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Prenatal DDT exposure and child adiposity at age 12: The CHAMACOS study

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ABSTRACT

Objective: Using data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort study, we assessed the association of *in utero* exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenylethylene (DDE) with child adiposity at age 12.

Methods: We included 240 children with *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE concentrations measured in maternal serum collected during pregnancy (ng/g lipid) and complete 12-year follow-up data. Age- and sex-specific body mass index (BMI) *z*-scores were calculated from CDC growth charts. Children with BMI *z*-scores ≥ 85th percentile were classified as overweight or obese.

Results: At 12 years, BMI *z*-score averaged 1.09 (± 1.03) and 55.4% of children were overweight or obese. Prenatal DDT and DDE exposure was associated with several adiposity measures in boys but not girls. Among boys, 10-fold increases in prenatal DDT and DDE concentrations were associated with increased BMI *z*-score (*o,p′*-DDT, adj-β = 0.37, 95% CI: 0.08, 0.65; *p,p′*-DDT, adj-β = 0.26, 95% CI: 0.03, 0.48; *p,p′*-DDE, adj-β = 0.31, 95% CI: 0.02, 0.59). Results for girls were nonsignificant. The difference by sex persisted after considering pubertal status.

Conclusions: These results support the chemical obesogen hypothesis, that *in utero* exposure to DDT and DDE may increase risk of obesity in males later in life.

1. Introduction

In utero exposure to endocrine disrupting chemicals has been hypothesized to increase risk of obesity in childhood and into adulthood (Heindel and vom Saal, 2009; Tang-Peronard et al., 2011; Heindel and Schug, 2013; Heindel et al., 2015). The organochlorine pesticide dichlorodiphenyltrichloroethane (DDT), and its primary breakdown product, dichlorodiphenyldichloroethylene (DDE), are persistent organic pollutants with known endocrine disruptor activity (Agency for Toxic Substances and Disease Registry, 2002). DDT has been shown to have estrogenic effects, while DDE acts as an androgen antagonist (Kelce et al., 1995; Klotz et al., 1996; Kojima et al., 2004).

In experimental studies, both DDT and DDE exposure are associated with adipose dysfunction. *In vitro*, DDT and DDE increase adipocyte differentiation and expression patterns of CCAAT enhancer binding protein-α and peroxisome proliferator-activated receptor-γ, the main transcription factors regulating the adipogenic process (Moreno-Aliaga and Matsumura, 2002; Kim et al., 2016). DDE has also been shown to increase basal free fatty acid uptake and adipokine (leptin, resistin, adiponectin) production in 3T3-L1 adipocytes (Howell and Mangum, 2011).

Several longitudinal birth cohort studies have examined associations between prenatal exposure to DDT or DDE and child adiposity (Valvi et al., 2012; Cupul-Uicab et al., 2013; Warner et al., 2013, 2014; Delvaux et al., 2014; Hoyer et al., 2014; Tang-Peronard et al., 2014; Agay-Shay et al., 2015). With follow-up ages ranging from 5 to 9 years, significant positive associations between prenatal DDT and/or DDE exposure and body mass index (BMI) *z*-score or overweight status have been reported in some (Valvi et al., 2012; Warner et al., 2014; Agay-Shay et al., 2015), but not all studies (Cupul-Uicab et al., 2013; Hoyer et al., 2014; Tang-Peronard et al., 2014). Inconsistent sex-specific effects have been noted, with associations reported in males only (Valvi et al., 2012; Delvaux et al., 2014; Tang-Peronard et al., 2014; Warner et al., 2014), females only (Delvaux et al., 2014; Tang-Peronard et al., 2014), and both males and females (Agay-Shay et al., 2015).

Using data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a longitudinal birth cohort in a California agricultural community, we previously reported sex-specific positive associations between prenatal DDT and DDE exposure and several adiposity measures at 9 years of age (Warner et al., 2014). Specifically, in boys, we reported that higher prenatal DDT concentrations were significantly associated with increased BMI *z*-scores. Also in

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boys, both prenatal DDT and DDE concentrations were associated with significantly increased waist circumference *z*-scores, and odds of increased waist circumference and overweight. In contrast, in girls, we found no evidence of associations between prenatal DDT and DDE concentrations and any measure of adiposity.

Here, we report results from follow-up of the CHAMACOS cohort at 12 years of age when almost all (93%) children had entered puberty based on assessment by the Tanner scale (Tanner, 1986). We assessed the association of maternal serum concentrations of *o,p´*-DDT, *p,p´*-DDT, and *p,p´*-DDE during pregnancy with child adiposity at age 12 including BMI *z*-score, waist circumference, percent body fat, and overweight or obesity status. We also examined whether these relationships were modified by child sex.

2. Methods

2.1. Study population

The CHAMACOS study is a longitudinal birth cohort study of environmental exposures and childhood growth and development. Details of the study population are presented elsewhere (Eskenazi et al., 2003). Pregnant women were recruited from prenatal clinics serving the farmworker population in the Salinas Valley, California between 1999 and 2000. Eligible women were at least 18 years of age, less than 20 weeks gestation, English- or Spanish-speakers, qualified for government-sponsored health insurance, and planned to deliver at the county hospital. The study was approved by the Institutional Review Boards at participating institutions. Prior to participation, we obtained written informed consent from all mothers, oral assent from children beginning at age 7, and written assent from children at age 12.

