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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.

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None of the authors have any conflicts of interest.

Authors' contributions to manuscript

The authors' responsibilities were as follows – US, PG and PW were responsible for the data collection; AMV performed the biomarker analyses; FN performed the POP analyses; JLTP, BLH, TKJ, AMV, SM and HRA were involved in the design of the current study; JLTP analyzed data and wrote the first draft of the manuscript; BLH, TKJ and HRA supervised the work; and all authors were involved in data interpretation, revising the manuscript, and they approved the final version of the manuscript.

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.

Keywords

Persistent organochlorine pollutants; insulin; leptin; metabolic markers; prenatal exposure

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≥25) (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.

SUBJECTS AND METHODS

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, as the feasibility of obtaining fasting blood samples from pre-scholars is problematic.

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.

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.

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 used HCB concentrations determined in breast milk samples (n = 483). Serum analyses were conducted by gas chromatography with electron capture detection at Department of Environmental Health, University of Southern Denmark (Heilmann et al., 2006). Milk analyses were performed by similar methodology at the Department of Health, State Agency for Health and Occupational Safety of Schleswig-Holstein, Germany. To avoid problems with congeners not assessed and concentrations below the detection limit, a simplified concentration of the sum of PCBs (ΣPCB) was calculated as the sum of congeners PCBs 138, 153, and 180 multiplied by 2 (Grandjean et al., 1995). The ΣPCB, DDE and HCB concentrations were expressed in relation to the total lipid concentration in the sample (Grandjean et al., 1995).

Statistics

The correlation between PCB and DDE concentration in maternal serum and milk was assessed using Pearson's correlation test. Because of the high correlation between concentrations in maternal serum and milk (ΣPCB: r = 0.78; DDE: r = 0.90), missing PCB and DDE serum data were calculated from the milk results using the average ratio (ΣPCB: 0.88; DDE: 0.93) between the two. To assess the robustness of the conclusions derived from this method, multiple imputation analysis was applied as a sensitivity analysis. The missing values for HCB (n = 37) were derived from multiple imputation analysis.

To examine (eventually non-linear) dose-response relationships, the exposure variables were treated as categorical variables (quartiles). The exposure variables, PCBs, DDE and HCB, were log-transformed to approach a Gaussian distribution of the residuals. Linear trends across quartiles of POPs were calculated by treating the categories in each model as continuous variables.

Exploratory multiple linear regression analyses of associations between POPs and all the metabolic markers (insulin, leptin, PAI-1 (total), resistin, visfatin, glucagon and GIP) were performed with log-transformed outcomes to approach a Gaussian distribution. PCBs, DDE and HCB were in these analyses used as continuous variables.

Logistic regression analysis was used to examine associations between prenatal PCBs, DDE and HCB (in quartiles) and odds ratios for having serum concentrations of insulin and leptin above the 75th percentile. For these analyses, insulin and leptin were transformed from continuous to binary variables (75th percentile for insulin: 401 (girls), 406 (boys) and leptin: 2,753 (girls), 1,579 (boys) [in pg/mL],). Variables were coded 0 for low biomarker level and 1 for high biomarker level.

To assess whether insulin and leptin mediated our previous findings of increased adiposity among 7-year-old girls with high POP levels and overweight mothers, we included insulin and leptin, respectively, using 3 dummy variables describing the quartiles in a fully adjusted linear regression model on relations between POPs and BMI or waist circumference to determine any attenuation of the previously obtained beta estimates.

All the analyses were conducted for the whole cohort and separately for girls and boys.

Covariates were identified from *a priori* considerations of relevant factors that may influence the outcome variables and were then excluded stepwise if they did not modify the odds ratios by > 10%. Accordingly, covariates retained in the final models were: maternal age, maternal pre-pregnancy BMI and parity. Birth weight, socioeconomic status, maternal smoking, alcohol consumption and breast-feeding status were not included in the final adjustments, because they did not modify the odds ratios by more than 10%. The analyses were performed both with and without BMI at 5 years of age (as continuous variable) in the models to investigate whether BMI mediated the possible association between exposure to POPs and the metabolic biomarkers. Because the blood samples were non-fasting, time of day for sampling was included as an obligatory covariate.

