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Determinants of serum levels of perfluorinated alkyl acids in Danish pregnant women

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ABSTRACT

Humans are exposed to perfluorinated alkyl acids (PFAAs) from food, drinking water, air, dust, and consumer products. PFAAs are persistent and bio-accumulative. In the present study, we aimed to establish how the serum levels of PFAAs differ according to age, pre-pregnancy body mass index (BMI), previous miscarriages, educational level, country of birth, smoking, and alcohol intake. We included 1438 Danish pregnant nulliparous women from the Aarhus Birth Cohort. The women gave a blood serum sample between week 11 and 13 of pregnancy. Sixteen PFAAs were extracted from serum using solid phase extraction and analyzed by liquid chromatography/tandem mass spectrometry. Multivariable linear regression analysis was used to determine the associations between individual characteristics of the women and their levels of seven PFAAs that were detected in at least 50% of the samples.

The total concentration of the PFAAs ($\sum$PFAA) was higher in older women. On average, normal weight women had a higher $\sum$PFAA level than underweight, overweight, and obese women. Higher levels were also observed for women without previous miscarriages, women with a high educational level, women born in Denmark (as opposed to women born elsewhere but currently living in Denmark), non-smokers, and women who consumed alcohol before or during pregnancy. These associations were similar for all the studied PFAAs, although the levels of perfluoroundecanoic acid varied more across the categories of age, BMI, education, smoking, and alcohol consumption than any other PFAAs measured.

1. Introduction

Perfluorinated alkyl acids (PFAAs) are anthropogenic compounds which have been used as surfactants for more than sixty years (Schultz et al., 2003). Humans are exposed to PFAAs and their precursors directly from consumer products (e.g. impregnated carpets, furniture, clothing, and food packaging) (D'Eon et al., 2009; Guo et al., 2009; Trier et al., 2011; Vestergren and Cousins, 2009), or from contaminated food, drinking water, air, or dust (Bjorklund et al., 2009; Haug et al., 2011; Holzer et al., 2008; Wilhelm et al., 2015). Food items such as fish, meat (including beef, pork, lamb, and organ meat), milk, potatoes, snacks, and eggs are major sources of PFAA exposure for non-occupationally exposed individuals (Bjermo et al., 2013; Eriksen et al., 2011; Halldorsson et al., 2008; Haug et al., 2011; Long et al., 2015; Vestergren and Cousins, 2009).

Long-chain PFAAs, including perfluorinated sulfonic acids (PFSAs) with six or more carbon atoms and perfluorinated carboxylic acids (PFCAs) with eight or more carbon atoms, are very persistent and bio-accumulative (Bjerregaard-Olesen et al., 2016). The production and use of many long-chain PFAAs, includ-

Abbreviations: BMI, body mass index; IQR, interquartile range; LOQ, limit of quantification; PFAA, perfluorinated alkyl acids; PFBS, perfluorobutane sulfonate; PFCA, perfluorinated carboxylic acid; PFDA, perfluorodecanoate; PFDoA, perfluorododecanoate; PFDS, perfluorodecane sulfonate; PFHpA, perfluoroheptanoate; PFHpS, perfluoroheptane sulfonate; PFHxA, perfluorohexanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, Perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFOSA, perfluorooctane sulfonamide; PFPeA, perfluoropentanoate; PFSA, perfluorinated sulfonic acid; PFUnA, perfluoroundecanoate; PFTrA, perfluorotridecanoate; PFTeA, perfluorotetradecanoate.

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ing perfluorooctane sulfonate (PFOS, C8) and perfluorooctanoic acid (PFOA, C8), are being phased out in the Western countries, whereas manufacturers from other countries, including China, have increased their production (Land et al., 2015). In our recent study involving 1533 Danish pregnant nulliparous women, we found that the levels of the seven most abundant PFAAs in serum decreased with between 6 and 15% per year between 2008 and 2013 (Bjerregaard-Olesen et al., 2016).

Most studies have suggested that PFAA concentrations decrease during pregnancy (Glynn et al., 2012; Jain 2013) and with parity (Berg et al., 2014; Brantsaeter et al., 2013; Fei et al., 2007), whereas the relation with other characteristics of the pregnancy and the pregnant woman is less well described. There have been reports of associations between PFAA levels and age (Berg et al., 2014; Bjermo et al., 2013; Cho et al., 2015; Fei et al., 2007; Gribble et al., 2015; Jain 2013; Kristensen et al., 2013; Long et al., 2012; Olsen et al., 2005), BMI (Brantsaeter et al., 2013; Fei et al., 2007; Ji et al., 2012), educational level (Bjermo et al., 2013; Brantsaeter et al., 2013), smoking (Cho et al., 2015; Eriksen et al., 2011; Fei et al., 2007), race (Calafat et al., 2007; Kato et al., 1999), and alcohol intake (Bjermo et al., 2013; Eriksen et al., 2011), but the results were inconsistent, even with regards to the direction of the associations.

In the present study, we investigated the associations between the levels of the most abundant PFAAs and characteristics of Danish pregnant nulliparous women, their lifestyle, and their pregnancy.

2. Materials and methods

2.1. Participants and samples

The study included women from the Aarhus Birth Cohort who gave birth at Aarhus University Hospital, Aarhus, Denmark. Information about the Aarhus Birth Cohort and Biobank, a questionnaire, a consent form, and a postage-paid envelope for response were sent to the pregnant women in early pregnancy (Mortensen et al., 2013). Depending on the availability of lab technicians, between 45% and 48% of the women planning to give birth at Aarhus University Hospital were included in the Aarhus Birth Cohort Biobank (Mortensen et al., 2013). The blood samples were processed within 2 h after blood draw and stored at −80° C (Mortensen et al., 2013).

In the present study, we included women who joined the Aarhus Birth Cohort between 2008 and 2013 and 1) were nulliparous, as the PFAA levels have been shown to be affected by reproductive history (i.e. parity, time since most recent pregnancy, and recent breastfeeding duration) (Brantsaeter et al., 2013); 2) donated a blood sample in the first trimester of pregnancy, as PFAA concentrations have been shown to decrease during pregnancy (Fei et al., 2007; Glynn et al., 2012; Jain, 2013); and 3) filled out a questionnaire with information about height, pre-pregnancy weight, previous miscarriages, educational level, country of birth, smoking, alcohol consumption etc. In total, 1533 women were selected for the study (Table 1) by random sampling from 2853 women who fulfilled the inclusion criteria during the investigated time period (Bach et al., 2015a). Ninety-five women were excluded due to missing data on the included variables. Thus, the analyses included a total of 1438 women.

All participants provided written consent to the storing of their blood samples in the biobank, and further consented that the samples and information could be used for approved research. The study was approved by the Danish National Committee on Health Research Ethics (reference M-20110054) and the Danish Data Protection Agency (reference 2011-41-6014). Data from the Aarhus Birth Cohort of pregnant women were previously used to evaluate the associations between PFAA levels and time to pregnancy (Bach et al., 2015b) and indices of fetal growth (Bach et al., 2015a) as well as the time trends between 2008 and 2013 (Bjerregaard-Olesen et al., 2016).

2.2. Chemical analysis

The quantitative chemical analysis was conducted at the Department of Environmental Science, Aarhus University. We analyzed 16 PFAAs (perfluorobutane sulfonate [PFBS], perfluorohexane sulfonate [PFHxS], perfluoroheptane sulfonate [PFHpS], PFOS, perfluorodecane sulfonate [PFDS], perfluorooctane sulfonamide [PFOSA], perfluoropentanoic acid [PFPeA], perfluorohexanoic acid [PFHxA], perfluoroheptanoic acid [PFHpA], PFOA, perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], perfluoroundecanoic acid [PFUnA], perfluorododecanoic acid [PFDoA], perfluorotridecanoic acid [PFTrA], and perfluorotetradecanoic acid [PFTeA]) using a method based on solid phase extraction and liquid chromatography/tandem mass spectrometry. The analytical performance specifications and details of the method were given elsewhere (Bach et al., 2015c; Bjerregaard-Olesen et al., 2016).

2.3. Statistics

For the samples with PFAA concentrations below the limit of quantification (LOQ), we replaced the concentrations by the LOQ/2. Only the PFAAs with levels above the LOQ for at least 50% of the samples were included in the statistical analyses. In addition to providing data on the single compounds, the analyses were also performed for the summed concentration of three groups of PFAAs: 1) the PFAAs with a sulfonic acid or sulfonamide functional group (i.e. the PFSAs) 2) the PFAAs with a carboxylic acid functional group (i.e. the PFCAs) and 3) all the PFAAs as follows:

$$\Sigma PFSA = c(PFBS) + c(PFHxS) + c(PFOS) + c(PFDS) + c(PFOSA)$$

$$\Sigma PFCA = c(PFPeA) + c(PFHxA) + c(PFHpA) + c(PFOA) + c(PFNA) + c(PFDA) + c(PFUnA) + c(PFDoA) + c(PFTrA) + c(PFTeA)$$

$$\Sigma PFAA = \Sigma PFCA + \Sigma PFSA$$

. . .where c is the serum level (ng/mL) of the PFAA presented in the parenthesis. The PFAA levels were natural log-transformed to approximate a normal distribution.

The variables analyzed in relation to PFAA serum levels during pregnancy were: age at delivery (≤25; 26–29; and ≥30 years), pre-pregnancy BMI (<18.5; 18.5-24.9; 25–30; >30 kg/m^2), previous miscarriages (0; 1; 2; ≥3 miscarriages), educational level (*low:* municipal primary and lower secondary school; *lower middle:* upper secondary school or 1–2 years of vocational training; *higher middle:* additional 3–4 years of education; *high:* >4 years of additional education), the pregnant women's country of birth (Denmark; other Europe; rest of the world), smoking status (non-smoker; smoker until pregnancy; smoker during pregnancy), and drinking habits (no alcohol intake; intake only before pregnancy; intake during pregnancy).

We calculated relative PFAA levels for each category of the included variables compared to the reference groups by back-exponentiation from the linear parameter estimates (β) using multivariable linear regression analyses. *P*-values for the linear associations (trends) across the categories were estimated for those variables where there was a distinct order across the categories (i.e. all except country of birth). We alternately treated each of the variables as the independent (exposure) variable. Previous studies have reported that PFAA levels vary across categories of age (Bjermo et al., 2013; Brantsaeter et al., 2013; Fei et al., 2007), BMI (Brantsaeter et al., 2013; Fei et al., 2007), reproductive history (Berg et al., 2014; Brantsaeter et al., 2013; Fei et al., 2007),