Prenatal exposure to persistent organic pollutants and markers of obesity and cardiometabolic risk in Spanish adolescents

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ABSTRACT

Background: Prenatal exposure to persistent organic pollutants (POPs) has been linked to cardiometabolic (CM) risk factors in childhood, but there are no studies evaluating the persistence of these associations into adolescence, a period of relevant changes in endocrine-dependent organ systems and rapid increases in lean and fat mass. We examined the associations of prenatal POP exposures with body mass index (BMI) from age 4 to 18 years, and with other CM risk markers in adolescence.

Methods: We analysed 379 children from the Spanish INMA-Menorca birth cohort study with measured cord blood POP concentrations. We calculated BMI z-scores at ages 4, 6, 11, 14 and 18 years using the WHO growth reference. Body fat % was measured at 11 and 18 years and waist-to-height ratio (WHtR) and blood pressure (BP) at 11, 14 and 18 years. We measured CM biomarkers in fasting blood collected at age 14 years and calculated a CM-risk score as the sum of the sex-, and age-specific z-scores for waist circumference, mean arterial BP, homeostatic model assessment of insulin resistance, fasting blood triglycerides, and high-density lipoprotein cholesterol (LDL-C) (n = 217). Generalised estimating equations and multivariate linear regression models assessed the associations with repeated and single time-point measures, respectively.

Results: Hexachlorobenzene (HCB) exposure in the third tertile, compared to the first tertile, was associated with higher BMI (β = 0.24; 95% CI: 0.01, 0.47) and WHtR z-score (β = 0.27; 95% CI: 0.04, 0.51). A continuous increase in HCB was associated with an elevated body fat % (β per 10-fold increase = 4.21; 95% CI: 0.51, 7.92), systolic BP (β = 0.32; 95% CI: 0.02, 0.64) and diastolic BP z-score (β = 0.32; 95% CI: 0.02, 0.62) across all ages, and with higher CM-risk score (β = 1.59; 95% CI: 0.02, 3.18) and lipid biomarkers (total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL-C) at 14 years. Dichlorodiphenyltrichloroethane (p,p’-DDT) exposure was non-monotonically associated with BMI and systolic BP, p,p’-DDE and Σ-polychlorinated biphenyls (PCBs) (sum of congeners 118, 138, 153, 180) were not associated with adiposity or BP. p,p’-DDT exposure was associated with an increased CM-risk score, and ΣPCBs concentrations with LDL-C in all adolescents and with total cholesterol only in girls (p-sex interaction = 0.05).

Abbreviations: BMI, body mass index; BP, blood pressure; CM, cardiometabolic; DAG, directed acyclic graph; p,p’-DDE, p,p’-dichlorodiphenyldichloroethane; p,p’-DDT, p,p’-dichlorodiphenyltrichloroethane; EDCs, endocrine-disrupting chemicals; GAM, generalised additive models; GC, gas chromatography; GEE, generalised estimating equations; HCB, hexachlorobenzene; HDL-C, high-density lipoprotein cholesterol (LDL-C); HOMA-IR, homeostatic model assessment of insulin resistance; INMA, Infancia y Medio Ambiente; LDL-C, low-density lipoprotein cholesterol; LOD, limit of detection; LOQ, limit of quantification; MAP, mean arterial pressure; PCBs, polychlorinated biphenyls; POP, persistent organic pollutant; T2D, type II diabetes mellitus; WHO, World Health Organization; WHtR, Waist-to-Height Ratio.

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1. Introduction

Over the past four decades the prevalence of obesity, insulin resistance, dyslipidemia, and hypertension - the main risk factors for adult metabolic syndrome - has increased worldwide in adults and children (NCD Risk Factor Collaboration (NCD-RisC), 2020, 2017a, 2017b, 2016). Lifestyle factors, such as a poor-quality diet and sedentarism, and genetic susceptibility are primary contributors to the development of the metabolic disease (Ruiz et al., 2019). However, these factors alone cannot explain their current global trends (Heindel et al., 2015a).

The ‘metabolic disruptor hypothesis’ postulates that endocrine-disrupting chemicals (EDCs), including persistent organic pollutants (POPs), may promote metabolic changes predisposing an organism to obesity, type II diabetes mellitus (T2D), or other aspects of metabolic syndrome, especially if exposure occurs during sensitive windows of foetal and child development (Grun and Blumberg, 2006; Heindel et al., 2017; Heindel et al., 2015). This hypothesis is currently supported by data from in vivo and in vitro studies and to a lesser extent from observational studies (Heindel et al., 2017). POPs include carbon-based chlorinated lipophilic pesticides [i.e. dichlorodiphenyltrichloroethane (p,p'-DDT), its prime metabolite dichlorodiphenyldichloroethane (p,p'-DDE), and hexachlorobenzene (HCB)] as well as industrial chemicals (polychlorinated biphenyls (PCBs)). They accumulate in animal and human lipid-containing tissues like adipose tissue and, similar to other POPs, may promote metabolic changes predisposing an organism to metabolic syndrome, especially if exposure occurs during sensitive windows of foetal and child development (Grun and Blumberg, 2006; Heindel et al., 2017). However, due to their environmental persistence and the slow rate of biodegradation (between 3 and 15 years of half-lives) (Milbrath et al., 2009), these chemicals are still detected in human lipid-containing tissues like adipose tissue and, similar to other EDCs, may disrupt critical biological processes of the endocrine and metabolic systems through mimicking or blocking the action of natural hormones (Heindel et al., 2017). The production and use of these chemicals have been banned (PCBs, HCB) or restricted (p,p'-DDT) under the Stockholm Convention (2004). However, due to their environmental persistence and the slow rate of biodegradation (between 3 and 15 years of half-lives) (Milbrath et al., 2009), these chemicals are still detected in international biomonitoring study populations, including pregnant women and children (Porta et al., 2008). Contaminated food, especially animal products, is the primary source of human exposure (Porta et al., 2008). In early life, maternal concentrations of POPs are transmitted to the child through the placenta, and postnatally, via breast milk (Carrizo et al., 2006).