Effect modification by sex on the association between prenatal POP-exposure and insulin concentration at 5 years of age was examined because of some suggestion from previous studies that EDC exposure may have sex-dimorphic effects (Frye et al., 2012) and previous observations of a female obesogenic susceptibility to EDCs (Tang-Péronard et al., 2011). There is furthermore some evidence suggesting that obesity may modify the association between POPs and metabolic diseases (Lee et al., 2006), and for this reason we also examined the possible effect modification by BMI at 5 years of age. Finally, as we have previously reported an interaction between POP exposure and maternal pre-pregnancy BMI on the POP-related effect on later obesity in the child, we also assessed effect modification by maternal pre-pregnancy BMI. Effect modification was examined by including an interaction term with the exposure variable in the logistic regression analyses (POPs as continuous variables) investigating insulin and leptin ($p < 0.10$ used to define interactions).

Statistical significance was assumed when $p < 0.05$ (two-sided). All analyses were performed using STATA software, version 12 (STATA Corp., Texas).

RESULTS

Table 1 shows the main characteristics of the participants according to sex. The girls had higher serum concentrations of leptin than the boys. All other variables were essentially similar for boys and girls.

For boys, the median for ΣPCB was 1.19 μg/g lipid (range 0.22–15.48), DDE 0.57 μg/g lipid (0.04–11.41), HCB 0.04 μg/g lipid (0.01–0.26), and for girls 1.21 μg/g lipid (0.07–8.31), 0.58 μg/g lipid (0.06–5.18), 0.04 μg/g lipid (0.01–0.18), respectively (Supplemental Figure 1).

We did not observe any interactions between prenatal POP exposure and sex for serum concentrations of insulin and leptin (all $p>0.10$), but because of the evidence of EDC exposure related sex-dimorphic effects (Frye et al., 2012) and since we previously found a sex-specific association between POP exposure and obesity development in the same cohort (Tang-Péronard et al., 2014), the results are presented separately for boys and girls. Associations between prenatal POP exposure and serum concentrations of insulin and leptin were not modified by maternal pre-pregnancy BMI or BMI at 5 years of age (all $p>0.10$).

Multiple linear regression analyses did not show significant associations between prenatal exposure to POPs and serum concentrations of any of the biomarkers (Results of PCBs, DDE and HCB were essentially similar. Results of PCB analyses shown in Supplemental Table 1).

In the logistic regression analyses, after adjusting for child's BMI at 5 years of age, maternal age, parity, maternal pre-pregnancy BMI, and time of day for blood sampling, 5 year-old girls prenatally exposed to high levels of PCBs, DDE and HCB were significantly more likely to have non-fasting insulin concentrations above the 75th percentile (PCB 4^{th} quartile: OR=3.74; 95% CI: 1.36, 10.27. DDE 4^{th} quartile: OR=2.74; 95% CI: 1.08, 6.94. HCB 4^{th} quartile: OR=1.86; 95% CI: 0.99, 3.47) (Table 2). Adjusting for time of day for blood sampling had no influence on the results and no associations were observed in the

population overall (Supplemental Table 2). We did not observe any attenuation of the associations between prenatal POP exposures and adiposity among the 7-year-old girls with overweight mothers. As such, results were essentially similar before and after adjustment for insulin and leptin at 5 years of age. (Results of PCBs, HCB and DDE were essentially similar, and the results of PCB analyses are shown in Supplemental Table 3).

DISCUSSION

In this birth cohort study, girls with the highest prenatal POP exposure were more likely to have non-fasting serum concentrations of insulin above the 75th percentile at 5 years of age. This finding supports our hypothesis that exposure to POPs during a critical period of development, may program the offspring to be more susceptible to metabolic disorders later in life. We did not observe any associations with leptin, or other of the metabolic markers, and furthermore no significant associations were found among the boys. Additionally, we found no indication of a mediating effect of insulin or leptin of the association between any of the POPs and later weight gain.

To our knowledge, no previous studies in humans have prospectively investigated associations between prenatal exposure to POPs and biomarkers of metabolic disturbances during childhood. Although research points to the prenatal period as the most vulnerable period for exposure to EDCs (Newbold et al., 2007), our results are in line with other epidemiological prospective studies in adult populations that have reported associations between exposure in adulthood to PCBs, DDE, HCB, and risk of development of T2D (reviewed in (Lee et al., 2014)) (Lee et al., 2014; Lee et al., 2010; Wu et al., 2013). Associations between exposure to PCBs, DDE and insulin resistance, dyslipidemia, T2D and metabolic syndrome in adult populations have also been reported in cross-sectional studies (Airaksinen et al., 2011; Grandjean et al., 2011; Lee et al., 2007a; Lee et al., 2007b; Lee et al., 2014; Lee et al., 2011; Uemura et al., 2009). Furthermore, studies in adult rodents support a link between postnatal exposure to PCBs, DDT/DDE and later insulin resistance and associated metabolic disorders (Gray et al., 2013; Ibrahim et al., 2011; Ruzzin et al., 2010).

