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Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy

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ABSTRACT

Background: Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have consistently been associated with higher cholesterol levels in cross sectional studies. Concerns have, however, been raised about potential confounding by diet and clinical relevance.
Objective: To examine the association between concentrations of PFOS and PFOA and total cholesterol in serum during pregnancy taking into considerations confounding by diet.
Methods: 854 Danish women who gave birth in 1988–89 and provided a blood sample and reported their diet in week 30 of gestation.
Results: Mean serum PFOS, PFOA and total cholesterol concentrations were 22.3 ng/mL, 4.1 ng/mL and 7.3 mmol/L, respectively. Maternal diet was a significant predictor of serum PFOS and PFOA concentrations. In particular intake of meat and meat products was positively associated while intake of vegetables was inversely associated (P for trend <0.01) with relative difference between the highest and lowest quartile in PFOS and PFOA concentrations ranging between 6% and 25% of mean values. After adjustment for dietary factors both PFOA and PFOS were positively and similarly associated with serum cholesterol (P for trend ≤ 0.01). For example, the mean increase in serum cholesterol was 0.39 mmol/L (95%CI: 0.09, 0.68) when comparing women in the highest to lowest quintile of PFOA concentrations. In comparison the mean increase in serum cholesterol was 0.61 mmol/L (95%CI: 0.17, 1.05) when comparing women in the highest to lowest quintile of saturated fat intake.
Conclusion: In this study associations between PFOS and PFOA with serum cholesterol appeared unrelated to dietary intake and were similar in magnitude as the associations between saturated fat intake and serum cholesterol.

1. Introduction

Perfluoroalkyl acids (PFAAs) are synthetic chemicals consisting of a fully fluorinated alkyl chain of varying length with a functional group attached. These compounds do not occur naturally in the environment but because of their unique properties to repel both oil and water they have been synthesized and used in a variety of consumer products resulting in wide spread environmental presence. Dietary exposures, possibly through indirect contamination from food packaging materials, have been suggested to be the dominant source of PFAAs exposure in humans (D'eon and Mabury, 2011; D'Hollander et al., 2010; Fromme et al., 2009). Of these compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are found in highest concentrations in humans and because of their persistent nature and potential toxicity, these compounds may influence human health (Grandjean et al., 2012; Halldorsson et al., 2012; Lau et al., 2007; Vested et al., 2013).

Cross sectional studies have relatively consistently observed a positive association between serum concentrations of PFOS and

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PFOA and total cholesterol. These studies include both occupationally exposed workers, subjects from communities with high exposure levels (Costa et al., 2009; Frisbee et al., 2010; Olsen et al., 2003; Sakr et al., 2007a, 2007b; Steenland et al., 2009) and the general population (Eriksen et al., 2013; Nelson et al., 2010) including pregnant women (Jain, 2013; Starling et al., 2014). However, several studies have reported no association (Emmett et al., 2006; Olsen et al., 2000, 2012). Given the observational nature of these studies it remains unclear whether these relations may be causal or driven by other factors. Diet is one obvious candidate as diet may influence both serum cholesterol and PFAA concentrations (Halldorsson et al., 2008; Lichtenstein et al., 1999). Although some studies have accounted for dietary fat intake (Eriksen et al., 2013; Nelson et al., 2010) the importance of this adjustment is usually not reported and other dietary factors than fat are rarely accounted for. Furthermore the strength of the association between PFAAs and serum cholesterol varies considerably between studies and the overall clinical significance in relation to more established risk factors such as age and BMI has been disputed (Kerger et al., 2011). Thus, the aim of this study was to examine in a cohort of 854 pregnant women associations between serum concentrations of PFAAs and total cholesterol with particular focus on the influence of diet; and to compare the effect estimates for these associations with other known risk factors such as high saturated fat intake.

