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Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population

Yaning Fu [a,b], Tieyu Wang [a], Quanliang Fu [c], Pei Wang [a,b], Yonglong Lu [a,*]

[a] State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Red Cross Hospital of Yuanyang, Yuanyang County, Henan 453500, China

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ABSTRACT

Perfluoroalkyl acids (PFAAs) have been used in a variety of products for many years and have been detected worldwide in human serum. Previous studies have suggested the potential effects of PFAAs on serum lipids. To investigate the associations between serum concentrations of PFAAs and serum lipid levels, 133 participants were randomly selected from the people coming for health check-up in Yuanyang Red Cross Hospital of Henan, China. Linear regression analysis revealed that perfluoro-octanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA), with a median concentration of 1.43, 0.37, and 0.19 ng/mL, respectively, were positively associated with total cholesterol (TC). Those in the highest quartile of PFOA exposure had ln-TC levels 0.24 mmol/L higher than those in the lowest quartile. For PFNA and PFDA, effect estimates were 0.25 and 0.16 mmol/L, respectively. A positive association between high-density lipoprotein cholesterol (HDLC) and PFDA was found, and there was a 0.18 mmol/L increase of HDLC for the top PFDA quartile compared with the lowest quartile. PFOA and PFNA were positively associated with low-density lipoprotein cholesterol (LDLC). Ln-LDLC levels of people in both top PFOA and PFNA quartiles were 0.33 mmol/L higher than those in the lowest quartiles. Logistic regression analysis indicated that increased PFOA and PFOS quartiles were positively associated with an increased risk of abnormal TC and LDLC when controlling for no confounding factors.

1. Introduction

As a class of synthetic chemicals with surface-active properties, perfluoroalkyl acids (PFAAs) have been widely used as surfactants and surface protectors for over 50 years in many applications, including lubricants, paints, polishes, fire-fighting foams, food packaging and so on (Lau et al., 2007). In recent years, PFAAs have attracted great attention internationally due to their global occurrences in environment and biota (Giesy and Kannan, 2001; Meesters and Schroder, 2004), as well as their toxicity (Lau et al., 2007) and bioaccumulation (Martin et al., 2004). PFAAs have also been found in potential human exposure pathways, such as food (Tittlemier et al., 2006), drinking water (Skutlarek et al., 2006), indoor air and dust (Shoeib et al., 2005), which hence raise great public-health concerns.

PFAAs have been detected from human blood or serum samples in general population from many countries (Kannan et al., 2004; Fromme et al., 2009), including China (So et al., 2006; Pan et al., 2010). PFAAs are slowly eliminated in the human body (Lau et al., 2007). Unlike many other persistent organic pollutants accumulating in lipids, PFAAs bind to proteins in the liver and serum (Conder et al., 2008). The arithmetic mean serum half-lives of perfluoro-octane sulfonate (PFOS) and perfluoro-octanoic acid (PFOA) in humans are estimated to be 5.4 years [95% confidence interval (CI), 3.9–6.9 years] and 3.8 years (95% CI, 3.1–4.4 years), respectively (Olsen et al., 2007). Although the half-lives for perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUdA) in humans have not been estimated, longer-chain compounds are generally assumed to have longer half-lives, which has been proven by animal studies (Martin et al., 2003).

Previous toxicological studies have shown that PFAAs have various adverse health effects, including the potential to affect circulating concentrations of cholesterol (Olsen et al., 2009; DeWitt et al., 2012; Lin et al., 2013; Seacat et al., 2002). On daily exposure to PFOS at doses of 0.75 mg/kg body weight/day over a six-month period, cynomolgus monkeys exhibited a marked decrease in serum TC at serum PFOS concentrations approximately 150,000 ng/mL (Seacat et al., 2002). Moreover, animal studies have revealed that PFAAs bind to peroxisome proliferator-activated

* Corresponding author. Fax: +86 10 62918177.
E-mail address: yllu@rcees.ac.cn (Y. Lu).