The exact mechanisms behind a diabetogenic effect of POPs are currently unknown but may depend on the exposure level. Suggested mechanisms include interference with key regulatory genes involved in mitochondrial function and lipid homeostasis, as proposed by Ruzzin et al. (Ruzzin et al., 2010) based on in vivo and in vitro studies.

The higher insulin levels indicate that impaired insulin signaling may be an early effect of prenatal POP exposure as this effect occurred prior to increase in BMI, and waist circumference from age 5 to 7 years in the girls with overweight mothers from the same cohort (Tang-Péronard et al., 2014). Sensitivity analyses did not reveal any differences in the insulin levels among the highest exposed girls according to maternal pre-pregnancy BMI (data not shown), however, it may be speculated that some of the causal pathway linking POPs to increased weight and waist circumference, may depend on insulin. Indeed, a high fasting plasma insulin concentration has earlier been found to predict weight gain among children 5 to 9 years of age (Odeleye et al., 1997), and particularly abdominal obesity has

been found to associate with the insulin resistance syndrome (Biddinger and Kahn, 2006). However, a mediating effect of insulin or leptin could not be confirmed in the present study. Nevertheless, our findings of high insulin levels with high prenatal exposures to POPs indicate a potential disturbance of glucose homeostasis and insulin resistance that may be one of the first signs of metabolic disruption (Schoeters et al., 2011) and later risk of T2D. Thus, evidence indicates that insulin resistance is followed by onset of T2D 10–20 years later (Shulman, 2000). Insulin levels have been found to correlate with central fat distribution (Bacha et al., 2003) and thus high insulin might be an effect of a high body fat content. However, adjusting for BMI at 5 years of age did not attenuate the associations between prenatal POP exposure and the high levels of insulin suggesting that POPs, and not body fat per se, may be responsible for the high insulin levels at this age. Interestingly, in a cross-sectional study of adults, higher plasma levels of POPs was reported to be a separate risk factor among metabolically abnormal obese phenotypes compared to metabolically healthy, but obese, people (Gauthier et al., 2014). BMI is not the most sensitive obesity marker for insulin resistance and adjusting for waist circumference at 5 years of age would have been more appropriate. This information was however not available. Adjusting for change in weight from birth to 5 years of age gave essentially similar estimates.

In contrast to the findings in the present study, an inverse association between serum PCBs and fasting insulin was previously reported in a cross-sectional study among Faroese non-diabetic residents aged 70–74 years with high dietary POP exposure (10). The apparent discrepancy between their findings and ours most likely is related to the differences in design (cross-sectional vs. prospective), to differences in timing of exposure (late in adult life vs. prenatally) or simply to the age difference at examination that likely reflects different pathogenic stages from stimulation of increased insulin secretion in young subjects followed by impaired β-cell function at old age when the β-cells no longer can compensate for the ambient insulin resistance. However, a recent cross-sectional study among healthy Danish children aged 8–10 years also found strong inverse associations between serum PCB and fasting insulin, despite a considerably lower PCB exposure (5). Taken all together, the potential effects of POPs on insulin secretion and glucose homeostasis may be very complex. As early life exposure to POPs is suggested to have a more profound influence on increased risk of metabolic diseases later in life (Barouki et al., 2012; Martin-Gronert and Ozanne, 2005), the fact that we measured POPs prenatally, may be the most likely explanation for the differing observations.

Previous research has documented insulin resistance to be associated with elevated leptin levels in non-diabetic subjects (Donahue et al., 1999; Kennedy et al., 1997). Moreover, leptin has recently received attention as a potential mediator of developmental programming of metabolic disorders later in life (Vickers and Sloboda, 2012). A down regulation of the *LEPR* (leptin receptor) gene was reported in children from a high PCB exposed population in Slovak (Ghosh et al., 2013). In accordance with this observation, concentrations of PCB101, PCB153 and PCB180 at levels detectable in human adipose tissue, caused increased leptin gene expression and a reduction of leptin receptor expression and signaling in mature 3T3-L1 adipocytes consistent with leptin-resistance (Ferrante et al., 2014). In rats, gestational exposure to doses of PCBs (Aroclor, mixture of PCB congeners) at levels

relevant for human exposure caused depressed leptin concentrations in 15-day-old offspring but elevated leptin in 30-day-old animals (Provost et al., 2007).

