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Prenatal pesticide exposure associated with glycated haemoglobin and markers of metabolic dysfunction in adolescents

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ABSTRACT

Background: Pesticide exposure has been associated with increased risk of diabetes mellitus in adults, but potential effects of prenatal exposure on glucose regulation have not been investigated.

The aim of this study was to investigate if maternal occupational pesticide exposure in pregnancy was associated with glycated haemoglobin A1c (HbA1c) in adolescents and whether an association was modified by sex and paraoxonase-1 (*PON1*) Q192R polymorphism.

Methods: A prospective cohort study of children whose mothers were either occupationally exposed or unexposed to pesticides in early pregnancy. At age 10-to-16 years, the children (n = 168) underwent clinical examinations including pubertal stage assessment (accepted by 141 children) and blood sampling. *PON1* Q192R genotype was available for 139 children and 103 mothers.

The main outcome measure was HbA1c but other relevant biomarkers were also included.

Results: Prenatal pesticide exposure was associated with a 5.0% (95% confidence interval: 1.8; 8.2) higher HbA1c compared to unexposed children after adjustment for confounders. After stratification, the association remained significant for girls (6.2% (1.6; 11.1)) and if the child or the mother had the *PON1* 192R-allele (6.1% (1.6; 10.8) and 7.1% (2.0; 12.6), respectively). Besides, an exposure-related increase was seen for the leptin-to-adiponectin ratio, for plasminogen activator inhibitor type-1 in girls, and for interleukin-6 in children whose mothers had the R-allele.

Conclusion: Prenatal pesticide exposure was associated with higher HbA1c and changes in related biomarkers in adolescents. Our results suggest an adverse effect on glucose homeostasis and support previous findings from this cohort of an exposure-associated metabolic risk profile with higher susceptibility related to female sex and the *PON1* 192R-allele.

1. Introduction

Exposure to pesticides has been associated with an increased risk of diabetes mellitus (Evangelou et al., 2016). The evidence was strongest for persistent organochlorine pesticides, but occupational exposure to currently used non-persistent pesticides has also been associated with abnormal glucose regulation and increased risk of type 2 diabetes (T2D) in adults (Schreinemachers, 2010; Xiao et al., 2017). In experimental studies, early life exposure to low doses of some commonly used pesticides caused disrupted glucose and lipid homeostasis in adult rats (Hocine et al., 2016; Lassiter et al., 2010; Slotkin, 2011) but the potential impact of prenatal exposure on glucose homeostasis later in life has not been explored in human studies.

To investigate the potential health effects of prenatal pesticide exposure, we have followed a cohort of children whose mothers were employed in greenhouse horticulture during pregnancy. Some of the mothers were occupationally exposed to mixtures of pesticides in the first trimester before the pregnancy was recognized and preventive measures were taken. In this cohort we found an association between maternal pesticide exposure and body fat accumulation from birth to school age (6–11 years of age) in their children (Wohlfahrt-Veje et al., 2011). This association was mainly driven by children with a single nucleotide polymorphism in the paraoxonase-1 gene (*PON1*). Paraoxonase-1 is a high-density lipoprotein (HDL)-associated enzyme with

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antioxidant functions. A common polymorphism in the coding sequence, a glutamine (Q)/arginine (R) substitution at position 192, affects the antioxidant properties (Mackness and Mackness, 2015) and the R-allele has been associated with higher risk for coronary heart disease (Wang et al., 2011). We found that prenatally pesticide exposed children with the R-allele had larger abdominal circumference and higher body fat content, blood pressure, and serum concentrations of selected metabolic markers than unexposed children with the same genotype (Andersen et al., 2012; Jorgensen et al., 2015). The findings on body fat composition were confirmed by dual X-ray absorptiometry (DXA) at adolescence where especially android fat%, but also gynoid and total fat%, were positively associated with prenatal pesticide exposure. These associations were stronger for girls than for boys and also stronger if the child or the mother carried the R-allele (Tinggaard et al., 2016). Thus, our findings indicate disturbance of metabolic pathways in the children, and we therefor hypothesized that glucose regulation would also be impaired, possibly dependent on sex and *PON1* Q192R genotype. Glycated haemoglobin A1c (HbA1c) reflects average blood glucose concentration over the previous eight to 12 weeks (Nathan et al., 2007). To test the hypothesis, we investigated associations between prenatal pesticide exposure and HbA1c in blood samples collected at two clinical examinations at ages 10–15 and 11–16 years. To support our findings, we also measured metabolic and inflammatory biomarkers related to insulin sensitivity.

2. Methods

2.1. Study population

From 1996 to 2000 pregnant women working in greenhouse nurseries were recruited consecutively when they were referred to the Department of Occupational Health at Odense University Hospital, Denmark, for risk assessment of their working conditions and guidance for safe work practices during pregnancy. Their children (N = 203) were first examined at three months of age (Andersen et al., 2008) and then followed up at school age where 44 additional age-matched controls were included (Wohlfahrt-Veje et al., 2011). The present study includes data from two additional follow-up examinations during puberty when the children were between 10 and 16 years of age (Tinggaard et al., 2016).

