic acid (PFHpA), perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorooctane sulfonic acid (PFOS), perfluoroundecanoic acid (PFUdA), perfluorotridecanoic acid (PFTrA), and perfluorooctanesulfonamide (FOSA). A serum sample (0.5–2 mL) was spiked with 10 µL of a solution containing 1,000 ng/mL of 13C labeled PFAS analogues. Acetone (10 mL) and anhydrous calcium chloride (1.5 g) were added, the mixture was shaken for 10 min at 300 rpm and centrifuged at 3,000 rpm. The upper acetone phase was collected and evaporated to near dryness using a gentle stream of nitrogen. The residue was reconstituted in methanol (250 µL) and the resulting solution was centrifuged to remove solid particles. An extract aliquot (5 µL) was analysed using an Infinity 1290 ultra-performance liquid chromatograph (Agilent Technologies, Santa Clara, CA) coupled to a 6490A triple quadrupole mass spectrometer (Agilent Technologies). Chromatographic separation was achieved using a Kinetex Phenyl-hexyl 100A HPLC column (150 mm × 2.1 mm × 2.6 µm, Phenomenex, Torrance, CA) maintained at 40 °C and a mobile phase consisting of (A) aqueous ammonium acetate (2 mmol/L) and (B) methanol. Mobile phase composition and flow rate were linearly increased as follows: 0 min, 20% B, 0.35 mL/min; 2.5 min, 60% B, 0.30 mL/min; 6 min, 80% B, 0.40 mL/min; 8 min, 80% B, 0.40 mL/min. The limits of quantification (LOQ) varied with serum sample size and ranged from 0.013 to 0.050 ng/mL for PFHxS, PFDA, PFUdA, PFDoA, PFTrA, and FOSA; from 0.038 to 0.150 ng/mL for PFOA, PFNA and PFOS; from 0.028 to 0.113 ng/mL for PFBS; and from 0.125 to 0.500 ng/mL for PFHpA.

Statistical Analysis

The serum PFAS levels were described as the mean, standard error, standard deviation, and the geometric median, mean (GM)

with its 95% confidence interval. Concentrations lower than the LOQ were assigned a value of half of the LOQ. The differences among the subgroups were tested by the independent samples t-test. The data were analysed using Shapiro-Wilk test for normal distribution before statistical analysis and transformed by natural logarithm to achieve normality. Extended model approach in multiple linear regression models was used for identification of the associations between serum levels of PFASs and lipid profile components with outcome variables TC, HDL-C, LDL-C and TG. The models were controlled for age, gender, lipid modulation drugs use, fasting state, BMI, income level (income quintiles), alcohol intake, and smoking. The models were optimized using stepwise regression to exclude predictors that did not contribute to the prediction of the dependent variable. The relation between PFAS serum levels and metabolic syndrome occurrence was tested using a logistic regression model adjusted for age, sex, income level, smoking, and alcohol intake. Metabolic syndrome was determined upon the International Diabetes Federation criteria (25), i.e., min. 3 of these 5 indicators should occurred: blood pressure ≥ 130 and/or ≥ 85 and/or treatment; triglycerides ≥ 1.7 mmol/L (in case of non-fasting ≥ 2 mmol/L); HDL-C < 1 mmol/L in males, < 1.3 mmol/L in females and/or treatment; glycated haemoglobin ≥ 39 mmol/L (fasting glucose > 5.6 mmol/L) and/or diagnosed diabetes mellitus type 2; waist circumference > 94 cm in males and > 80 cm in females. Data were processed using IBM SPSS Statistics for Windows software, version 24 (IBM Corp., Armonk, N.Y., USA), and STATA software 14.2 (StataCorp LLC, Texas, USA).