The fact that we did not observe any associations between exposure to POPs and variations in leptin among girls or boys may simply be explained by the young age of the included population. Thus, the findings in the present study of a direct association between POPs and increased insulin levels may be speculated to contribute to hyperleptinemia later in life.

The present study has some limitations. Measurement of metabolic markers was not part of the initial study plan. Therefore fasting blood samples were not collected and the serum samples were not initially treated with protease inhibitors as recommended by the manufacture of the Luminex panel and time from sampling to freezing were not completely standardized. This may have affected the concentrations of some of the biomarkers. Besides, the sensitivity of the Luminex method is expected to be lower than for standard enzyme-linked immunosorbent assays (ELISA). However, in a recent study, we found that leptin and insulin concentrations determined by the Luminex (same panel as applied in the present study) and ELISA showed an acceptable agreement, although the absolute concentration for leptin differed systematically by a factor of approximately four. The difference tended to increase at high leptin levels (Jorgensen et al., 2015) and this cannot be excluded to have affected the absence of significant associations for leptin. To our knowledge, measurements from the Luminex panel have not been compared with standard ELISA methods for the other biomarkers in the panel and therefore we have chosen only to present the data for these biomarkers without further statistical analysis or interpretation of the results.

Another limitation is that the metabolic biomarkers were not measured in blood at fasting conditions. Fasting samples were not available from the children, as metabolic biomarkers were not a part of the initial study plan. While serum concentrations of insulin are markedly affected by composition and time from recent meals, serum concentrations of leptin seem less affected. However, estimates of insulin resistance measured in non-fasting blood seem to correlate well with values obtained in fasting blood samples (Hancox and Landhuis, 2011), thus suggesting that non-fasting blood samples can be used. On the other hand we cannot exclude possible bias related to taking the blood samples in the fasting state has remained. However, most likely such bias would have been random, which would tend to attenuate the associations. The fact that we still find significant associations would favor true associations. On the other hand, a bias related to using non-fasting insulin could be non-random, if overweight girls consume more carbohydrates than lean girls. That could result in greater increases in insulin, and attenuated associations would then emerge, and a statistical positive association between POPs and insulin concentrations may in that case not reflect a true causal effect. Also, even if we adjusted models for maternal BMI and child BMI, these covariates are just proxies of adiposity and residual confounding may remain. Hence, even in this prospective study, we cannot firmly conclude that observed associations reflect true causal effects.

Interaction analyses require large power, and this might explain why, in the present study, the analysis of interaction between prenatal POP exposure and sex on insulin level, did not reach significance, although associations appeared stronger among girls than boys in sex-

stratified analysis. A sex-selective effect is, however, consistent with the recent findings in the same cohort, where an association between prenatal exposure to POPs and elevated adiposity measurements was present among girls only (Tang-Péronard et al., 2014). Higher susceptibility to metabolic disturbances among females after POP exposure has previously been reported (Halldorsson et al., 2012; Tang-Péronard et al., 2011; Tang-Péronard et al., 2014; Wang et al., 2008), although the mechanism behind remains unknown.

Because humans are exposed simultaneously to a variety of highly correlated environmental pollutants with endocrine disrupting abilities (Vandenberg et al., 2012), the high levels of insulin may be a result of exposure to a combination of pollutants (Kortenkamp, 2007).

It should also be noted, that our findings of an association between prenatal exposure to POPs and high levels of insulin among girls at 5 years of age, was observed in a population with rather high dietary POP exposure from seafood (Grandjean et al., 2011; Longnecker et al., 2003). A major strength of the study is the homogenous well-described cohort (Grandjean et al., 2001; Steuerwald et al., 2000) and the prospectively design of the study, which enabled us to conclude on the association between exposure to POPs in utero and subsequent metabolic disturbance.

In conclusion, we found that prenatal exposure to PCBs, DDE and HCB was associated with elevated non-fasting insulin levels among 5-year-old girls. No associations were found among boys. These findings might indicate that exposure to POPs during a critical developmental period may alter the susceptibility among female offspring to develop metabolic dysfunction later in life. Although our study has some limitations and we cannot exclude residual confounding, these findings may have important public health implications due to the ubiquity of exposure to POPs and the high prevalence of metabolic disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The Ethical Committee of the Faroe Islands approved the study protocol.

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 pollutants.

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Highlights

1. Prenatal POP exposure was associated with elevated insulin levels among 5-year-old girls
2. No associations between prenatal POP exposure and insulin were observed among boys
3. A mediating effect of insulin or leptin on later obesity in girls was not observed
4. These findings support an association between POP exposure and metabolic dysfunction

Table 1

Maternal and child characteristics according to sex. Faroese Cohort 1996–2001, (N=520)[1].

