Reproductive Toxicology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort

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ARTICLE INFO

Article history:
Received 27 August 2016
Accepted 30 August 2016
Available online xxx

Keywords:
Perfluorinated compounds
Endocrine disruption
Reproduction
Anogenital distance
Birth weight
Gestational length

ABSTRACT

In the Odense child cohort, serum concentrations of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) were measured in 638 pregnant women. Birth weight, head and abdominal circumferences and gestational age were determined. Anogenital distance (AGD), the distance from the anus to the genital organs, and penile width were measured 3 months after expected date of birth in 511 children. PFOS, PFHxS, PFNA and PFDA were associated with a decreased AGD in girls (p-trend < 0.05) after adjusting for age and weight-for-age standard deviation score. PFOS in the highest quartile was associated with a 2.8 mm (95% confidence intervals −4.5; −1.1) reduction in AGD in girls. No such tendencies were seen in boys. However, a tendency toward increased birth weight in girls and reduced birth weight in boys suggests that sex-dimorphic effects may occur from endocrine disrupting effects of these substances.

1. Introduction

Perfluoroalkyl substances (PFASs) are synthetic chemicals produced in high quantities [25]. Their unique properties of high stability and low surface tension make them grease and water repellant and therefore suitable for use in a wide range of consumer products like food packaging, textiles, outdoor clothes, footwear and carpets; they are also used in paints, lubricants, waxes and in firefighting foams [25,30]. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) have been most widely used; they are persistent in the environment and bio-accumulate [25]. They can be detected in human serum in Western populations, and they cross the placenta [25,29]. PFASs have a long elimination half-life –PFOS, 5.4 years and PFOA, 3.8 years [31]. In recent years, the use and production of PFOA and PFOS have been phased out due to the growing knowledge about their adverse health effects [25]. However, they have been replaced with the shorter and longer chained PFASs (e.g perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA)) [14] whose adverse effects have been studied less extensively.

Among other important adverse effects, PFASs may have endocrine disrupting properties [24]. PFASs influence the expression of estrogen-responsive genes in animal studies [3], and PFAS-induced changes in sex hormone biosynthesis have been reported in vitro [9]. PFASs have been shown to interfere with the estrogen receptor in human in vitro studies [22]. PFAS exposure has been associated with fetal growth, and a recent review found that maternal exposure to PFOA and PFOS was associated with low birth weight, although the results were equivocal, perhaps due to differences in exposure and sex-dimorphic effects [2].

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http://dx.doi.org/10.1016/j.reprotox.2016.08.019
0890-6238/

Anogenital distance (AGD; distance from anus to genitals) is routinely used in animal toxicology studies and is sensitive to anti-androgenic exposure. In rodents AGD has been shown to reflect the amount of androgen to which a male fetus is exposed in early development; males have longer AGD than females, and higher *in utero* androgen exposure results in longer AGD [38]. Numerous studies have shown that prenatal phthalate exposure shortens male AGD in rodents and humans [13,20]. High prenatal exposure to testosterone has also been associated with longer AGD in female rodent offspring, suggesting masculinization effects, and the changes were found to be indicators of permanent alteration in AGD in the adult female rodent [40]. To our knowledge no human investigations have studied the effects of maternal PFAS exposure on AGD in the offspring, but PFASs exposure in wild male mink has been associated with shorter AGD [33].

We therefore investigated the association between maternal PFAS exposure, including the less studied perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA) and perfluorohexane sulfonic acid (PFHxS), and birth outcomes (birth weight, head and abdominal circumference and gestational length) and AGD at three months of age.

2. Materials and methods

2.1. Study population

This study is based on data from the ongoing Odense Child Cohort (OCC). All women residing in the Municipality of Odense in the Region of Southern Denmark, who were pregnant between January 2010 and December 2012, were invited to participate. The pregnant women were recruited at a voluntary information meeting introducing the ultrasound examinations at Odense University Hospital (OUH) or at their first antenatal visit at gestational week 8–16. Serum samples were collected at recruitment and stored in freezers at the Odense Patient data Explorative Network (OPEN). A total of 6707 pregnant women were eligible, 4017 were approached, and 2874 (42.9%) were successfully enrolled in the cohort with an informed consent form. The pregnant women filled in a questionnaire about general health and lifestyle. Participants were better educated, smoked less and were more often of Danish origin than non-participants [23]. As of November 2014, 2448 singleton children were active members of the cohort.

2.2. Birth outcome, AGD and covariates

We obtained information from birth records about parity, maternal smoking, maternal pre-pregnancy BMI, gestational age (days) at birth and birth measures such as birth weight (grams), head circumference (cm), and abdominal circumference (cm). Midwives assessed birth size right after parturition. Maternal ethnicity and education were obtained from a questionnaire completed in early pregnancy.

Three months after expected date of birth (median age 3.5 months, range 2.1–6.8 months) the children were invited to a clinical examination including measurements of length, weight and AGD. In boys, a short AGD was measured with a Vernier caliper from the center of the anus to the posterior base of the scrotum (AGD_{as}) and a long AGD was measured from the center of the anus to the cephalad insertion of the penis (AGD_{ap}). Penile width was also measured. Correspondingly, in girls a short AGD was measured from the center of the anus to the posterior fourchette (AGD_{af}) and a long AGD from the center of the anus to the top of the clitoris (AGD_{ac}). The genital measures were performed blindly and repeated three times in each child, and an arithmetic mean was calculated. All technicians attended training sessions and supervision. AGD measurements for the first 46 girls were excluded due to low accuracy. The coefficient of variation (CV) for AGDs was below 10% (3% on average for AGD_{as}, 2% for AGD_{ap}, 4% for AGD_{af} and 3% for AGD_{ac}) for all the triplicate AGD measurements, except for AGD_{af}, in which two girls had CVs of 0.10 and 0.14, respectively.

2.3. PFAS measurements

PFASs were measured in serum samples obtained at gestational week 5–12 (median 10 weeks) from a subset of 649 pregnant women. The first 200 samples were selected at random among women recruited in 2010, whereas an additional 449 were selected in 2011–2012 among those women who had available information from questionnaires, birth records and a clinical 3-month examination of the child. Three mothers from 2010 did not satisfy the latter criteria, and a total 638 children were therefore eligible for analyses of associations between birth outcomes and PFASs. The analysis of serum PFAS concentrations was performed by online solid phase extraction followed by liquid chromatography and triple quadropole mass spectrometry (LC–MS/MS). The quantified PFASs are comprised of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) [16]. The Limit of Quantification (LOQ) was 0.03 ng/mL for all the reported compounds. Non-detectable concentrations were found only in a small number of samples and were replaced with half of the limit of quantification LOQ (i.e. 0.015 ng/mL) United States Environmental Protection Agency, 2000. Results from the first 200 samples and further descriptions of PFAS measurements have been reported elsewhere [37].

2.4. Ethics

The study complied with the Declaration of Helsinki and was approved by the health research ethics committee system in Denmark (S-20090130) and the Danish Data Protection Agency (j.no. 2008-58-0035).

2.5. Statistics

PFASs distributions were skewed and were therefore divided into quartiles, but also entered as continuous variables after transformation by the natural logarithm to approach normality. Differences in serum-PFAS concentrations according to characteristics of the pregnant women were first tested by Kruskal Wallis test.

Multiple linear regression analysis was used to analyze the associations between maternal PFASs exposure and birth outcomes (birth weight, head and abdominal circumference and gestational age), AGD and penile width at 3-months of age adjusted for potential confounders. We tested for linear trends across PFASs quartiles in regression models by means of ordinal PFASs quartiles using integer values from 1 to 4. Confounders included in multivariable models were factors known a priori to be important predictors of birth outcomes or AGD. The AGD measurements, penile width and birth outcomes were left untransformed due to acceptable normal distributions of the residuals. The analyses of birth outcomes were adjusted for gestational age (days), parity (primiparous/multiparous), maternal smoking during pregnancy (yes/no), pre-pregnancy BMI (<20, 20–25, 25+ kg/m2), maternal ethnicity (mother or maternal parents born in Western countries (yes/no)), maternal education separated into primary (minimum 9th grade), secondary (minimum high school or equivalent), and tertiary (minimum bachelor degree or equivalent). Maternal education and ethnicity were omitted from the final model, as they were

Please cite this article in press as: D.V. Lind, et al., Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort, Reprod Toxicol (2016), http://dx.doi.org/10.1016/j.reprotox.2016.08.019