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Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status

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ABSTRACT

Polyfluoroalkyl substances (PFASs) are a group of common chemicals that ubiquitously exist in wildlife and humans. However, few studies have researched the effect of PFASs on reproductive hormones in adolescents. To provide information in this regard, we recruited 225 Taiwanese adolescents aged 13–15 years from 2009 to 2010 to investigate the relationship between serum PFASs (PFOS, PFOA, PFBS, PFDA, PFDoA, PFHxA, PFHxS, PFNA and PFTA) and reproductive hormone concentrations using a cross-sectional study design. Results showed PFOS and PFTA levels were highest among the PFASs, with a median concentrations of 29.9 (interquartile range: 13.0–43.8) ng/mL and 6.0 (0.6–25.9) ng/mL in males, and a median concentrations of 28.8 (14.8–42.6) ng/mL and 4.5 (0.3–18.4) ng/mL in females. After adjustment for confounding factors, nonsignificant associations between PFASs and reproductive hormone were found except for PFNA with ln(estradiol) ($\beta = 0.2060$, 95%CI: 0.0016, 0.4105). When stratified by sex, more significant associations were found in males than in females. Among males, PFASs were negatively associated with ln(testosterone) level for PFOS ($\beta = -0.0029$, 95%CI: -0.0055, -0.0003), PFDA ($\beta = -0.2565$, 95%CI: -0.4135, -0.0994), PFHxA ($\beta = -0.3095$, 95%CI: -0.5942, -0.0248), and PFNA ($\beta = -0.4233$, 95%CI: -0.6998, -0.1467). Furthermore, male participant ln(estradiol) levels were positively associated with PFOA ($\beta = 0.0921$, 95%CI: 0.0186, 0.1656), and PFHxS ($\beta = 0.0462$, 95%CI: 0.0020, 0.0905). Among females, a significant relationship was found only for PFDoA with ln(testosterone) ($\beta = -0.0119$, 95%CI: -0.0227, -0.0010). In conclusion, this study showed higher levels of PFASs coincide with lower testosterone and higher estradiol levels, and more significant associations of PFASs with reproductive hormone were found in males than in females.

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1. Introduction

Reproductive hormones determine sex differences and control organ function and skeletal muscle growth. Testosterone and estradiol, which are steroid hormones, are primarily secreted from testes in males and ovaries in females (Cheng and Mruk, 2010; Wall et al., 2014). Small amounts of both hormones are released by the adrenal glands in both sexes (Shea et al., 2014). During puberty, serum reproductive hormones rise dramatically and insufficient levels will induce adverse health conditions, such as infertility (Patel et al., 2015), loss of bone density (Morley et al., 1997; Schow et al., 1997), obesity (Pinola et al., 2012), and depression (Barrett-Connor et al., 1999).

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of synthetic chemicals which are widely used in various

Abbreviations: PFASs, polyfluoroalkyl substances; PFBS, perfluorobutane sulfonate; PFHxS, perfluorohexane sulfonate; PFOS, perfluorooctane sulfonate; PFHxA, perfluorohexane acid; PFHpA, perfluoroheptanoic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFTA, perfluorotetradecanoic acid; 95%CI, 95% confidence intervals.

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manufacturing and industrial processes (Rahman et al., 2014).The combination of carbon and fluorine atoms in the aliphatic carbon backbone of these substances, in which fluorine has replaced hydrogen atoms, allows PFASs to resist degradation and makes them extremely persistent in environmental and in biological samples (Lindstrom et al., 2011).The potential harmful effects of PFASs to human health have raised concern. Until now, most PFASs toxicity studies have investigated developmental deficits (Lau et al., 2003), neurotoxicity (Mariussen, 2012), and immune system function (Grandjean et al., 2012).

Several studies have found that the PFAS affect the endocrine system in vivo and in vitro (Jensen and Leffers, 2008). Animal studies have demonstrated that a higher exposure to PFOA concentrations is associated with lower testosterone levels and higher estradiol levels in male adult rats (Biegel et al., 1995). For PFOS, higher exposure was associated with lower estradiol levels in male monkeys (Seacat et al., 2002) and decreased testosterone levels in male fish (Arukwe et al., 2013). Furthermore, PFNA is associated with a dramatic decrease in testosterone levels and an elevation in estradiol levels in rats (Feng et al., 2009).

Epidemiological evidence is limited and not consistent. In a study of 2292 children aged 6–9 years old, Lopez-Espinosa et al. (2016) reported significant associations of reproductive hormone levels with certain PFASs. The study demonstrated there were significant inverse associations of testosterone levels with PFOA and PFOS in boys, and PFOS in girls, as well as significant inverse associations of estradiol levels with PFOS concentration in girls. The same author previously reported delayed puberty in children is correlated with PFOS and PFOA levels (Lopez-Espinosa et al., 2011). Additionally, a negative association between serum PFOS concentration and testosterone levels in young Danish men (median age 19 years, $n = 247$) was reported by Joensen et al. (2013). In a separate study 25,957 adult women in the same Danish community, PFOS and PFOA concentrations were positively associated with earlier menopause, and PFOS concentrations were associated with lower estradiol levels (Knox et al., 2011). However, no association was found between PFOA or PFOS concentrations and testosterone and estradiol levels in Danish men (median age, 19 years) (Joensen et al., 2009). Olsen et al. (1998) found no association between PFOA or PFOS concentrations and testosterone and estradiol levels in American workers.