RESULTS

The serum lipid profile and other metabolic syndrome parameters, such as blood pressure and the body mass indicators, were analysed for 168 CZ-EHES survey participants. The descriptive characteristics are presented in Table 1. There were 64 males and 104 females in the CZ-EHES/HBM group; the mean age was 46.6 years ranging from 25 to 64 years. There were more alcohol consumers among males (83%) than females (69%), but on the contrary, more females (29%) than males (19%) were smokers. The mean TC value was 5.1 ± 0.08 mmol/L with 43.5% cases that can be classified as acceptable (< 5.0 mmol/L). The mean LDL-C value amounted to 3.0 ± 0.07 with 48.8% cases classified as acceptable (< 3.0 mmol/L). The mean HDL-C reached the value of 1.5 mmol/L. The body mass index (BMI) ranged from 19.4 to 41.5 in males and from 18.7 to 49.8 in females, with the means amounted to 28.6 and 27.3, respectively.

The prevalence of the metabolic syndrome indicators is presented in Tables 2 and 3. Metabolic syndrome was determined in

Table 1. *Descriptive characteristics of CZ-EHES/HBM study participants (N = 168)*

Characteristics	Males n = 64 n (%)	Females n = 104 n (%)	Total n (%)
Age (years), mean (SD)	45.6 (1.4)	47.3 (1.1)	46.6 (0.9)
Education level			
≤ high school	43 (67)	69 (66)	112 (67)
> high school	21 (33)	35 (34)	56 (33)
Income quintile			
1st–3rd income quintile	27 (43)	52 (50)	79 (47)
4th–5th income quintile	36 (57)	52 (50)	88 (53)
Alcohol consumption at least once a month	53 (83)	72 (69)	125 (74)
Smokers, current	12 (19)	30 (29)	42 (25)
Body mass index (kg/m^2)			
< 25	14 (22)	42 (40)	56 (33)
25–30	29 (45)	35 (34)	64 (38)
> 30	21 (33)	27 (26)	48 (29)
Blood pressure*			
Optimal (< 120 and < 80 mmHg)	8 (13)	41 (39)	49 (30)
Normal (120–129 and/or 80–84 mmHg)	14 (22)	14 (14)	28 (17)
High/normal (130–139 and/or 85–89 mmHg)	10 (16)	9 (9)	19 (11)
Hypertension (≥ 140 and/or ≥ 90 mmHg) and/or use of antihypertensive drugs	32 (50)	40 (39)	72 (43)
Glycated haemoglobin HbA1c**			
Normal (20–38 mmol/mol)	39 (61)	73 (70)	112 (67)
Prediabetes (39–47 mmol/mol)	19 (30)	22 (21)	41 (25)
Diabetes (≥ 48 mmol/mol and/or treatment of diabetes type 2)	6 (10)	9 (9)	15 (9)

*Categories of blood pressure according to the ESC/ESH guidelines (26)
**Categories of HbA1c according to the American Diabetes Association (27)

Table 2. *Characteristics of serum lipid profile and metabolic syndrome components*

	n	Mean (SD)	Min	Max
Total cholesterol (mmol/L)	168	5.1 (1.0)	3.0	8.3
LDL-cholesterol (mmol/L)*	166	3.0 (0.9)	0.8	5.9
HDL-cholesterol (mmol/L)	168	1.5 (0.4)	0.6	2.6
Triglycerides (mmol/L)	165	1.4 (0.8)	0.1	4.9
Fasting	54	1.2 (0.6)	0.4	2.8
Non-fasting	111	1.5 (0.8)	0.1	4.9
Systolic BP (mmHg)	168	125.0 (18.2)	83.5	176.0
Diastolic BP (mmHg)	168	83.6 (10.9)	57.0	115.5
Body mass index (kg/m^2)	168	27.8 (5.9)	18.7	49.8
Waist circumference (cm)				
Males	64	99.1 (96.5)	72.0	135.0
Females	104	86.7 (84.5)	61.5	130.0

*Calculated; BP – blood pressure, SD – standard deviation

Table 3. *Prevalence of metabolic syndrome components* (N = 168)*

Component	n (%)
Elevated blood pressure and/or treatment	83 (49)
Elevated triglycerides and/or blood lipid treatment	35 (21)
Reduced HDL-C	17 (10)
Elevated glycated haemoglobin and/or diabetes mellitus diagnose	56 (34)
Elevated waist circumference	104 (62)
Use of antihypertensive drugs	36 (21)
Use of lipid modulation drugs	16 (10)
Diagnose of diabetes	18 (11)

*Definitions based upon IDF definitions (25). For the criteria of metabolic syndrome indicators, see section Statistical analysis.