Of 601 women who were initially enrolled, 527 were followed through delivery of a singleton liveborn child who survived the neonatal period, and 417 provided a serum sample during pregnancy for DDT and DDE analysis. Of these, complete follow-up interview, puberty assessment, and anthropometric measurements were available for 240 children at age 12. The children included in the analysis did not differ significantly from those who were excluded due to missing prenatal exposure measures or 12-year anthropometric data in terms of maternal sociodemographic characteristics (education, marital status, income), maternal pre-pregnancy BMI or child birthweight, maternal serum DDT and DDE levels, or child overweight status (data not shown).

2.2. Procedure

Details of the study methods are presented elsewhere (Eskenazi et al., 2003, 2004). Briefly, women were interviewed in English or Spanish using structured questionnaires twice during pregnancy, after delivery, and when children were 6 months, 1, 2, 3½, 5, 7, 9, 10½, and 12 years old. During each interview, information collected included family sociodemographics, maternal characteristics, medical histories, child-based developmental milestones, and diet and behavioral information. Mothers were weighed and height was recorded. Beginning at the 9-year visit, clinical Tanner staging was conducted by trained research staff. Children were considered to have entered puberty if they were stage 2 + for breast development for girls or stage 2 + for pubic hair or genital development for boys.

At each visit, children were weighed and measured. At 12-years, barefoot standing height was measured to the nearest 0.1 cm using a stadiometer. Standing weight to the nearest 0.1 kg and percent body fat were measured using a bioimpedence scale (Tanita TBF-300A Body Composition Analyzer). For calculation of percent body fat, the scale was set to child mode and the manufacturer's algorithm, validated for children 7 years and older, was used. Waist circumference was measured to the nearest 0.1 cm by placing a measuring tape around the abdomen at the level of the iliac crest, parallel to the floor. Both height and waist circumference were measured in triplicate and averaged for analysis.

2.3. Serum DDT and DDE concentrations

Maternal serum samples were collected by venipuncture at about 26 weeks gestation (n = 215) or delivery (n = 25). Serum *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE concentrations were measured at the Centers for Disease Control and Prevention by isotope dilution gas chromatography-high resolution mass spectrometry methods (Barr et al., 2003) and reported on a whole-weight basis (pg/g). The limit of detection (LOD) was 1.3, 1.5, and 2.9 pg/g serum for *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE, respectively. For values below the LOD, a serum level equal to one-half the detection limit was assigned (Hornung and Reed, 1990). Lipid-adjusted values (ng/g) were calculated by dividing *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE on a whole-weight basis by total serum lipid content, estimated by a "summation" method (Phillips et al., 1989).

2.4. Statistical analysis

Lipid-adjusted concentrations of *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE were $\log_{10}$-transformed and analyzed as continuous variables. Age- and sex-specific BMI (kg/m^2) *z*-scores and percentiles for each child were calculated using 2000 Centers for Disease Control and Prevention growth charts (Kuczmarski et al., 2002). Children who were ≥ 85th percentile but < 95th percentile for their age and sex were classified as overweight, and those who were ≥ 95th percentile were classified as obese. Age- and sex-specific waist circumference *z*-scores and percentiles for each child were calculated using NHANES III reference data for Mexican-American children (Cook et al., 2009). Children who were in the 90th percentile or higher were classified as having increased waist circumference (Zimmet et al., 2007).

For all outcomes, we used generalized additive models with a 3-degrees-of-freedom cubic spline to evaluate the shape of the exposure-response curves in the full sample, and in boys and girls separately. None of the digression from linearity tests was significant ($p \leq 0.15$), suggesting the relationships were linear. We examined the relationship of maternal serum DDT and DDE concentrations with continuous outcomes (BMI *z*-score, waist circumference *z*-score, percent body fat) using multivariable linear regression, and with binary outcomes (overweight (≥ 85th *versus* < 85th percentile), obesity (≥ 95th *versus* < 95th percentile), increased waist circumference (≥ 90th *versus* < 90th percentile)) using multivariable Poisson regression with a robust variance estimator. Due to the high prevalence of binary outcomes, we used a Poisson link function to estimate the relative risk (RR) instead of the odds ratio.

Based on our review of the obesity literature and directed acyclic graphs, we considered the following variables as potential confounders. Maternal variables considered included country of birth, race, years in the United States at childbirth, education level, marital status, household socioeconomic status, pre-pregnancy BMI, weight gain during pregnancy, age at delivery, smoking during pregnancy, BMI at 12-year visit, and household food insecurity. Child variables considered included age, sex, birthweight, birth order, low birth weight (< 2500 g) and preterm (< 37 weeks) status, breastfeeding duration, and pubertal status at 12-year visit. Based on the hypothesized causal relationships between these variables, the minimal set of confounders for adjustment were maternal pre-pregnancy BMI (continuous), years in the United States at childbirth (continuous), and puberty stage (ordinal). We considered effect modification by child sex in all analyses by including a cross-product term between exposure and sex. Interaction *p*-values < 0.2 were considered significant.

We also examined the relationship between maternal serum DDT and DDE concentrations and 2- to 12-year BMI *z*-scores longitudinally, using generalized estimating equations (GEE) with robust standard errors and exchangeable correlation structure. We applied GEE to account for correlations among repeated outcome measures from the same