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# Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls

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ABSTRACT

*Background:* Several persistent organochlorine pollutants (POPs) possess endocrine disrupting abilities, thereby potentially leading to an increased risk of obesity and metabolic diseases, especially if the exposure occurs during prenatal life. We have previously found associations between prenatal POP exposures and increased BMI, waist circumference and change in BMI from 5 to 7 years of age, though only among girls with overweight mothers.

*Objectives:* In the same birth cohort, we investigated whether prenatal POP exposure was associated with serum concentrations of insulin and leptin among 5-year-old children, thus possibly mediating the association with overweight and obesity at 7 years of age.

*Methods:* The analyses were based on a prospective Faroese Birth Cohort ($n=656$), recruited between 1997 and 2000. Major POPs, polychlorinated biphenyls (PCBs), *p,p′*-dichlorodiphenyldichloroethylene (DDE) and hexachlorobenzene (HCB), were measured in maternal pregnancy serum and breast milk. Children were followed-up at the age of 5 years where a non-fasting blood sample was drawn; 520 children (273 boys and 247 girls) had adequate serum amounts available for biomarker analyses by Luminex® technology. Insulin and leptin concentrations were transformed from continuous to binary variables, using the 75th percentile as a cut-off point. Multiple logistic regression was used to investigate associations between prenatal POP exposures and non-fasting serum concentrations of insulin and leptin at age 5 while taking into account confounders.

*Results:* Girls with highest prenatal POP exposure were more likely to have high non-fasting insulin levels (PCBs 4th quartile: OR=3.71; 95% CI: 1.36, 10.01. DDE 4th quartile: OR=2.75; 95% CI: 1.09, 6.90. HCB 4th quartile: OR=1.98; 95% CI: 1.06, 3.69) compared to girls in the lowest quartile. No significant associations were observed with leptin, or among boys. A mediating effect of insulin or leptin on later obesity was not observed.

*Conclusion:* These findings suggest, that for girls, prenatal exposure to POPs may play a role for later development of metabolic diseases by affecting the level of insulin.



*Abbreviations:* DDE, *p,p′*-dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; PAI-1, plasminogen activator inhibitor; PCBs, polychlorinated biphenyls; POPs, persistent organochlorine pollutant

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## 1. Introduction

The prevalence of obesity and metabolic diseases has substantially increased during the past decades, although the increase in childhood obesity in developed countries appears to have slowed down since 2006 (Ng et al., 2014). Lifestyle and genetic factors are likely to only partially explain the increase and it has been hypothesized that exposure, especially during early-life, to chemicals with endocrine disrupting abilities (EDCs) may increase susceptibility to obesity and metabolic diseases later in life (Casals-Casas and Desvergne, 2011; Lillycrop and Burdge, 2011; Thayer et al., 2012). This hypothesis is supported by epidemiological as well as experimental research linking chemical exposures to obesity, metabolic syndrome and type 2 diabetes (T2D) (Hectors et al., 2013; Lee et al., 2014; Tang-Péronard et al., 2011; Thayer et al., 2012). In epidemiological cross-sectional studies in adults, persistent organochlorine pollutants (POPs) such as polychlorinated biphenyls (PCBs), *p,p*-dichlorodiphenyldichloroethylene (DDE) and hexachlorobenzene (HCB) have been associated with dysmetabolic effects, including visceral obesity, insulin resistance, metabolic syndrome and type 2 diabetes (T2D) (Goncharov et al., 2008; Lee et al., 2011; Vasiliu et al., 2006). These findings are supported by experimental studies in rodents demonstrating abdominal obesity and insulin resistance after postnatal POP exposure (Ibrahim et al., 2011; Ruzzin et al., 2010). A recent review concluded that especially T2D seems strongly associated with POP exposure and that POPs may be a separate risk factor of T2D development, probably in interaction with other risk factors like obesity (Lee et al., 2014). Although most of the POPs were banned in the 1970's, humans are still exposed to these substances through consumption of fat-containing food, especially top predators in the marine food chain, due to high lipophilicity and slow metabolic degradation of POPs (Pelletier et al., 2003).