62 subjects; that is about one third (36.9%) of the CZ-EHES/HBM group. The most commonly identified components were increased waist circumference (61.9%) and elevated blood pressure (49.4%), followed by elevated blood glucose (33.5%).

Of the 11 measured PFASs, the serum levels of PFHxS, PFOA and PFOS were detected above the limit of quantification (LOQ) in 100% of the samples; PFDA and PFNA were found over LOQ in almost all samples (99.4% and 98.8%, respectively); PFUdA serum levels overreached the LOQ in 94% of the samples. The serum levels of PFBS, PFHpA, PFDoA, PFTrA were in more than 64% of cases above the LOQ, and therefore they were considered improper for quantification and further analysis. FOSA was detected in none of the serum samples.

The most dominant was PFOS with the mean value amounting to 4.814 ng/ml. Males had significantly higher levels of PFOS (5.220 ng/mL vs. 4.565 ng/mL, $p=0.025$) and PFHxS (0.554 ng/mL vs. 0.397 ng/mL, $p<0.001$) than females. For all quantified PFASs, the higher mean values were found in the subgroup of the older participants (aged 45–64 years) in comparison with the younger subgroup (aged 25–44 years); the differences were significant except for PFOS and PFUdA (PFHxS $p=0.005$; PFOA $p<0.001$; PFNA $p<0.001$; PFDA $p=0.012$). When tested the PFAS levels between the subgroups with lower than university education and higher education (university), the latter subgroup had higher PFAS mean levels, although not significantly. Similarly, 4th–5th income quintile subgroup showed higher PFAS values than 1st–3rd income quintile subgroup without significant difference. The subgroup of participants with overweight and obesity did not differ significantly compared to normal weight subgroup except for PFUdA, where higher levels were found in the subgroup with normal weight. The characteristics of the serum PFAS levels in the participants and their subgroups are presented in Table 4.

After adjustment for covariables, the fitted regression models testing the associations between the PFAS serum levels and particular serum lipid profile components revealed a significant positive association between serum PFHxS and blood TC ($p=0.005$) as well as LDL-C ($p=0.008$) levels. Significant positive association was also found between PFDA and HDL-C ($p=0.010$) levels. No association was detected between triglycerides and the examined PFASs. The linear regression coefficients with p-values are shown in Table 5. No association with serum PFASs was found in case of metabolic syndrome prevalence, negative association was observed particularly in PFOS (Table 6).

DISCUSSION

In this study, we explored the associations between serum PFASs prevalent in Czech adults and the lipid profile indicators as well as metabolic syndrome prevalence. The sample donors were the participants of the National Health Examination Survey CZ-EHES 2019.

The spectrum of the most frequently PFASs found in general adult population in our study was the same as in blood donors investigated by Sochorová et al. (21). The median serum values were similar when comparing Czech blood donors and CZ-EHES 2019 general adult population for PFOA, PFDA and PFNA (0.756 ng/mL vs. 0.686 ng/mL, 0.145 ng/mL vs. 0.150 ng/mL, 0.325 ng/mL vs. 0.365 ng/mL, respectively), but lower for PFOS, PFUdA and PFHxS (2.43 ng/mL vs. 3.44 ng/mL, 0.058 ng/mL vs. 0.083 ng/mL, and 0.184 ng/mL vs. 0.400 ng/mL, respectively). The lower values of these PFASs in blood donors could have resulted from the different composition of the population sample.

The associations found are consistent with various cross-sectional studies, which generally show a trend toward a positive association between PFASs and serum lipids (3). We found significant positive associations between PFHxS and total and LDL-cholesterol. Similarly to USA NHANES data (19) or Chinese data on adults (9, 11), we also found significant positive association between serum PFDA and HDL-C. We observed no association between triglycerides and any of the investigated PFASs, similarly to Fu et al. among Chinese adults (9). On the other hand, Christensen et al. (12) reported the highest levels of detected serum PFHxS to be associated with elevated TG. Negative association between PFOS and TG was found by Liu et al. (7). In the Czech study on the exposure of young male professional firefighters in relation to liver function and serum lipids CELSPAC-FIREexpo study (28) the authors observed a significant negative association of PFAS mixture with TG. They found a positive association between the mixture of serum PFASs together with mixture of