In the Spanish INMA birth cohort studies, we have previously reported that prenatal exposure to p,p'-DDT, p,p'-DDE, HCB and potentially PCBs may increase the risk for rapid weight gain in infancy (Mendez et al., 2011; Valvi et al., 2014) and elevated body mass index (BMI) later in childhood (Agay-Shay et al., 2015; Smink et al., 2008; Valvi et al., 2012). Positive associations between prenatal exposure to p,p'-DDE and p,p'-DDT with BMI and other obesity-related outcomes in children, such as waist-to-height ratio (WHR) and waist circumference, have also been reported in other longitudinal birth cohorts (Coker et al., 2018; Delvaux et al., 2014; Heggeseth et al., 2015; Iszatt et al., 2015; Tang-Péronard et al., 2014, p. - Vafeiadi et al., 2015; Warner et al., 2017; Warner et al., 2014). Fewer cohorts found null associations between p,p'-DDE or p,p'-DDT and childhood obesity (Capul-Uicab et al., 2013; Garced et al., 2012; Hoyer et al., 2014; Karlsen et al., 2017; Lauritzen et al., 2015). Findings for the association with prenatal PCBs have been less consistent (Capul-Uicab et al., 2013; Delvaux et al., 2014; Hoyer et al., 2014; Iszatt et al., 2015; Karlsen et al., 2017; Lauritzen et al., 2018; Tang-Péronard et al., 2014; Vafeiadi et al., 2015; Verhulst et al., 2009) as well as with prenatal HCB exposure (Capul-Uicab et al., 2013; Karlsen et al., 2017; Lauritzen et al., 2018; Vafeiadi et al., 2015).

A major limitation of the current state of evidence is that the effects of prenatal POP exposure on cardiometabolic (CM) traits other than indirect measures of obesity have been rarely explored. To our knowledge, only one previous study has assessed associations of prenatal POP exposures with other CM risk factors beyond child anthropometry (Vafeiadi et al., 2015). Further, no studies have assessed whether the associations shown between prenatal POPs exposure and childhood obesity and CM traits persist later in puberty, a sensitive period characterised by relevant changes in hormones with an essential role in metabolism and weight homeostasis and rapid developmental increases in lean and fat mass. As CM risk factors tend to track from childhood and adolescence into adulthood (Chen and Wang, 2008; Franks et al., 2010; Juhola et al., 2011; Juonala et al., 2011; Wright et al., 2010), the early identification of adolescents who are at risk of developing metabolic syndrome in later life is important. Therefore, we aimed to explore the persistency of the previously reported POP associations with BMI throughout adolescence and further, to assess the association with other CM markers (body fat %, blood pressure, lipids, and insulin resistance), as well as a combined CM-risk score, in adolescents from the Spanish INMA-Menorca birth cohort.

2. Methods

2.1. Study population

Women presenting for antenatal care were recruited into the INMA (INFancia y Medio Ambiente – Environment and Childhood) birth cohort set up in the Spanish Island of Menorca between April 1997 and June 1998 (n = 482). The protocol of the INMA study has been reported elsewhere (Guxens et al., 2012). Mothers were included if they (i) were resident in the study area, (ii) were at least 16 years old, (iii) had a singleton pregnancy, (iv) did not follow any assisted reproduction programme, (v) wished to deliver in the reference hospital and (vi) had no communication barrier. POP levels were measured in cord blood samples of 405 mothers, and their children were followed periodically until they reached the age of 18 years old. Of these, 379 children (78.6% of the initially enrolled cohort) had available data of at least one anthropometric (BMI, waist circumference) or blood pressure (BP) outcome between the age of 4 and 18 years and were included in the present study (Fig. 1). This study was approved by the ethics committee of the Hospital del Mar Research Institute (IMIM), and all mothers and adolescents signed written informed consent.