	Girls (n=247)				*Boys (n=273)*				
		Percentile				Percentile			
Characteristics	**Median**	**5th**	**75th**	**95th**	**Median**	**5th**	**75th**	**95th**	***P***
Metabolic markers (ng/mL)									
Insulin	162	1.4	401	1,211	133	1.2	406	1,221	0.33
Leptin	1,650	483	2753	6,430	889	307	1,579	3054	<0.001
Prenatal POPs (μg/g lipid)									
PCBs	1.2	0.4	2.0	3.7	1.2	0.4	2.0	4.2	0.32
DDE	0.6	0.1	1.0	2.3	0.6	0.1	1.0	2.2	0.24
HCB	0.04	0.02	0.05	0.1	0.04	0.02	0.05	0.09	0.96
BMI 5 y (kg/m^2)	16	14.1	16.9	18.7	15.9	14	16.6	18.1	0.56
Maternal age at child's birth (y)	30	21	33	37	30	21	33	38	0.19
Maternal pre-pregnancy BMI (kg/m^2)	23.4	18.8	26.3	30.5	22.8	18.8	25.5	31.2	0.26
Parity (n)	1	0	2	3	1	0	2	3	0.1

[1] Anova and Pearson chi-squared tests. PCB and DDE concentration in maternal serum (μg/g lipid). The PCB concentration was based on the sum of PCB congeners 138, 153, and 180. HCB in maternal milk (μg/g lipid). Non-fasting serum concentrations of insulin and leptin (ng/mL) measured at 5 years of age. PCBs, polychlorinated biphenyls; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene. At 5 y of age 11% of boys and 14% of girls were overweight and 1.4% of boys and 3% of girls were obese (IOTF definition); 31.5 % of the mothers were overweight and 7.9 % obese (WHO definition).

Table 2

Odds ratio for having insulin and leptin levels above the 75th percentile at 5 years of age after prenatal exposure to POPs, according to sex. Faroese Cohort 1996–2001, (N=520)[1].

	Girls (n=247)		*Boys (n=273)*	
	Insulin (ng/mL)	**Leptin (ng/mL)**	**Insulin (ng/mL)**	**Leptin (ng/mL)**
POPs in quartiles	**Odds Ratio (95% CI)**			
PCBs				
1	1.00	1.00	1.00	1.00
2	2.59 (0.99, 6.81)	0.68 (0.29, 1.60)	0.57 (0.25, 1.33)	0.63 (0.28, 1.44)
3	2.00 (0.74, 5.44)	0.48 (0.20, 1.18)	0.84 (0.38, 1.87)	1.20 (0.55, 2.59)
4	3.74 (1.36, 10.27)	0.61 (0.24, 1.53)	1.13 (0.51, 2.48)	0.78 (0.34, 1.79)
P for trend	0.03	0.23	0.56	0.93
DDE				
1	1.0	1.0	1.0	1.0
2	2.02 (0.83, 4.93)	0.99 (0.44, 2.22)	1.12 (0.51, 2.48)	0.87 (0.39, 1.94)
3	0.99 (0.37, 2.64)	0.46 (0.18, 1.14)	0.75 (0.32, 1.72)	1.00 (0.45, 2.20)
4	2.74 (1.08, 6.94)	0.75 (0.31, 1.82)	1.56 (0.71, 3.44)	1.01 (0.45, 2.28)
P for trend	0.10	0.29	0.44	0.91
HCB				
1	1.0	1.0	1.0	1.0
2	1.24 (0.68, 2.24)	0.58 (0.29, 1.15)	0.98 (0.58, 1.65)	0.97 (0.53, 1.77)
3	1.13 (0.62, 2.06)	0.57 (0.29, 1.13)	1.04 (0.62, 1.76)	1.11 (0.60, 2.06)
4	1.86 (0.99, 3.47)	0.68 (0.34, 1.36)	1.36 (0.80, 2.30)	1.25 (0.67, 2.36)
P for trend	0.09	0.34	0.24	0.44

[1] PCB and DDE concentration in maternal serum, μg/g lipid (log-transformed); The PCB concentration was based on the sum of PCB congeners 138, 153, and 180. HCB in maternal milk, μg/g lipid (log-transformed). Non-fasting serum concentrations of insulin and leptin (ng/mL) measured at 5 years of age. Logistic regression, adjustments made for maternal age, parity, maternal pre-pregnancy BMI, BMI at 5 years of age and time of day for blood sampling. PCBs, polychlorinated biphenyl(s); DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene.