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Interaction between paraoxonase 1 polymorphism and prenatal pesticide exposure on metabolic markers in children using a multiplex approach

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ABSTRACT

Prenatal environmental exposures may influence the risk of cardio-metabolic diseases later in life. This study used a multiplex approach to investigate non-fasting serum levels of metabolic markers in a cohort of school-aged children for whom associations between prenatal pesticide exposure and body fat content and blood pressure were previously found to be dependent on paraoxonase1 (*PON1*) Q192R genotype. In children with the *PON1* 192 R-allele, leptin, glucagon, and plasminogen activator inhibitor-1 (PAI-1) were positively associated with prenatal pesticide exposure. For *PON1* 192 QQ-homozygote children none of the biomarkers were significantly affected by prenatal pesticide exposure. In children with the R-allele, leptin was associated with both body fat measures and prenatal pesticide exposure and seems to mediate body fat accumulation in exposed children. These findings support our previous results of an adverse cardio-metabolic risk profile associated with prenatal pesticide exposure in children with the *PON1* 192 R-allele.

1. Introduction

Chemical exposures during vulnerable periods of early-life have been suggested to contribute to obesity development by interfering with central or peripheral regulation of critical pathways involved in energy balance and adipose tissue development [1–5]. Especially fetal exposure is suspected to increase the risk of obesity in adult life as developmental programming of metabolic signaling pathways may be disturbed [6,7]. Regulation of food intake, energy expenditure, and fat storage is complex and involves the interaction of hormones and other signal substances produced in the neuroendocrine and digestive systems as well as in adipose tissue [8]. Adipose tissue is now regarded as an endocrine organ that produces and secretes a number of bioactive substances including adipokines (e.g., resistin, visfatin, PAI-1, interleukin-6) that have pro- or anti-inflammatory activities, and the peptide hormones leptin and adiponectin that play a central role in energy homeostasis [9,10]. Imbalance in the expression of these adipokines seems related to obesity-induced chronic low-grade inflammation contributing to metabolic dysfunction and insulin resistance and thereby to development of type 2 diabetes, metabolic syndrome and cardiovascular disease [10–12].

In animal studies, low perinatal exposures to some neurotoxic or endocrine disrupting chemicals that caused adult obesity also resulted in altered serum levels of insulin, lipids, leptin and other adipokines [13–18]. In one study, alterations in metabolic markers were evident prior to apparent obesity suggesting that these may be useful as early markers of obesogenic effects [16]. Only few prospective human studies have investigated associations between prenatal exposure to environmental chemicals and later obesity development. These studies indicate that exposure to some persistent pollutants *in utero* may predispose to weight gain later in life [19,20]. A recent study demonstrated a positive association between maternal serum concentration of perfluorooctanoate (PFOA) in pregnancy and risk of overweight or obesity at 20 years of age in female, but not male, offspring. Furthermore, maternal PFOA was also positively associated with serum concentrations of insulin and leptin and inversely associated with adiponectin concentrations in female offspring [21].

We recently found an association between maternal occupational exposure to a mixture of modern non-persistent pesticides in early pregnancy and body fat accumulation from birth to school

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age in their children [22]. Further investigations revealed that this association was driven solely by children from the cohort with a common polymorphism in the *PON1* gene coding for the enzyme paraoxonase 1 [23]. Paraoxonase 1 is a high density lipoprotein (HDL)-associated enzyme known to detoxify oxon derivatives of some organophosphates [24] and to possess anti-oxidative and anti-inflammatory properties [25], thus protecting against cardiovascular disease [26]. A common polymorphism in the coding sequence, where glutamine (Q) is substituted with arginine (R) at position 192, is in some studies associated with cardiovascular risk [27]. We found that prenatally pesticide exposed children carrying the *PON1* 192R-allele had larger abdominal circumference and higher body fat content, BMI Z-scores, higher blood pressure, and serum concentrations of leptin and IGF-1 at school age compared to unexposed children [23]. These effects are all related to increased risk for development of metabolic syndrome and cardiovascular disease later in life. For children with the *PON1* 192QQ genotype, none of these variables were affected by prenatal pesticide exposure.