2.2. Prenatal POPs assessment

Concentrations of p,p'-DDT, p,p'-DDE, HCB, and the PCB congeners 28, 52, 110, 118, 138, 153, 180 were measured in cord blood using gas chromatography (GC) with electron capture detection (Hewlett-Packard 6890 N GC-ECD; Hewlett-Packard, Avondale, PA, USA) and GC coupled to chemical ionisation negative-ion mass spectrometry (Hewlett-Packard 5973 MSD) in the Department of Environmental Chemistry (IDAE-CSIC) in Barcelona (Spain) (Carrizo et al., 2007; Valvi et al., 2012). Details on the sample extraction and clean up can be found elsewhere (Grimail et al., 2010). The limits of detection (LOD) and quantification (LOQ) for all POPs ranged between 0.007 (p,p'-DDT) and 0.323 (HCB) ng/mL, and 0.011 and 0.485 ng/mL, respectively (Table S1). PCB congeners 28, 52 and 101 were excluded from this analysis because their percentages of values above the LOQ were less than 70% (Valvi et al., 2012). Since PCB-153 has been suggested as a good marker for overall exposure to PCBs (Hagmar et al., 2006), we calculated the sum of PCB congeners 118, 138, 153, and 180 to create one single variable (∑PCBs).
2.3. Anthropometric measurements

All anthropometric measurements were measured by trained staff using a standard protocol (i.e., in light clothing and without shoes) at ages 4 years (n = 240), 6 years (n = 360), 11 years (n = 335), 14 years (n = 273), and 18 years (n = 224) for height and weight, and at ages 11 years (n = 335), 14 years (n = 273), and 18 years (n = 225) for waist circumference (Fig. 1). We calculated BMI (weight/height2) age- and sex-specific z-scores using the World Health Organization (WHO) Growth reference (de Onis et al., 2009; de Onis et al., 2007). Overweight was defined as a BMI z-score equal to or above the WHO age- and sex-specific 85th percentile. Waist circumference was measured using an inelastic tape (SECA model 201) at the midpoint between the right lower rib and the iliac crest after gentle expiration. We used waist circumference (cm) and height (cm) to calculate the waist-to-height ratio (WHtR) as a proxy of central obesity (Ashwell et al., 2012). We derived age-, and sex-specific WHtR z-scores, as the standardised residuals from regression models of WHtR as the dependent variable, and age and sex as the predictors (Eisenmann, 2009).

Tetrapolar bioelectric impedance analyses were conducted using the RJL device at ages 11 (n = 334) and 18 years (n = 219). Fat-free mass at 11 and 18 years old, was determined by using the Horlick et al. (2002) equation. Body fat was obtained by subtracting the fat-free mass from measured total body weight. Body fat % was calculated as body fat (kg) divided by the total body weight (kg) and multiplied by 100.

2.4. Blood pressure (BP)

Systolic and diastolic BP was measured using an automatic digital monitor (OMRON 705-CPII) at ages 11 years (n = 335), 14 years (n = 273), and 18 years (n = 225) of age. After five minutes of rest, three consecutive measurements were taken with one-minute time intervals between them. We took the average of the second and third measurements to reduce measurement error, and like WHtR, we used regression models to derive BP z-scores standardised by age, sex, and height.

2.5. Blood biomarkers

Serum samples were collected at fasting conditions from adolescents at 14 years (n = 219) and were stored at −20 °C until analysis was carried out at the CQS laboratory in Madrid (Spain) using standard procedures. We measured biomarkers related to childhood obesity and insulin resistance, including fasting total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, insulin, and glucose. Total cholesterol was determined by cholesterol esterase and oxidase, HDL-C and LDL-C were measured by a direct method (particle elimination, cholesterol esterase and colourimetry) and triglycerides were quantified by lipase/glycerol kinase and colourimetric analysis. Insulin and glucose were analysed with hexokinase and luminescence immunoassay, respectively. Homeostatic model assessment for insulin resistance (HOMA-IR), a marker of insulin sensitivity validated in adolescents (Atabek and Pirgon, 2007; Keskin et al., 2005), was calculated as fasting insulin (μIU ml −1) × fasting glucose (mmol l −1)/22.5 (Keskin et al., 2005).

2.6. CM risk score

We generated a continuous score of CM risk by summing the z-scores specific for age and sex of five individual CM markers commonly used for the definition of adult metabolic syndrome: waist circumference, mean arterial pressure (MAP), HDL-C, triglycerides, and HOMA-IR. We used MAP instead of accounting for both systolic and diastolic BP to deal with the significant correlation shown between the two BP variables, as suggested previously (Eisenmann, 2008). HDL-C was summed after values were multiplied by −1 because it is inversely associated with CM risk. In the absence of a widely accepted definition for metabolic syndrome in children and adolescents, similar CM risk scores are increasingly used in epidemiological studies and have been shown to associate...
with risk factors of metabolic syndrome and to track CM risk from adolescence into adulthood (Giesemann, 2008). The CM score was calculated for 216 subjects who had all five CM risk factors measured (Fig. 1).

2.7. Covariates

Information on the following covariates was obtained through interviewer-administered questionnaires answered by mothers at recruitment: maternal age, educational level, country of origin, parity (first child or not), smoking during pregnancy ("yes" if they smoke at the moment of the questionnaire), self-reported pre-pregnancy BMI (weight (kg)/height (m)²) and social class (using the UK Registrar General’s 1990 classification according to parental occupation by ISCO88 code). We classified professional/managerial/technical occupations as nonmanual; skilled, partially skilled, and unskilled occupations as manual; and unemployed and housewives as unclassified. Infant sex, gestational age, birth weight and type of delivery were collected by clinical records. Exclusive breastfeeding duration and gestational diabetes were self-reported by mothers at follow-up visits after birth.