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receptors (PPARs), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and liver X receptor (LXR) (Elcombe et al., 2010; Bjork et al., 2011; Elcombe et al., 2012). Nuclear receptors play a key role in lipid metabolism and adipogenesis, raising the concern that PFAAs may disrupt lipid regulation (Maher et al., 2008; Fang et al., 2012). In addition, another study utilizing the APOE*3-Leiden.CETP mouse model showed that PFOS may reduce TC by impairing lipoprotein production (Bijland et al., 2011). Compared with the effects found in animals, studies in humans have reported inconsistent associations between PFAAs and serum lipids (Frisbee et al., 2010a; Nelson et al., 2010; Fisher et al., 2013). Nelson et al. (2010) investigated four types of PFAAs and reported that people in the highest PFOA quartile had TC levels 0.25 mmol/L higher than those in the lowest quartile, while Fisher et al. (2013) found no significant association between cholesterol levels and PFOS or PFOA levels. One study on American children and adolescents, Frisbee et al. (2010a) found that between the first and fifth quintiles of PFOA, there was a 0.12 and 0.10 mmol/L increase of TC and LDLC, respectively. In a longitudinal assessment of workers involved in the demolition and disposal of perfluoroalkyl manufacturing plants, Olsen et al. (2012) observed no adverse associations between changes in PFOA, PFOS, and serum lipids. In another longitudinal study, although it was questioned by Burstyn (2013) for its clinical relevancy, Fitz-Simon et al. (2013) found that there was a tendency for people with greater declines in serum PFOA or PFOS to have greater LDLC decrease. For a person whose serum PFOA or PFOS fell by half, the predicted fall in LDLC was 3.6% or 5%, respectively

The results of previous epidemiological studies are not consistent. Most of these studies have focused on PFOS and PFOA, and the influence of other PFAAs on serum lipids is seldom discussed. Most previous studies have been performed in populations of European ancestry, and few are reported in the Chinese population. In the present study, we aimed to investigate potential associations between serum concentrations of eight PFAAs and four serum lipid levels in a Chinese population.

2. Materials and methods

2.1. Study population

All of the participants in this study were recruited from the Yuanyang County of Henan Province, China. Henan, with a population of over 94 million, is the largest provincial economy among inland provinces of China. From October–November 2011, 133 participants aged from 0 to 88 years old were randomly selected from the people coming for health check-up in Yuanyang Red Cross Hospital. In the meantime, serum samples were collected from the participants. Briefly, the serum was immediately separated from approximate 5 mL whole blood and then stored at −20 °C prior to further analysis. In addition, telephone interviews were also performed to collect detailed information of each participant, including lifestyle and demographic factors. However, no information on cholesterol lowering medication was obtained. The study was approved by the Red Cross Hospital from Yuangyang County and written informed consent was obtained from each participant.

2.2. Analysis of serum lipids

TC, triglyceride (TG), high-density lipoprotein cholesterol (HDLC) and LDLC are commonly used in clinical and epidemiologic studies. Concentrations of TC, TG, HDLC and LDLC in sera of all participants were directly determined using a BS-800 Chemistry Analyzer (Mindray, Shenzhen, China) at the clinical laboratory of the Yuanyang Red Cross Hospital.

2.3. Chemical analysis

A total of 12 PFAAs were analyzed in serum samples, including perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), PFOS, perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUdA and perfluorododecanoic acid (PFDoA).

PFAAs were extracted from serum with methyl tert-butyl ether in forming an ion pair with tetrabutylammonium hydrogen sulfate. Extracts were evaporated to dryness under a stream of high-purity nitrogen, dissolved in 1 mL of mobile phase, and then analyzed using an Agilent 1290 high performance liquid chromatography system (Palo Alto, CA, USA) coupled with an Agilent 6460 Triple Quadrupole mass spectrometer (Santa Clara, CA, USA). The analysis was operated in multiple reaction monitoring (MRM) mode with the electrospray ionization (ESA) in negative mode. Detailed methods are provided in supplementary materials.