Studies investigating the impact of PFASs on human reproductive health are limited and controversial, especially in adolescents. Thus, the aim of the present study was to assess the associations between PFASs concentrations and the levels of reproductive hormones in adolescents aged 13–15.

2. Methods

2.1. Study participants

The study participants were from the entire control cohort of the Genetics and Biomarkers study for Childhood Asthma (GBCA) in Taiwan. This sample of 225 healthy adolescents (102 boys and 124 girls aged 13–15 years during 2009–2010), was selected from seven public schools in the Taipei City of Northern Taiwan (Zeng et al., 2015). Each school contributed a population of children who had no personal or family history of asthma. The response rate was 72%, and the age of participants ranged from 11.9 to 15.1 years (mean ± SD, 13.6 ± 0.7 years). After receiving written informed consent from the adolescents and their parents, the participants were surveyed to collect information concerning demographics and environmental exposures. Information regarding the smoking status and smoking history of each participant's adult household members and adult visitors was collected. Serum samples were collected for each child after 8 h of fasting. A trained technician measured the height, weight, waist circumference and blood pressure of each child. The study protocol was approved by the Institutional Review Board of the National Taiwan University Hospital Research Ethics Committee and was in compliance with the principles outlined in the Helsinki Declaration (Declaration of Helsinki 1990).

2.2. Covariates

Information regarding demographic characteristics such as age, sex, parental education, environmental tobacco smoke (ETS) exposure and exercise were collected via a self-reported questionnaire. ETS information was collected from the current and past household smoking status of each participant's adult household members and regular household visitors. Regular exercise was defined as 'yes' if the participant has exercised at least 1 h per day in the past year excluding physical education in the school, and 'no' if they have not. Trained study staff recorded the weight and height of the participants and calculated the body mass index (BMI; weight in kg per height in m^2).

2.3. Serum reproductive hormone determination and serum PFAS measurements

Clinical laboratory tests were performed at an accredited clinical diagnostic laboratory. The primary outcome of interest was serum reproductive hormone levels including testosterone and estradiol. Serum was extracted from red blood cells, stored in tubes, and chilled prior to being shipped to an analytical laboratory. We measured reproductive hormones in serum by immunoluminometric assay with an Architect random access assay system (Abbott Diagnostics, AbbottPark, IL). The limit of detection (LOD) for estradiol and testosterone were 18 pmol/L and 0.23 nmol/L, respectively. Volumes used for analyses were 50 μL for testosterone and estradiol. All the measurements were duplicated and the average of the two values was calculated and used as the concentrations of each sample. The intra-assay coefficients of variation of these measurements were all below 10%, and the inter-assay coefficients of variation were all below 15%. A total of 67% of the blood samples were drawn before 12:00 h, and 33% between 12:00 and 17:00 h.

PFASs were measured from 0.5 mL of serum using Agilent high-performance liquid chromatography (HPLC) in tandem with an Agilent 6410 Triple Quadrupole (QQQ) mass spectrometer (MS/MS) (Agilent, Palo Alto and Santa Clara, CA). Detailed information about standards and reagents, sample preparation and extraction, instrumental analysis, quality assurance and quality control, and recovery experiments in the present study is provided in Supplementary material and is described elsewhere (Hansen et al., 2001). Ten PFASs were analyzed in serum samples: perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), PFOS, perfluorohexanoicacid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorododecanoic acid (PFDoA), and perfluorotetradecanoic acid (PFTA). The limit of quantification (LOQ) for PFOS, PFOA and PFNA was 0.03 ng/mL, for PFBS and PFHxS was 0.07 ng/mL, for PFDA and PFDoA was 0.1 ng/mL, for PFHpA and PFHxA was 0.05 ng/mL, and for PFTA was 0.02 ng/mL. All the measurements were duplicated and the average of the two values was calculated and used as the concentrations of each sample. For statistical tests, concentrations of PFASs below the LOQ were assigned a value equal to the LOQ divided by the square root of 2. Nearly half serum PFHpA levels were below the LOQ, therefore PFHpA was not included in the present statistical analyses.

2.4. Statistical analysis

Statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA). Data were tested for normality (Q-Q plots) and homogeneity (Bartlett's test for unequal variances). Appropriate transformations were made as needed. Continuous variables with acceptable normality and homogeneity were given as the mean ± SD, otherwise, they were given as median and quartile 1(Q1)–quartile 3(Q3). The *t*-test was used to compare the normal

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