To investigate associations between exposure to environmental chemicals, obesity development and potential mechanisms, serum concentrations of metabolic markers may constitute a valuable tool. Individual ELISA measurements of these markers are costly, time consuming and require large sample volumes if several markers are measured. A multiplex approach that simultaneously determines the concentration of several metabolic markers may provide a good alternative with respect to time and costs while at the same time providing a pathway oriented approach. We therefore used the Luminex-100 system to analyze a standard panel of multiple metabolic markers including; C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, PAI-1, visfatin and resistin in the above mentioned cohort of children in which some were prenatally exposed to pesticides. The aim of the study was to (1) investigate whether a multiplex (Luminex) approach was suitable to determine serum levels of metabolic markers in stored samples and (2) examine whether these markers were related to differences in anthropometric measures observed between children with the *PON1* 192 R-allele and QQ homozygotes in relation to prenatal pesticide exposure.

2. Materials and methods

2.1. Study population

Recruitment, characteristics, exposure categorization, and clinical examinations of the children in this cohort have previously been described in detail [22,23,28]. In brief, pregnant greenhouse-workers were enrolled during gestational week 4–10 of their pregnancies and categorized as high, medium, or not exposed to pesticides by two toxicologists (independently). Pesticide exposed women were subsequently moved to work functions without exposure or went on paid leave. At age 6–11 years 177 children underwent a standardized clinical examination, in which height, body weight, thickness of skin folds, and other anthropometric parameters were measured [22].

2.2. Laboratory analysis

Venous non-fasting blood samples were obtained (between midmorning and late afternoon) from 145 out of 177 children. *PON1*-genotype for Q192R (rs662) was successfully determined for 141 of the children as previously described [23]. For preparation of serum, blood samples collected into tubes without anticoagulants (VF-109SP, Terumo) was centrifuged at 3000 g for 10 min between 45 and 60 min after sampling and frozen in aliquots at −80 °C immediately after. Serum concentrations of insulin and leptin were determined for 134 of the children (80 exposed and 54 unexposed) using commercial ELISA hormone kits from RayBio using 100 μl serum for each analysis (RayBio Human Insulin ELISA Kit and Human Leptin ELISA Kit, cat no. ELH-insulin-001 human and ELH-leptin-001 human, AH Diagnostics, Aarhus, Denmark) as described previously [23].

2.3. Determination of metabolic marker levels by multiplex

Levels of metabolic markers were determined in serum samples from 136 children. For 134 of the children, insulin and leptin concentrations were also determined by ELISA. The levels of ten metabolic markers were analysed in a total volume of 50 μl serum using the multiplex assay Bio-Plex Pro Assay system (Bio-Rad, Hercules, CA) with the 171-A4C01M for Diabetes 12-plex Complete Kit according to the manufacturer's instructions. This allows simultaneous determination of: C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, plasminogen activator inhibitor 1 (PAI-1), visfatin and resistin in a 96 well plate. In brief, standards and samples were diluted in serum diluent (from the Kit) and added to a 96 well filter plate. Plates were incubated at room temperature (RT) for 2 h with antibodies chemically attached to fluorescent-labeled micro-beads. After three filter washes (with buffer provided in the Kit), premixed detection antibodies were added to each well and incubated for 30 min at RT. Following three washes, premixed streptavidin–phycoerythrin was added to each well and incubated for 10 min at RT followed by three washes. Then beads were re-suspended and the reaction mixture was quantified using a fluorescence-based detection. One serum sample was included in each run as an internal standard in order to allow adjustment for inter-run variation.

2.4. Ethics

The study was conducted according to the Helsinki II Declaration with written informed consent by all mothers and oral consent by the children and was approved by the Regional Scientific Ethical Committee for Southern Denmark and the Danish Data Protection Agency.

2.5. Statistics

Multiplex data were automatically processed and analyzed by Bio-Plex Manager software (Bio-Rad, Herculex, CA). For visfatin 30 of 136 samples were below limit of detection (LOD) and for glucagon 13 of 136 samples were below LOD. However, the samples were included in the data analysis with a value corresponding to LOD/2 for visfatin or glucagon, respectively. The insulin and leptin concentrations measured by both the ELISA and multiplex method were compared by Deming regression analysis to account for imprecision in both methods [29] and a Bland-Altman plot [30].

The sum of four skinfolds (mm) was calculated as the sum of: triceps + subscapular + biceps + flank skinfolds (mm). Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters (kg/m^2). Age and gender specific BMI Z-scores were calculated (child value minus mean value for gender and age divided by standard deviation for gender and age) using a Danish reference population [31].

Logarithmic transformation of the variables was used when necessary to approach a normal distribution and was required for abdominal circumference, sum of four skin folds, and serum concentrations of leptin, insulin (both ELISA and multiplex measurements), C-peptide, ghrelin, GLP-1, glucagon, and PAI-1.

Since only few children had the RR genotype, those with the QR ($n = 48$) and RR ($n = 11$) type were combined as one group in the