Both insulin and leptin resistance, reflected by high serum concentrations, are implicated in the pathogenesis of T2D and metabolic syndrome (Kahn et al., 2006; Tong et al., 2005). Leptin is mainly produced in adipose tissue and the serum concentration is correlated to body fat mass. However, among obese individuals with metabolic disturbances such as insulin-resistance serum leptin levels increase independently of body fat mass (Fischer et al., 2002) indicating leptin resistance. Studies have shown that elevated levels of insulin track from childhood into adulthood (Bao et al., 1996; Nguyen et al., 2010). The main objective of the current study was therefore to investigate in a cohort from the Faroe Islands, whether prenatal exposure to PCBs, DDE or HCB was associated with high serum concentrations of insulin and leptin already among 5-year-old children. We recently reported, based on data from the same cohort, that prenatal exposure to POPs was associated with increased BMI and waist circumference among highly POP-exposed 7-year-old girls with overweight mothers (BMI $\geq$ 25 kg/m$^2$) (Tang-Péronard et al., 2014). A further objective of the current study was therefore to investigate whether elevated levels of insulin or leptin at 5 years of age were mediators of this association among the girls at 7 years of age.

## 2. Subjects and methods

### 2.1. Subjects

The population included in the present study represents a birth cohort from the Faroe Islands, where a frequent intake of seafood is associated with relatively high exposure to PCBs, DDE and HCB (Grandjean et al., 1995). The cohort consists of 640 singleton pregnant women, who gave birth between November 1997 and March 2000.

Obstetric variables, including date of birth, birth weight, parity and maternal age were obtained from obstetrical and medical records. Information on pre-pregnancy weight and height, socio economic status, maternal smoking and alcohol use during pregnancy were self-reported. Detailed follow-up examinations were scheduled for the whole cohort when the children were approximately 5 and 7 years of age. The clinical examinations, including measurement of height and body weight, took place from early morning to late afternoon and took an average of two hours for each subject. Body weight was measured in kg on an electronic weight to the nearest single digit after the decimal point. A maternal interview on the child's current health and past medical history, including duration of breast-feeding (exclusive and total, in months), was included in the examination. At the end of the examination, a non-fasting blood sample was drawn.

Out of the 640 cohort members, 60 cohort members did not participate in the 5-year examination. The main reasons were: 14 opted out of examination, did not want to participate any longer; 30 children were living abroad; 14 children did not want to participate at 5 years-examination, 2 children had died (undiagnosed primary carnitine deficiency $N=1$, SUCLA2-deficiency $N=1$). Besides, 13 children were excluded because they had been diagnosed with a chronic disorder and for 47 women and children blood samples for POP and biomarker analyses were not available, leaving a final sample of 520 (81% of original cohort). Of the children re-examined at age 7, a total of 75 girls had overweight mothers. This group was included in a subanalysis of mediation effects; for more details on this cohort see (Tang-Péronard et al., 2014).

The Ethical Review Committee serving the Faroe Islands as well as the US Institutional Review Board approved the study protocol, and written informed consent was obtained from all mothers.

### 2.2. Metabolic markers

The Luminex® technology was applied to quantify the serum levels of biomarkers. A multiplex human pro-diabetes panel (Bio-Plex, assay #171-A7001M) from BioRad (BioRad Laboratories, Symbion Science Park, Copenhagen, Denmark) was applied. In addition to insulin and leptin, the multiplex panel also included measurements of PAI-1 (total), resistin, visfatin, ghrelin, glucagon, GIP, C-peptide and GLP-1 (in pg/mL). Analysis was performed on a Luminex IS100® platform (Luminex Corporation, Austin, TX, USA), and fluorescence signals were analyzed using the Bioplex software ver. 5.0 (Bio-Rad laboratories, CA, USA). A total of 520 serum samples were analyzed blinded to POP content. A volume of 12.5 μL serum was used for the analysis. The intra-run variation was less than 20% and 34% for leptin and insulin, respectively. An internal standard based on a pooled sample of serum was included on each of the 16 plates allowing adjustment for inter-run variation. Since the serum samples were not initially prepared by adding inhibitors to protect degradation of GLP-1, ghrelin, and C-peptide, as recommended by the manufacturer, these three biomarkers were not included in the data-analysis.

### 2.3. Measurement of exposure

Exposure to PCBs, DDE and HCB was assessed by analysis of biological samples obtained at the prospective clinical examinations. Maternal serum was obtained at the last antenatal examination in the 34th week of pregnancy and transition milk was sampled 4–5 days after parturition. Prenatal PCB and DDE exposure was determined from analyses of maternal serum (ΣPCB: $n=383$; DDE: $n=384$) and from breast milk if serum values were missing (ΣPCB: $n=137$; DDE: $n=136$). Because all serum concentrations of HCB in this study were close to or below LOD we