Details of the study, including recruitment procedure and exposure assessment, have been described previously (Andersen et al., 2008; Wohlfahrt-Veje et al., 2011). Briefly, the mothers were categorized as occupationally exposed or unexposed to pesticides based on detailed information about working conditions for the previous three months obtained from interview at enrollment (gestational weeks 4–10) and supplemented by telephone interview of the employers. All exposure assessments were performed independently by two toxicologists with expertise in working conditions in greenhouse horticultures and completed before the first examination of the children. Women categorized as exposed went on paid leave or were moved to work functions with less or no pesticide exposure shortly after enrollment. Hence, the exposure classification relates to the early weeks of the first trimester before study enrollment. For all women, the main work functions were nursing and handling of plants, which had some times been treated with pesticides. More than 100 different pesticide formulations were used in the greenhouses and the women were often exposed to mixtures of various insecticides, fungicides, and growth regulators.

Information on life-style factors during pregnancy was collected by an interview-assisted questionnaire at the first examination of the child. Socioeconomic status (SES) was grouped into five groups ranked 1(high) to 5 (low) based on parental education and occupation (Hansen, 1978). The group of the highest ranked parent living with the child was used. Information on gestational age at birth, birth weight, and birth length was obtained from obstetric records. Weight-for-gestational age (WGA) was calculated according to Marsal et al. (1996).

Invitations for this study were sent to 243 eligible children/families (one child had died and three families had moved abroad). Overall, 168 children participated in at least one of two follow-up examinations that took place from October 2011 to January 2012 and from March to June 2013.

The study was conducted according to the Helsinki II Declaration with written informed consent by one or both parents as approved by The Regional Scientific Ethical Committees for Southern Denmark (S-20070068) and The Danish Data Protection Agency (1996–1200-154, 2007–41-0956).

2.2. Clinical examination and blood sampling

The clinical examination included anthropometry and assessment of pubertal stage according to Tanner and Marshall as previously described (Tinggaard et al., 2016). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Total body fat percentage (body fat%) was calculated from skinfolds by the Slaughter equation as previously described (Tinggaard et al., 2016). The same paediatrician (JT) performed all clinical examinations blinded to information about maternal pesticide exposure.

Venous blood samples were collected in EDTA-coated and uncoated vials. Erythrocyte fractions separated from EDTA-treated samples and serum separated from uncoated vials were stored at − 80 °C until analysis. Blood samples were obtained from 157 children and from 122 of their mothers. In an attempt to get overnight fasting samples, we tried to schedule one of the two examinations to take place in the morning, either before school start or in weekends. This way, we obtained fasting blood samples from 124 children.

One child with diagnosed type 1 diabetes mellitus was excluded from the study. Since puberty affects insulin resistance and related metabolic biomarkers (Hannon et al., 2006), 15 children who did not accept assessment of pubertal stage were also excluded, thus leaving 141 children with available blood samples.

2.3. Laboratory analyses

2.3.1. PON1 genotyping

DNA was isolated from buffy coats and *PON1* Q192R (rs662) was genotyped by a Taqman-based allelic discrimination assay as previously described (Christiansen et al., 2004). The *PON1* genotype was successfully determined in 122 mothers and 40 additional children for whom the genotype was not determined at previous examinations.

2.3.2. Measurement of HbA1c and metabolic and inflammatory biomarkers

Besides HbA1c, we selected biomarkers identified a priori to be associated with insulin sensitivity (Festa et al., 2002; Finucane et al., 2009; Friedrich et al., 2012) including markers of glucose regulation (insulin, c-peptide, insulin-like growth factor 1 (IGF-1)), adipocyte function (leptin, adiponectin), inflammation (interleukin 6 (IL6), tumour necrosis factor alpha (TNFα), high-sensitivity C-reactive protein (hs-CRP), plasminogen activator inhibitor type-1 (PAI-1)), and dyslipidemia (total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TG)).

HbA1c was analysed in the erythrocyte fraction using high performance liquid chromatography (HPLC) (TOSOH G8) with inter-assay CV below 5%. All other biomarkers were analysed in serum. Insulin, c-peptide, leptin, adiponectin, TNFα, and IL6 were determined by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, R&D Systems, Minn., USA) and PAI-1 by an ELISA kit (Invitrogen from Thermo Fisher, Carlsbad, Ca, USA). All inter-assay CVs were below 10%. IGF-1 was analysed by chemiluminescence immunoassay (IDS-iSYS) with inter-assay CV below 7.2%. Hs-CRP was analysed by immunoturbidimetry using a Roche/Hitachi Modular P Analyzer ACN 217(Tina-Quant, Roche/Hitachi, Mannheim, Germany), with an inter-assay CV below 6%. Total-cholesterol, HDL, LDL, and TG