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Dietary predictors and plasma concentrations of perfluorinated alkyl acids in a Singapore population

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HIGHLIGHTS

- 13 PFAAs in 270 plasma samples from Singapore Prospective Study Program were analyzed.
- Intakes of seafood and meat were associated with increased PFAAs, whereas grains and soy products had inverse associations.
- Plasma Omega-3 fatty acids were correlated with various long-chain PFAAs.

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ABSTRACT

Perfluorinated alkyl acids (PFAAs), a family of man-made organofluorinated compounds, have drawn much attention due to their ubiquitous existence in the environment and their bioaccumulation potential. Here, we examined the plasma concentrations of thirteen PFAAs in a healthy population (N = 270) in Singapore, and investigated the association between major food groups and plasma PFAA concentrations. We detected eight types of PFAAs in more than 75% of all samples (N = 270), and their median concentrations ranged from 0.05 to 8.34 ng mL^{-1}. Age- and gender-related differences were observed for the three dominant PFAAs, i.e., perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonate (PFHxS) and perfluorooctanoate acid (PFOA), with concentrations being higher in men and older adults. Multiple linear regression analyses showed that fish, shellfish, red meat and poultry were associated with increased PFAAs concentrations in plasma, whereas grains and soy products showed inverse associations with PFAAs. Further, significant correlations were observed between various long-chain PFAAs and plasma concentrations of omega-3 fatty acids, suggesting seafood was a significant source of these PFAAs, within this population. Future studies on diet exposure to PFAAs are encouraged to focus more on the effects on diet pattern.

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1. Introduction

Perfluorinated alkyl acids (PFAAs) are a family of man-made organofluorine compounds that consist of a carbon backbone in various lengths and a charged functional moiety, which confer these substances with certain properties such as thermal and chemical stability, amphiphilicity and surfactant properties. With these desirable properties, they have a wide range of usage in industrial and consumer products. On the other hand, their high stability has led to the persistence of PFAAs in the environment, as well as in wildlife and humans. Certain long-chain PFAAs can stay in the human body for years with estimated half-lives up to 4–8 years (Olsen et al., 2007).

Extensive amount of biomonitoring data on human blood concentrations of PFAAs have shown their ubiquitousness in humans worldwide. There has been substantial studies demonstrating various toxicities of these compounds to mammalians, such as liver toxicity, disruption of lipids metabolism, reproductive toxicity, developmental toxicity, endocrine toxicity, and immunotoxicity (Lau et al., 2007; Stahl et al., 2011). Some of these effects are supported by many epidemiological studies where significant or

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suggestive associations were observed between certain PFAAs and adverse outcomes, including high cholesterol (Nelson et al., 2010) (Steenland et al., 2009), thyroid hormone disruption (Wen et al., 2013; Lewis et al., 2015), hyperuricemia (Steenland et al., 2010), elevated homocysteine and hypertension (Min et al., 2012), lower birth weight and size (Fei et al., 2007), although these evidence remains limited.

Although PFAAs have been widely detected in various environment and biota media, the sources of human exposure are not fully clear. Suggested pathways include air, household dust, drinking water, diet and food packaging (Stahl et al., 2011). Amongst these, diet is regarded as an important source based on studies that measured food composite samples from the UK (FSA, 2006), Spain (Ericson et al., 2008), and Canada (Tittlemier et al., 2006, 2007). Determination of the correlations between self-reported food intake and plasma/serum concentrations of PFAAs also suggests that foodstuffs are significant determinants of human exposure to PFAAs (Falandysz et al., 2006; Halldorsson et al., 2008; Rylander et al., 2010). However, they only covered a very limited geographical regions and information from Asia with different dietary habits is generally lacking.

Singapore is not an agricultural country, which imports over 95% of the foods from different parts of the world. A recent study reported that PFAAs was detected at low ng L^{-1} levels in the rivers and reservoirs in Singapore, however, the levels were negligible in drinking water (Nguyen et al., 2012). The objective of the present study was thus to investigate whether dietary intake is an important source of PFAAs exposure in the general population of Singapore. In here, the profiles and concentrations of PFAAs in plasma of a healthy Singapore population were assessed to identify the significant food predictors among major food groups, if any, and if dietary preference (high seafood, high meat, low grain) could affect the exposure. To further investigate the potential dietary influence, correlations between plasma fatty acids and concentrations of PFAAs were also evaluated.