2. Methods

2.1. Study population

In Aarhus Denmark, 965 women with singleton pregnancies were recruited into a birth cohort between April 1988 and January 1989. The study has been described in detail elsewhere (Olsen et al., 1995). In brief, the women were attending the same antenatal center covering a geographically well-defined area of the city. At a routine midwife visit in gestational week 30 an interview was conducted which covered medical history, anthropometry, diet and lifestyle as well as socioeconomic factors. Blood samples were also taken and immediately separated into serum, plasma, and erythrocytes and frozen at −20 °C. Information on maternal health and pregnancy outcomes were extracted from hospital records and the Danish Medical Birth Registry. The study was approved by the Danish Data Protection Agency and the Danish Council of Ethics (Reference no. 20070157), and all participants gave written consent before inclusion in the study.

2.2. Cohort attrition

Of the 965 pregnant women participating in the study, blood samples were available for 873 women. Among these women, information on diet was missing for 19 women resulting in 854 being available for analyses (88.5% of the original cohort).

2.3. Exposure variables

Diet was assessed by a self-administered semi-quantitative food frequency questionnaire mailed to the women prior to the midwife visit in gestation week 30 combined with the face-to-face interview on dietary habits during the antanatal visit. The questionnaire focused on intake (frequency and estimated portion sizes) of easily quantified food items consumed during the previous three months. In the interview the women were asked systematically about various other less frequently consumed food items covering all main food groups using photographs to assess portion sizes. Food and nutrient intake was then quantified by combining the dietary information collected with both standard recipes and the national food composition database (www.foodcomp.dk).

2.4. Biochemical measurements

Serum concentrations of PFAAs were measured at the Department of Analytical Chemistry at the Norwegian Institute of Public Health in Oslo. Analytical procedures have been described elsewhere (Haug et al., 2009). Limit of quantification (LOQ) for the 19 PFAAs analyzed ranged between 0.05 and 0.20 ng/mL serum. For quantification of PFOS the total area of the linear and branched isomers was integrated. Of the 19 PFAAs analyzed 10 were below LOQ for more than 70% of the samples and those compounds were excluded from our analyzes. PFOS and PFOA were above LOQ in all samples (0.05 ng/mL for both compounds). Quality of the analytical procedure was monitored by analyzing in-house quality control samples as well as human serum samples from an intercomparison exercise (AMAP ring test for persistent organic pollutants in human serum, 2009, round 2 2009). Coefficient of variation was 11% for PFOA and 4.4% for PFOS for the in-house quality control samples. Total serum cholesterol was measured using a standard enzymatic method on a COBAS 8000 (Roche Diagnostics USA).

2.5. Statistical analysis

Associations between maternal diet and lifestyle characteristics and serum concentrations of PFOA, PFOS and cholesterol were evaluated using linear regression analyses. Same procedures were also used for examining the association between maternal PFOS and PFOA concentrations with serum cholesterol. For continuous exposures a test for linear trend (*T*-test) was performed under the null hypothesis that the linear regression coefficient was not significantly different form zero. For dichotomous characteristics an *F*-test (type III) was used to test for differences between groups under the null hypothesis that mean levels across all categories were equal. Visual inspection of model residual suggested that use of untransformed concentrations of PFOS and PFOA was appropriate.

In order to account for potential influence of diet for the association between serum PFOS and PFOA and cholesterol these associations were first explored for different food groups. The food groups selected for analyses were dairy products, vegetables, fruits, meat and meat products, fish and fish products; and cereals and starch products. In terms of amount (grams per day), these groups represent the majority (~80% of weight) of foods consumed in our study population. Some of these food groups have also previously found to be associated with levels of PFOS and PFOA among women from the Danish National Birth Cohort (Halldorsson et al., 2008). In our analyses the food group variables were first examined as continuous in a multivariate linear regression analyses were they were mutually adjusted for each other. For those food group variables that were associated with of PFOS and PFOA concentrations, their associations were examined further by dividing intake into quintiles to account for non-linearity and to evaluate the magnitude of the association in more detail. On a nutrient level we also explored saturated fat intake as potential confounder for the association between PFOS and PFOA with serum cholesterol, as it is a well-established predictor of serum cholesterol (Lichtenstein et al., 1999). The effect estimates for serum PFOS, PFOA, and saturated fat intake in relation to total serum cholesterol were also compared.

Covariates were selected and included in our regression models were based on information provided in previous studies (Brantsaeter et al., 2013; Halldorsson et al., 2008). When examining the association between diet and serum PFOS and PFOA concentration