2.8. Statistical analysis

We log-transformed all cord blood POP concentrations to handle the right-skewed distributions. We, therefore, expressed β coefficients as the increase of 10-fold of the exposure is equal to a β* log (10) additive change in the mean of the outcome. We performed multiple imputations by chained equations of missing values in covariate data (<5%) to avoid loss of participants in the study (Spratt et al., 2010; Sterne et al., 2009). POP measurements below the LOQ were imputed using distribution-based multiple imputations by assuming a log-normal distribution of POPs and conditioning the imputation to the range (0, LOD) for values < LOD and (LOD, LOQ) for values < LOQ (Baccarelli et al., 2005). We generated five complete data sets by using the ice package for Stata (Royston, 2005), and estimates on each data set were combined using Rubin’s rules for multiple imputations (Little and Rubin, 2014). A detailed description of this procedure is provided in Table S2. Distributions in imputed datasets were similar to those observed (Table S3). We performed generalised additive models (GAMs) with the R package ‘mgcv’ to assess departures from linearity in the relationship between log-transformed POPs and each outcome. If the effective degrees of freedom were equal to 1, the relationship was closer to linear. Because a few GAM models showed evidence of non-linearity (Fig. S1-S2), we modelled POPs concentrations as both continuous and categorical variables using piecewise cut-offs.

We estimated associations between prenatal POPs exposure and repeated anthropometric and BP measures using generalised estimating equations (GEE) with an unstructured correlation matrix. To assess whether the exposure effect differs over time, an interaction term between age at outcome assessment (4 years, 6 years, 11 years and/or 14 years, and 18 years) and the POP exposure variable was included. Multivariable linear regression models were used to evaluate the associations between the POPs concentrations and the CM biomarkers and risk score at 14 years old.

Covariates included in the multivariate-adjusted GEEs and linear regression models were selected based on prior knowledge and directed acyclic graphs (DAGs) using DAGitty software (Shrier and Platt, 2008). Prior knowledge was acquired from previous analyses conducted in this cohort (Smink et al., 2008; Valvi et al., 2012) and evidence from other prospective studies (Ribartuzes et al., 2011; Ong et al., 2002; Vafeiadi et al., 2015). We additionally examined if maternal diet variables (daily intake of fruits and nuts, meat, fish, dairy products and vegetables) confounded the associations; since coefficient estimates did not change, these variables were not retained. Therefore, final multivariate models included the following maternal characteristics at pregnancy: parity history (nulliparous/multiparous), pre-pregnancy BMI (kg/m²), education (less secondary/secondary completed), socioeconomic status (non-manuals/manuals/unclassified), smoking (yes/no), and age (years) (Fig. S3). We additionally adjusted by child’s sex and age (years) at outcome assessment in the models without standardized outcomes. Gestational diabetes, delivery type, gestational duration, birth weight, and breastfeeding duration may be mediating factors in the association of prenatal POP exposures and child CM outcomes at later ages (Fig. S3). Therefore, we did not adjust associations for these variables as we are interested in the total effect of prenatal POP exposures on CM outcomes (VanderWeele, 2009).

We performed a sensitivity analysis by splitting up the PCBs into dioxin-like (118) and non-dioxin like (sum of 138, 153, 180 congeners) PCBs, as they have shown to exhibit different modes of action in experimental studies (Kim et al., 2011). We stratified by sex to obtain sex-specific estimates, and we tested sex-interactions by inserting cross-product terms (POP*sex) in the statistical models, as POP effects have been suggested to differ by sex in previous studies (Valvi et al., 2012; Warner et al., 2017). Multipollutant models adjusted for the four main exposure variables (p,p’-DDT, p,p’-DDE, HCB and ΣPCBs) were also performed to evaluate whether associations for each POP are confounded by the effect of other POPs. We assessed collinearity with variance inflation factors (VIFs) for each model. Since all VIFs were less than 2 (Kim, 2019), none of the exposures was removed from the multipollutant models. The statistical package STATA version 15.0 (Stata Corporation, College Station, TX, USA) and R version 4.0.2 (R Foundation, Vienna, Austria) were used for statistical analyses.

3. Results

3.1. Study population characteristics

Children with at least one outcome measured from 4 to 18 years old (n = 379) and its subgroup of adolescents with biomarkers measured at 14 years (n = 219) had similar offspring and maternal characteristics (Table 1). Table S4 shows that children included in the study (n = 379) were similar to those excluded (because of missing exposure or outcome data, n = 103), except for higher birth weight. In the full population, about half of the children (49.3%) were females, and almost 40% were breastfed for more than six months (Table 1). Almost all mothers were predominantly Spanish (97%), 21% of them had pre-pregnancy overweight, 58% were nulliparous, and 6% developed gestational diabetes during pregnancy (Table 1). The highest POP concentration on average was found for p,p’-DDE, followed by HCB, ΣPCBs, and p,p’-DDE (Table 1). The correlation between these compounds ranged from 0.15 (between p,p’-DDT and ΣPCBs) to 0.46 (between p,p’- DDT and p,p’-DDE) (data not shown). The prevalence of overweight was 28–29% at 4, 6 and 11 years and 23% and 20% at 14 and 18 years, respectively (Table 2).