2.4. Quality assurance and quality control

To check the contamination occurred during the extraction process, procedural blanks were prepared at an interval of every 10 samples. Moreover, background contamination was monitored using solvent blanks containing acetonitrile and Milli-Q water (2:3, v/v) for every 20 samples. In addition, duplicate injections and calibration check standards were performed for every 20 samples to assure the precision and accuracy of each run. Concentrations of serum extracts were determined via nine-point matrix-matched calibration curves ranging from 0.01 to 100 ng/mL, which were performed by adding mixed PFAA standard solutions into blank newborn bovine serum. The regression coefficients (r^2) of calibration curves for all the target analytes were higher than 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) were defined as three-fold and ten-fold greater than the signal-to-noise ratio, respectively. The recovery and reproducibility of the serum sample extraction were determined on six replicate analyses of 0.5 mL of newborn bovine serum containing 2 ng of each PFAA standard. No PFAA contamination was found above the LOD in newborn bovine serum. Table S1 summarizes the LOD, LOQ and matrix spike recoveries for all the target chemicals.

2.5. Statistical analysis

Statistical analyses were conducted using SPSS Statistics v19.0 (SPSS Inc., Chicago, IL). PFPeA, PFDoA, PFBS and PFHxS were detected in less than 75% of samples (63.91%, 34.59%, 30.08% and 26.32%, respectively). Therefore, results for PFPeA, PFDoA, PFBS and PFHxS were not further discussed in our study. Concentrations of PFAAs were expressed as median or mean ± standard deviation. Value below the LOD was assigned as a proxy value of an LOD/2.

TC, TG, HDLC and LDLC were used as the outcome variables in linear regression analyses. TC, TG and LDLC were ln-transformed to satisfy the normality criteria. Exposure was modeled in quartiles of PFAA concentrations. Each quartile was compared with the reference group (the first quartile). The effect estimates and the corresponding 95% confidence intervals (CIs) were presented. PFAA category was considered as a linear predictor in the quartile analyses.

Binary logistic regression analyses were conducted to assess the odds ratio of abnormal lipids with increasing PFAA quartile. The serum lipid categorization for adults was based on the Guidelines on Prevention and Treatment of Blood Lipid Abnormality in Chinese Adults (Zhao, 2008), while its categorization for children was based on the Expert Consensus for Prevention and Treatment of Dyslipidemia in Children and Adolescents (Xiang and Du, 2009). For TC, values higher than 5.18 mmol/L in adults or 4.40 mmol/L in children were classified as abnormal. For TG, values higher than 1.70 mmol/L were classified as abnormal. For HDLC, values lower than 1.04 mmol/L were classified as abnormal. For LDLC, values higher than 3.37 mmol/L in adults or 2.85 mmol/L in children were classified as abnormal. Logistic regression analyses were performed using PFAA quartile dummy variables, in which the first quartile was considered as the reference group. Age, gender and body mass index (BMI) were included in adjusted models as covariates to assess their confounding influence.

3. Results

Table 1 lists general characteristics of participants in the present study. The participants consisted of 80 males and 53 females. The median age was 30 years, ranging from 0.3 to 80 years. The BMI ranged from 15.3 to 35.6, with a median value of 23.5.

Among the 12 analyzed PFAAs, eight compounds were detected in more than 75% of the samples (78.20% for PFBA, 76.69% for PFHxA, 98.5% for PFHpA, 100% for PFOA, 97.74% for PFNA, 82.71% for PFDA, 81.20% for PFUdA and 100.00% for PFOS). PFOS was detected with the greatest median concentration (1.47 ng/mL, range: 0.08–10.20 ng/mL), followed by PFOA (1.43 ng/mL, range: 0.32–39.46 ng/mL) and PFNA (0.37 ng/mL, range: 0.02–4.18 ng/mL), and they accounted for about 80% of the total PFAAs. Significant correlations were detected between PFBA and PFOS,