Table 4. *Characteristics of the serum PFAS levels (ng/mL) (N = 168)*

		PFHxS	PFOA	PFNA	PFOS	PFDA	PFUdA
Total	Median	0.400	0.686	0.365	3.439	0.150	0.083
	Mean	0.457	0.865	0.414	4.814	0.193	0.096
	SE	0.021	0.05	0.019	0.410	0.012	0.005
	SD	0.271	0.647	0.243	5.312	0.155	0.067
	GM	0.387	0.698	0.353	3.503	0.155	0.076
	95% CI	0.354–0.423	0.631–0.770	0.323–0.386	3.127–3.924	0.141–0.171	0.068–0.085
Males n = 64	Median	0,532	0.690	0.408	4.154	0.156	0.081
	Mean	0.554*	0.84	0.431	5.220*	0.193	0.093
	SE	0.035	0.072	0.025	0.642	0.019	0.008
	SD	0.277	0.578	0.199	5.135	0.152	0.065
	GM	0.488	0.721	0.389	4.127	0.159	0.075
	95% CI	0.428–0.556	0.632–0.821	0.346–0.437	3.522–4.837	0.137–0.185	0.063–0.089
Females n = 104	Median	0.328	0.688	0.344	3.051	0.148	0.079
	Mean	0.397*	0.880	0.403	4.565*	0.193	0.098
	SE	0.025	0.068	0.026	0.532	0.015	0.007
	SD	0.250	0.689	0.268	5.428	0.157	0.070
	GM	0.336	0.684	0.333	3.167	0.152	0.076
	95% CI	0.230–0.376	0.594–0.787	0.294–0.377	2.715–3.694	0.134–0.174	0.066–0.088
Age 25–44 n = 75	Median	0.321	0.523	0.283	3.051	0.134	0.076
	Mean	0.385*	0.696*	0.352*	4.305	0.165*	0.086
	SE	0.024	0.067	0.028	0.630	0.016	0.007
	SD	0.211	0.584	0.242	5.487	0.135	0.058
	GM	0.333	0.567	0.303	3.095	0.138	0.069
	95% CI	−0.293–0.378	0.493–0.651	0.270–0.341	2.619–3.656	0.121–0.156	0.059–0.081
Age 45–64 n = 93	Median	0.448	0.864	0.415	3.862	0.176	0.086
	Mean	0.514*	1.001*	0.464*	5.225	0.216*	0.105
	SE	0.031	0.069	0.025	0.538	0.017	0.008
	SD	0.300	0.667	0.237	5.169	0.165	0.073
	GM	0.437	0.825	0.400	3.871	0.171	0.082
	95% CI	0.387–0.493	0.722–0.942	0.353–0.453	3.318–4.518	0.148–0.197	0.070–0.095
≤ High school n = 112	Median	0.419	0.693	0.369	3.439	0.142	0.076
	Mean	0.448	0.846	0.41	4.651	0.192	0.093
	SE	0.250	0.054	0.022	0.455	0.015	0.006
	SD	0.267	0.584	0.239	4.944	0.162	0.069
	GM	0.379	0.702	0.345	3.432	0.149	0.071
	95% CI	0.339–0.423	0.626–0.786	0.308–0.387	2.992–3.937	0.131–0.170	0.061–0.082
> High school n = 56	Median	0.358	0.662	0.357	3.488	0.156	0.092
	Mean	0.474	0.906	0.422	5.170	0.195	0.102
	SE	0.039	0.108	0.036	0.849	0.02	0.009
	SD	0.280	0.763	0.253	6.020	0.141	0.063
	GM	0.405	0.688	0.372	3.662	0.168	0.087
	95% CI	0.346–0.473	0.562–0.843	0.326–0.425	2.974–4.509	0.147–0.193	0.073–0.102