Pearson’s correlation analysis showed strong positive correlations between BMI z-score and WHR z-score at all ages (r > -0.82). Correlations of body fat % with BMI z-score and WHR z-score decreased with age (0.79 and 0.82 respectively, at 11 years, and 0.63 and 0.62 respectively, at 18 years) (Tables S5-S7). Within-age correlations between BP (systolic and diastolic) and anthropometric measurements were significant at 14 years (r between 0.25 and 0.35), whereas they were small and at some cases non-significant at 11 and 18 years old (Tables S5-S7). Total cholesterol and LDL-C were the most strongly correlated CM biomarkers (r = 0.89) (Table S6). HDL-C was inversely correlated with all markers except for total cholesterol, and HOMA-IR correlated positively and significantly with all biomarkers (Table S6). CM risk correlated moderately and significantly with anthropometric and BP outcomes and POPs concentrations showed that relationships with all study outcomes were linear with few exceptions.
β blood p,p\textsubscript{0.02, 0.64} (Table 3) and with higher diastolic BP (for a 10-fold increase, with higher systolic BP (for a 10-fold increase in HCB, β 0.22; 95% CI: 0.03, 0.41; β 0.29; 95% CI: 0.08, 0.50, respectively). A continuous increase in HCB concentrations was associated with higher systolic BP (for a 10-fold increase in HCB, β 0.32; 95% CI: 0.02, 0.64) (Table 3) and with higher diastolic BP (for a 10-fold increase, β = 0.32; 95% CI: 0.02, 0.62) (Table 3). Conversely, p,p\textsubscript{-DDT} concentrations were negatively associated with both systolic and diastolic BP z-score, but these only reached statistical significance for p,p\textsubscript{-DDT} in the second tertile and systolic BP (Table 3). Effect estimates did not change notably in complete cases analyses (data not shown). In unadjusted models, most coefficients were of larger magnitude (Table S8) and were attenuated mostly after adjustment of the maternal pre-pregnancy BMI models, most coefficients were of larger magnitude (Table S8) and were notably in complete cases analyses (data not shown). In unadjusted models, most coefficients were of larger magnitude (Table S8) and were noted in complete cases analyses (data not shown). In unadusted models, most coefficients were of larger magnitude (Table S8) and were attenuated mostly after adjustment of the maternal pre-pregnancy BMI covariate.

The associations between POPs and BMI, WHR and body fat % were largely consistent over the ages at follow-up (p for interaction > 0.10).
reached statistical significance with LDL-C (for a 10-fold increase in PCB-118 and HOMA-IR in the sensitivity analysis (for a 10-fold increase in HDL-C were not associated with any of the measured POPs (Table 4). Significantly associated with any of the CM outcomes. HOMA-IR and PCBs, CM risk scores in children at 14 years (for a 10-fold increase, association with PCB-118, nevertheless, we observed a positive association between dioxin-like PCB-118 and HCB and triglycerides, and LDL-C concentrations (β = 14.47 mg/dL; 95% CI: 2.31, 21.54); (β = 11.93 mg/dL; 95% CI: 2.31, 21.54); (β = 11.62 mg/dL; 95% CI: 3.84, 19.39), respectively) (Table 4). 3PCBs only reached statistical significance with LDL-C (for a 10-fold increase in 3PCBs, β = 11.44 mg/dL; 95% CI: 0.02, 22.89) and p,p'-DDE was not significantly associated with any of the CM outcomes. HOME-IR and HDL-C were not associated with any of the measured POPs (Table 4). Nevertheless, we observed a positive association between dioxin-like PCB-118 and HOME-IR in the sensitivity analysis (for a 10-fold increase in PCB-118, β = 0.37; 95% CI: 0.02, 0.71) (Table S14). Total cholesterol and LDL-C levels showed a stronger positive association with 3PCBs in girls (for a 10-fold increase, (β = 26.02 mg/dL; 95% CI: 8.77, 43.24) and (β = 24.71 mg/dL; 95% CI: 20.01, 29.41), respectively) than in boys (β = -3.64 mg/dL; 95% CI: -23.62, 16.37) and (β = -2.65 mg/dL; 95% CI: -19.99, 14.69), respectively) (p-interaction = 0.05) (Table S15). Including all four POPs in one multivariable model reduced some of the effect estimates and the following associations were no longer statistically significant: p,p'-DDT and HCB with CM-risk score, HCB and triglycerides, and 3PCBs and LDL-C (Table S16).

4. Discussion

This is the first birth cohort study examining the long-term effects of prenatal exposure to POPs on obesity and other CM risk factors in adolescents. We found that prenatal exposure to p,p'-DDT and HCB was associated with increased BMI, other adiposity measures, and BP in childhood and adolescence, and a higher CM risk at 14 years. These findings provide prospective evidence that in utero exposure to POPs increases the risk for metabolic disorders in the first years of life and that these effects may persist through age 18 years.