2. Materials and methods

2.1. Study population

The participants in our study were part of the Singapore Prospective Study Program (SP2), a population-based study conducted between 2004 and 2007 in a multiethnic population (Chinese, Malay and Indian) in Singapore. Detailed population selection and methodology for SP2 have been described in previous studies (Wu et al., 2009; Chiang et al., 2011). Investigators administered a life style and a detailed Food Frequency Questionnaire (FFQ) at the participants' homes. 7744 participants completed the questionnaire, from a total of 10747 participants. Following this, all participants were invited to attend a health examination for additional tests and collection of blood samples, of which 5163 completed.

Only ethnic Chinese were selected for this study as they make up over 70% of the resident Singapore population and generally do not have strong dietary restrictions due to religion or cultural believes. After restricting the scope of our study to Chinese participants, we also excluded participants who were more than 80 years old, current smokers and drinkers as smoking or drinking might be a confounding factor to PFAAs exposures, and those who had missing data on age or BMI. Due to current constraints in funding, we randomly selected 300 persons within strata of high fish consumption, high meat consumption, and low fish and meat consumption from the final remained participants (N = 2458) for the current analysis. However, 30 participants had insufficient plasma samples for measurements, resulting in a total number of 270 participants.

Venous blood was drawn and collected in plain and fluoride oxalate tubes and was stored at 4 °C before processing. The maximum time of storage prior to processing was 4 h (Nang et al., 2009). After centrifugation, the supernatant, designated plasma was separated and transferred to a clean tube. Thereafter, the plasma samples were stored at −80 °C.

Ethics approval was obtained from the institutional review board of the National University of Singapore. Informed consent was obtained from all participants before the study was conducted.

2.2. Dietary data assessment

A trained interviewer ascertained dietary information of the subjects over the past one month via the administration of the semi-quantitative FFQ at the subjects' home. 159 items were specified in the questionnaire. For each of these food items, the respondent was asked to report intake as frequency per day, per week, per month, or rarely/never, and they were instructed about portion sizes expressed in terms of cups, tubs, and slices, and appropriate measuring utensils were supplied. Dietary intake from the FFQ was converted to grams per day (g d^{-1}) by multiplying self-reported frequency of intake by portion size. The FFQ was validated against three 24-h recalls and selected biomarker studies (Deurenberg-Yap et al., 2000). The correlation coefficients for energy or nutrient intakes assessed with the FFQ and 24-h recalls ranged from 0.46 to 0.58, which suggests that the FFQ reflected the participants' diet reasonably well (Deurenberg-Yap et al., 2000).

2.3. Analysis of PFAAs and quality assurance

2.3.1. Chemicals

Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), perfluoro-n-octanoic acid (PFOA), perfluoro-n-nonanoic acid(PFNA), perfluoro-n-decanoic acid (PFDA), perfluoro-n-undecanoic acid (PFUnDA), perfluoro-n-dodecanoic acid(PFDoDA), perfluoro-n-tridecanoic acid (PFTrDA), perfluoro-n-tetradecanoic acid (PFTeDA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS) were purchased from Merck Schuchardt (Hohenbrunn, Germany), and the purities were all above 96%. Perfluoron-[1,2,3,4−$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2−$^{13}C_2$]hexanoic acid (MPFHxA), perfluoro-n-[1,2,3,4,5−$^{13}C_5$]nonanoic acid (MPFNA), perfluoro-n-[1,2−$^{13}C_2$] decanoic acid(MPFDA), perfluoro-n-[1,2−$^{13}C_2$]dodecanoicacid (MPFDoDA), sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate (MPFHxS) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Perfluoro-n-[1,2,3,4−$^{13}C_4$]octanoic acid (MPFOA), and sodium perfluoro-1-[1,2,3,4,5,6,7,8−$^{13}C_4$]octanesulfonate (MPFOS) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA), with purities above 98%. All these compounds were used as standards.

2.3.2. Sample preparation

The plasma samples preparation procedure was similar to the method described by Hansen et al. (2001), with some minor modifications. Briefly, 0.2 mL of plasma, 20 μL of mixture of eight labeled internal standards (10 μg mL^{-1}), 1 mL of Na_2CO_3 (0.25 M), and 0.5 mL of tetra butyl ammonium hydrogen sulfate (TBAHs, 0.5 M) were added to a 15 mL polypropylene(PP) tube for extraction and mixed well. Methyl *tert*-butyl ether (MTBE, 3 mL) was then added to the solution, and the mixture was vortex for 1 min. The organic and aqueous layers were separated by centrifugation (Thermo Scientific, Waltham, MA, USA) at 3000 r min^{-1} for 6 min, and the organic layer was transferred to a second polypropylene tube. The aqueous layer was again extracted twice with MTBE. The

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