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		PFHxS	PFOA	PFNA	PFOS	PFDA	PFUdA
1st–3rd income quintile n = 79	Median	0.339	0.703	0.356	3.332	0.145	0.078
	Mean	0.428	0.787	0.378	4.722	0.171	0.089
	SE	0.031	0.052	0.021	0.699	0.013	0.007
	SD	0.275	0.459	0.187	6.217	0.115	0.064
	GM	0.352	0.665	0.335	3.342	0.144	0.069
	95% CI	0.304–0.406	0.580–0.762	0.299–0.375	2.832–3.943	0.126–0.164	0.059–0.082
4th–5th income quintile n = 88	Median	0.408	0.686	0.367	3.556	0.152	0.083
	Mean	0.479	0.938	0.445	4.886	0.212	0.103
	SE	0.028	0.083	0.03	0.471	0.019	0.008
	SD	0.266	0.777	0.282	4.415	0.182	0.070
	GM	0.419	0.730	0.370	3.634	0.165	0.081
	95% CI	0.375–0.467	0.630–0.845	0.323–0.424	3.097–4.264	0.143–0.191	0.067–0.095
BMI ≤ 25 (kg/m^2) n = 56	Median	0.354	0.768	0.370	3.409	0.170	0.090
	Mean	0.439	0.903	0.436	4.590	0.207	0.113*
	SE	0.035	0.092	0.037	0.544	0.022	0.101
	SD	0.259	0.688	0.279	4.071	0.167	0.076
	GM	0.376	0.737	0.373	3.480	0.167	0.088
	95% CI	0.323–0.437	0.625–0.870	0.322–0.432	2.864–4.229	0.140–0.198	0.071–0.108
BMI > 25 (kg/m^2) n = 112	Median	0.408	0.677	0.357	3.556	0.140	0.077
	Mean	0.465	0.846	0.403	4.926	0.186	0.088*
	SE	0.026	0.059	0.021	0.553	0.014	0.006
	SD	0.277	0.628	0.223	5.849	0.149	0.061
	GM	0.393	0.678	0.344	3.514	0.149	0.070
	95% CI	0.351–0.439	0.598–0.769	0.308–0.385	3.050–4.049	0.132–0.169	0.062–0.080

SE – standard error; SD – standard deviation; GM – geometric mean; 95% CI – confidence interval of GM; *statistically significant difference of the means between the subgroups

urine polycyclic aromatic hydrocarbons and total/LDL cholesterol by Bayesian weighted quantile sum regression.

The associations between serum PFAS levels and the metabolic syndrome prevalence are inconsistent across the studies. Our results are in concordance with various cross-sectional studies which report no associations (4, 7, 12, 13, 30, 31). Fisher et al. (29) explored data from the Canadian Health Measures Survey to investigate PFOA, PFOS and PFHxS and found no association with metabolic syndrome. Similar results were reported by Lin et al. (30) on a group of elderly Taiwanese in a cross-sectional study from 2016 to 2017. We observed rather negative associations although statistically insignificant which were reported also by Jeddi et al. namely for PFOS (31). Christensen et al. (12) examined the data from the National Health and Nutrition Examination Survey (NHANES) 2007–2014 and they found the decreasing risk of metabolic syndrome and its components in subjects with increasing PFAS levels, except only for PFNA. On the other hand, Yang et al. identified positive associations between serum PFASs and risk of metabolic syndrome in smaller group of adult Chinese males (11), but after adjustment only for age.

Although the positive associations between serum levels of lipids and PFASs have been found repeatedly in the epidemiological studies, the causality of these exposure-effect relationships is still not well known. The cross-sectional design of majority of the studies does not allow the causality of the associations to be traced. The discrepancy due to the opposite effects of the PFASs serum levels on blood lipid homeostasis in rodents and in humans is well described in the literature (32, 33). Furthermore, additional complications arise in observing associations. The joint intestinal reabsorption of bile salts and PFASs and uptake into the liver via shared pathways is a possible source of confounding, as inter-individual variability in reabsorption may suggest a positive association between serum PFAS levels and cholesterol, whose level is affected just by the reabsorption of bile acids (31).