4.1. POPs and adiposity

We found that prenatal p,p'-DDT and HCB concentrations were significantly associated with increased BMI during childhood and adolescence (from 4 to 18 years old), as well as WHR during adolescence. We also found a positive association between prenatal HCB and body fat % in adolescence. These findings confirm that the relationship between prenatal p,p'-DDT and HCB and BMI reported previously in this cohort at 6 years old persists at later stages (Smink et al., 2008; Valvi et al., 2012). HCB has also been associated with an increased risk of overweight in childhood and rapid growth in early infancy in other INMA-Spanish cohorts (Agay-Shay et al., 2015; Valvi et al., 2014) as well as in birth cohorts from the Faroe Islands and Greece (Karlén et al., 2017; Vafeiadi et al., 2015). Conversely, two cohorts from Belgium and the US reported non-significant associations of prenatal exposures to HCB and child anthropometry in childhood (Cupul-Uicab et al., 2013; Delvaux et al., 2014). Higher exposure levels of HCB in Menorca compared to these cohorts could partly explain these inconsistencies. Prenatal exposure to p,p'-DDT has been linked to a higher child BMI in many previous prospective studies (Coker et al., 2018; Delvaux et al., 2014; Heggeseth et al., 2015; Iszatt et al., 2015; Tang-Péronard et al., 2014; Vafeiadi et al., 2015; Warner et al., 2017; Warner et al., 2014), including the Spanish-INMA cohorts (Agay-Shay et al., 2015; Valvi et al., 2014; Valvi et al., 2012), but non-significant associations have been also reported in other studies (Cupul-Uicab et al., 2013; Garced et al., 2012; Hayer et al., 2014; Karlén et al., 2017; Lauritzen et al., 2018). In the prior INMA-Menorca cohort analysis of 344 children at age 6 years only, we found a non-monotonic association between prenatal p,p'-DDT exposure and the risk for overweight that was stronger and significant in girls only (Valvi et al., 2012). In the present longitudinal analysis, the only exception, a higher WHR z-score associated with p,p'-DDT was observed at age 14 years, but not at 11 or 18 years (p for interaction = 0.08) (Fig. 2B, Table S8). We observed the same patterns of association for systolic and diastolic BP z-scores (p for interaction = 0.01) (Fig. 2, Table S9). Overall, 18 years was the only age-point that did not show any statistically significant associations (Fig. 2A-E, Table S8). We found no associations between ΣPCBs and any of the outcomes assessed (Table 3). Dioxin-like PCB-118 had positive associations with BMI z-score and body fat % compared to non-dioxin-like congeners, but none of these associations was statistically significant (Table S10). We did not find statistical evidence of sex interaction (p interaction > 0.10) (Table S11). Estimates from the multipollutant model revealed stronger associations between p,p'-DDT and PCB y z-score, and between HCB and body fat %, systolic BP and diastolic BP (Table S12).

3.3. POPs associations with CM outcomes at 14 years

Increased prenatal HCB concentrations were associated with higher CM risk scores in children at 14 years (for a 10-fold increase, β = 1.59; 95% CI: 0.02, 3.18), as was p,p'-DDT exposure in the third tertile (β = 0.10; 95% CI: 0.11, 2.07) (Table 4). HCB exposure in the third tertile (>0.90 mg/mL) was also associated with higher total cholesterol, triglycerides and LDL-C concentrations ([β = 14.47 mg/dL; 95% CI: 5.73, 23.21]; [β = 11.93 mg/dL; 95% CI: 2.31, 21.54]; [β = 11.62 mg/dL; 95% CI: 3.84, 19.39], respectively) (Table 4). 3PCBs only reached statistical significance with LDL-C (for a 10-fold increase in 3PCBs, β = 11.44 mg/dL; 95% CI: 0.02, 22.89) and p,p'-DDE was not significantly associated with any of the CM outcomes. HOME-IR and HDL-C were not associated with any of the measured POPs (Table 4).
Fig. 2. Adjusted associations between prenatal cord blood concentrations of POPs and BMI z-score (A), WHtR z-score (B), Body Fat % (C), Systolic BP z-score (D), and Diastolic BP z-score (E) at each age of follow-up from 4 years until age 18 years. Generalized estimating equation models were adjusted for maternal characteristics (i.e. parity history, pre-pregnancy BMI, education, socioeconomic status, smoking, and age at pregnancy), and child’s follow up visit. Body fat % model was additionally adjusted by sex. Upper values represent the p-values of the age at follow-up interaction term.
associations with continuous BMI z-scores across all ages between 4 and 18 years did not reach the level of significance at either sex, but a pattern of non-significant positive associations was observed at all ages most of which were of the same magnitude as the associations seen for p,p'-DDT. Our findings for prenatal p,p'-DDT exposure are consistent with previous studies that found positive associations with WHtR in girls at 2 years (Coker et al., 2018), BMI in boys at 12 years (Warner et al., 2017), BMI growth trajectories (Heindel et al., 2015), BMI and overweight risk at 9 years (Warner et al., 2014). On the contrary, other cohorts reported non-significant associations with BMI at 5 years (Karlsen et al., 2017; Lauritzen et al., 2018) and 7 years (Cupul-Uicab et al., 2013).

We did not find a clear association of prenatal exposure to PCBs and adiposity apart from the non-monotonic positive association with childhood overweight at age 6 years in girls shown previously in the INMA-Menorca study (Valvi et al., 2012). Our findings are in line with previous INMA-studies (Valvi et al., 2014) and other longitudinal cohorts (Cupul-Uicab et al., 2013; Delvaux et al., 2014; Karlsen et al., 2017; Lauritzen et al., 2018; Vafeiadi et al., 2015), but not with the findings of one other Spanish-INMA subcohort that found a positive association between PCB-138 and child BMI (Agay-Shay et al., 2015), and the study by Tang-Péronard et al. an association between high levels of $\Sigma$PCBs (138, 153, 180) and an elevated BMI in girls (Tang-Péronard et al., 2014). Overall, the evidence from birth cohorts about the potential obesogenic role of PCBs exposure is inconclusive, which could be partially due to the different PCB exposure mixture and ranges across studies.

The underlying mechanism(s) of p,p'-DDT and HCB-induced adiposity in humans are understood only in part. New experimental studies have shown mixtures of POPs to increment weight and visceral fat in animals, as well as fat uptake and storage in adipocytes (Cano-Sancho et al., 2017). p,p'-DDT is suggested to be an oestrogen receptor $\alpha$ agonist and androgen receptor antagonist, while HCB an androgen receptor and oestrogen receptor-related $\gamma$ antagonist (Li et al., 2008). The pivotal role of oestrogens in regulating energy balance is well known, either by acting directly on the brain or through activation of oestrogen receptor on adipocytes (Mauvais-Jarvis et al., 2013). Several in vitro models showed that p,p'-DDT exposure increased lipid accumulation and adipocyte differentiation, by increasing the mRNA expression of
peroxisome proliferator-activated receptor γ (PPARγ), the master regulator of adipogenesis (Canoso et al., 2017).

4.2. POPs and BP

We found that p,p′-DDT and HCB were associated with higher BP in adolescence. Prenatal exposure to HCB and p,p′-DDE has also been associated with increased BP levels in Greek preschool children (Vafeiadi et al., 2015). In South Korea, circulating PCBs in 8-year-old children were related to higher BP levels after a one-year follow-up (Lee et al., 2016). In adults, exposure to p,p′-DDT, p,p′-DDE and PCBs have been associated with a higher risk for hypertension (Donat-Vargas et al., 2018; Goncharov et al., 2011; La Merrill et al., 2014; Lind et al., 2014; Valera et al., 2013) and stroke (Lim et al., 2018). HCB has also been linked to hypertension (Arrebola et al., 2015) and abnormal growth of the left ventricle in the elderly (Sjöberg Lind et al., 2013). Our study suggests that any impact of HCB and p,p′-DDE exposure on BP may already start early in life.

We did not detect sex interaction between DDT and BP, which could be due to methodological limitations, such as the modest sample size. However, based on prior knowledge, we hypothesized that the association between p,p′-DDT and high BP may be due to its effects on adiposity or its anti-androgenic activity that may directly affect BP levels, as low testosterone levels are linked to hypertension; and p,p′-DDE levels have been inversely associated with testosterone levels in men (Park et al., 2016). Experimental studies have also reported hypertension in animal models due to exposure to p,p′-DDT (La Merrill et al., 2016) and HCB (Castilla et al., 2018).

4.3. POPs and CM risk at 14 years old

Our study showed that prenatal exposure to p,p′-DDT and HCB was associated with an increased CM risk at 14 years. HCB was also associated with greater levels of serum lipids (total cholesterol, triglycerides, and LDL-C). We further observed an association between prenatal exposure to PCBs and LDL-C. This relationship was stronger in girls, who presented an association with total cholesterol as well. PCBs are known to exhibit estrogenic, antitoxicogenic, and antiandrogenic effects (Li et al., 2008), which may explain in part the sex-specific associations. These results differ from a previous study in which prenatal exposure to p,p′-DDE, HCB and PCBs (118, 138, 153, 156, 170, 180) was not associated with blood lipids (total cholesterol, HDL-C, and LDL-C) at 4 years old (Vafeiadi et al., 2015). None of the POPs was associated with HOMA-IR, except for the dioxin-like PCB-118, which may be due to its binding on the aryl-hydrocarbon receptor (Lee, 2011). A previous study conducted in the Faroe Islands reported positive associations between prenatal non-dioxin-like ZPCBs (138, 153, 180), p,p′-DDE and HCB with high insulin levels in 5–7-year-old girls (Tang-Péronard et al., 2015). Differences in POPs levels and low HOMA-IR levels in our population could explain, at least in part, the inconsistencies in findings across studies. Experimental studies have suggested that POPs and other EDCs may disturb blood lipids balance at different levels. First, by enhancing the liver synthesis of cholesterol and triglycerides (Bioll et al., 1998). Second, via down-regulation the expression of genes that regulate lipid metabolism (Ruzzin et al., 2010). High DDE (median, 1.04 ng/mL) was lower than the median exposure in other studies (Vafeiadi et al., 2015, 2.04 ng/mL; Cupul-Uicab et al., 2013, 24.59 ng/mL; Garced et al., 2012, 7.60 ng/mL; Lauritzen et al., 2018, 1.30 ng/mL). Conversely, HCB concentrations (0.68 ng/mL) were higher than those previously reported (Vafeiadi et al., 2015, 0.09 ng/mL; Cupul-Uicab et al., 2013, 0.24 ng/mL; Lauritzen et al., 2018, 0.10 ng/mL). Exposure to PCBs (0.55 ng/mL) was lower than previously reported levels in the USA (Cupul-Uicab et al., 2013, 2.74 ng/mL) and in the Faroe Islands (Karsen et al., 2017, 3.56 ng/mL) but somewhat higher than in another cohort in Greece (Vafeiadi et al. 2015., 0.32 ng/mL). Therefore, this cohort represents a general western population with little industrial activity but with high exposure to some POPs in the past. Strengths of this study include its prospective design with repeated outcome assessments, and its long follow-up period (from pregnancy to late adolescence). This study is also strengthened by detailed childhood adiposity and CM measurements. We included body fat %, which