Strength and Limitations

The strength of our study lies in linking of the human biomonitoring to the Health Examination Survey conducted according to the European uniform EHES manual. The anthropometric and health data were collected objectively by the trained medical professionals. For blood glucose status, we used glycated haemoglobin that is a substance produced in the body by non-enzymatical reaction (so-called glycation) of haemoglobin and glucose. While simple fasting blood glucose reflects the current value of the sugar level in blood, the HbA1c value provides information about the average blood sugar level (glycaemia) over a period of several weeks; it is so-called "long-term glycaemia".

Table 5. *Linear regression coeficients with 95% CI from linear regression model* of serum PFAS levels and serum lipid components*

	Coefficient	95% CI		p-value
		Lower	Upper	
Total cholesterol				
PFHxS	0.071	0.021	0.121	**0.005**
PFOA	0.021	−0.023	0.065	0.348
PFNA	0.030	−0.026	0.085	0.294
PFOS	−0.010	−0.049	0.028	0.600
PFDA	0.033	−0.011	0.077	0.143
PFUdA	0.041	−0.006	0.089	0.085
LDL-C				
PFHxS	0.117	0.031	0.202	**0.008**
PFOA	0.003	−0.079	0.085	0.944
PFNA	0.023	−0.077	0.122	0.653
PFOS	−0.015	−0.080	0.050	0.650
PFDA	0.037	−0.039	0.114	0.337
PFUdA	0.047	−0.031	0.125	0.234
HDL-C				
PFHxS	−0.016	−0.075	0.044	0.603
PFOA	0.037	−0.015	0.088	0.161
PFNA	0.059	−0.005	0.123	0.069
PFOS	−0.022	−0.066	0.022	0.319
PFDA	0.067	0.016	0.117	**0.010**
PFUdA	0.017	−0.039	0.074	0.549
Triglycerides				
PFHxS	−0.036	−0.191	0.118	0.643
PFOA	0.004	−0.140	0.149	0.953
PFNA	−0.056	−0.227	0.115	0.520
PFOS	0.075	−0.037	0.187	0.189
PFDA	−0.028	−0.168	0.112	0.692
PFUdA	−0.015	−0.165	0.136	0.847

*Adjusted for age, gender, lipid modulation drugs use, fasting status, BMI, income quintile, alcohol intake and smoking. Numbers in bold indicate statistically significant values.

Table 6. *Odds ratios with 95% CI of the logistic regression model* of serum PFAS levels and metabolic syndrome prevalence*

	OR	95% CI		p-value
		Lower	Upper	
Metabolic syndrome				
PFHxS	1.12	0.24	5.20	0.947
PFOA	0.89	0.22	3.60	0.870
PFNA	0.62	0.14	2.74	0.525
PFOS	0.34	0.10	1.15	0.083
PFDA	0.57	0.15	2.20	0.409
PFUdA	0.37	0.11	1.21	0.100

*Adjusted for age, sex, income quintile, smoking and alcohol intake

In addition to the benefits, linking large health surveys to human biomonitoring brings also limitations. Non-fasting state does not annoy the health survey respondents and does not discourage them from participating in the survey. Therefore, non-fasting blood lipid testing is currently common in population health studies. Examination of blood lipids without previous fasting does not significantly affect the results of TC, LDL-C and HDL-C, however, TG can be affected markedly (34). For this reason, values over 5 mmol/L tend to be excluded from screenings, and for these individuals it is recommended to repeat the sampling (34). However, in our study no sample overreached this TG value. To control the possible influence on the associations, the fasting state was introduced into the statistical models as an adjusting factor.

The population sample of this study is quite small considering the size of the Czech population. To prevent the data from being fragmented into little groups we used overweight and obesity as an adjustment instead of stratification variable, as it is preferred by Jain and Ducatman (19), who shows that using obesity as stratifying rather than adjusting factor can better prove the associations.

Limitations of this study include also cross-sectional character not allowing estimation of the causalities, and not performing the correction for multiple testing.

CONCLUSIONS

We found serum levels of some PFASs to be significantly associated with blood cholesterol but not with metabolic syndrome in the Czech adult population from CZ-EHES 2019. Considering so far inconsistent outputs of the associations among PFASs and metabolic disorder symptoms across the studies and insufficient knowledge of the ways of action of PFASs in humans, further investigation is still needed.

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Conflicts of Interest

None declared

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