Table 4

<table>
<thead>
<tr>
<th>CM outcomes</th>
<th>POP exposure level</th>
<th>p,p′-DDT β (95% CI)</th>
<th>p,p′-DDE β (95% CI)</th>
<th>HCB β (95% CI)</th>
<th>∑PCBs β (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR (n = 219)</td>
<td>T2</td>
<td>-0.08 (-0.34, 0.18)</td>
<td>0.01 (-0.27, 0.28)</td>
<td>0.02 (-0.25, 0.29)</td>
<td>0.03 (-0.23, 0.29)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.02 (-0.26, 0.30)</td>
<td>0.14 (-0.13, 0.41)</td>
<td>0.22 (0.07, 0.50)</td>
<td>0.07 (-0.22, 0.35)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL) (n = 219)</td>
<td>T2</td>
<td>-2.84 (-10.90, 5.22)</td>
<td>-0.40 (-8.90, 8.11)</td>
<td>7.79 (0.26, 15.84)</td>
<td>0.20 (-7.88, 8.29)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>1.21 (-7.62, 10.03)</td>
<td>1.36 (-6.97, 9.69)</td>
<td>14.47 (5.73, 23.21)</td>
<td>7.68 (-1.21, 16.38)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL) (n = 219)</td>
<td>T2</td>
<td>-0.74 (-7.07, 5.60)</td>
<td>-1.57 (-10.96, 7.83)</td>
<td>12.55 (16.67, 26.73)</td>
<td>11.93 (-1.01, 24.87)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>-2.45 (-13.03, 4.52)</td>
<td>7.04 (-2.17, 16.24)</td>
<td>5.95 (-2.90, 14.80)</td>
<td>1.68 (-7.15, 10.51)</td>
</tr>
<tr>
<td>HDL-C (mg/dL) (n = 219)</td>
<td>T2</td>
<td>-0.74 (-7.07, 5.60)</td>
<td>-1.57 (-10.96, 7.83)</td>
<td>12.55 (16.67, 26.73)</td>
<td>11.93 (-1.01, 24.87)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>-1.21 (-10.81, 8.38)</td>
<td>5.51 (-3.51, 14.52)</td>
<td>11.93 (2.31, 21.54)</td>
<td>7.68 (-2.24, 17.16)</td>
</tr>
<tr>
<td>LDL-C (mg/dL) (n = 219)</td>
<td>T2</td>
<td>-3.94 (-10.78, 2.90)</td>
<td>1.68 (-8.52, 11.90)</td>
<td>12.41 (-3.41, 28.23)</td>
<td>12.83 (-1.22, 26.87)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>-1.76 (-5.03, 1.50)</td>
<td>-0.19 (-3.64, 3.26)</td>
<td>1.56 (1.78, 4.90)</td>
<td>3.02 (-0.25, 6.29)</td>
</tr>
<tr>
<td>CM risk Score (n = 216)</td>
<td>T2</td>
<td>0.38 (-3.19, 3.95)</td>
<td>-0.34 (3.32, 4.33)</td>
<td>1.63 (2.00, 5.26)</td>
<td>-0.34 (-0.34, 2.66)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.83 (-1.73, 3.38)</td>
<td>-0.58 (-4.37, 3.22)</td>
<td>0.83 (4.97, 6.65)</td>
<td>-2.39 (-7.67, 2.86)</td>
</tr>
<tr>
<td>LDL-C (mg/dL) (n = 219)</td>
<td>T2</td>
<td>-1.23 (-3.89, 5.93)</td>
<td>-1.15 (-8.68, 3.68)</td>
<td>5.57 (1.59, 12.73)</td>
<td>-3.61 (-3.71, 14.39)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>0.22 (-7.61, 8.66)</td>
<td>1.35 (-6.02, 8.73)</td>
<td>11.62 (3.84, 19.39)</td>
<td>6.87 (-0.93, 14.68)</td>
</tr>
</tbody>
</table>

Note: ¶, non-linear association; CI, confidence interval; CM, cardiometabolic; HOMA-IR, homeostatic model assessment for insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Second (T2) or third tertile (T3) compared with first. *All linear regression models were adjusted for maternal characteristics (i.e. parity history, pre-pregnancy BMI, education, socioeconomic status, smoking, and age at pregnancy), and child’s sex and age.
6. Conclusion

This study suggests that the previously reported associations with child BMI of at least some of the POPs may persist later in adolescence and further, that prenatal POP exposure may be related to major risk factors for adult metabolic syndrome. These findings require further exploration in other settings and larger cohorts.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Supplementary data

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References


