

Prenatal Exposure to Persistent Organic Pollutants and Rapid Weight Gain and Overweight in Infancy

Damaskini Valvi^{1,2,3,4}, Michelle Ann Mendez⁵, Raquel Garcia-Esteban^{1,2,3}, Ferran Ballester^{3,6,7}, Jesús Ibarluzea^{3,8,9}, Fernando Goñi^{3,9,10}, Joan O. Grimalt¹¹, Sabrina Llop^{3,6}, Loreto Santa Marina^{3,8,9}, Esther Vizcaino^{11,12}, Jordi Sunyer^{1,2,3,4} and Martine Vrijheid^{1,2,3}

Objective: To examine the effects of prenatal exposure to persistent organic pollutants (POPs) on rapid growth in the first 6 months of life and overweight at 14 months of age.

Design and Methods: In a Spanish birth cohort study, the POPs dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs—congeners 153, 138, 180) were measured in maternal serum collected in the first trimester of pregnancy during 2003–2008. Rapid growth was defined as a z-score weight gain >0.67 SD between 6 months of age and birth. Overweight at 14 months was defined as a BMI z-score ≥85th percentile. Generalized linear models examined the association between POPs and rapid growth ($N = 1285$) and overweight ($N = 1198$).

Results: The analysis population included 24% rapid growers and 30% overweight infants. DDE and HCB were positively associated with rapid growth and with overweight. There was some indication that infant sex and exclusive breastfeeding duration may modify the effects of DDE, and that maternal pre-pregnancy BMI status may influence the effects of HCB. PCBs were not related to postnatal growth.

Conclusion: Prenatal exposure to DDE and HCB may be associated with early postnatal growth. Further research is needed to evaluate the persistence of these associations at older ages.

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Introduction

Obesity is a complex condition influenced by genetic predisposition and environmental risk factors with the imbalance between energy intake and energy expenditure as the primary cause (1). Recently it has been hypothesized that environmental chemical exposures may influence metabolic programming, especially if exposure occurs *in utero*. The “environmental obesogen hypothesis” postulates that early-life exposure to chemicals that could mimic or block the natural action of endogenous hormones may perturb the mechanisms involved in adipogenesis or energy storage and, thus, may increase

an individual’s susceptibility to obesity (2). A well-documented example of a chemical “obesogen” is the effect of prenatal tobacco exposure on childhood obesity (3). Up to 20 different chemical classes are now hypothesized to be obesogenic, including persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethylene (DDT) and its prime metabolite dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs) (4,5).

DDE, HCB, and PCBs are organochlorine compounds with lipophilic properties that bioaccumulate in animal and human adipose tissues

¹ Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain. Correspondence: Damaskini Valvi (dvalvi@creal.cat) ² Hospital de Mar Medical Research Institute (IMIM), Barcelona, Spain ³ CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain ⁴ Department of Experimental and Health Sciences, Pompeu Fabra University (UPF), Barcelona, Spain ⁵ Department of Nutrition, Gillings School of Public Health, University of North Carolina-Chapel Hill, North Carolina, USA ⁶ Centre for Public Health Research (CSISP-FISABIO), Valencia, Spain ⁷ School of Nursing, University of Valencia, Valencia, Spain ⁸ Public Health Division of Gipuzkoa, Basque Government, Gipuzkoa, Spain ⁹ Donostia Biomedical Research Institute (BIDONOSTIA), Gipuzkoa, Spain ¹⁰ Public Health Laboratory of Gipuzkoa, Gipuzkoa, Spain ¹¹ Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDEA-CSIC), Barcelona, Spain ¹² Department of Preventive Medicine and Public Health, University of Oviedo, Asturias, Spain

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for many years (6). DDT and HCB were used as pesticides in agriculture and PCBs were used extensively in industrial applications in the past. Their use was banned by the Stockholm convention (2004) because of their neurotoxic and potentially endocrine-disrupting effects in humans (7). Nevertheless, today lower levels of DDE, HCB, and PCBs are still detected in human populations all around the world (6). Humans are mainly exposed to POPs through diet (6) and exposure may also occur from the mother to the child through the bloodstream via placenta and later via breastfeeding (8).

There is limited prospective evidence linking prenatal POP exposure to childhood or adult obesity (reviewed in Refs. 4,9). Prenatal DDE exposure has been associated with elevated BMI in infancy (10,11) and later childhood (12), with weight-for-height in puberty (13) and with elevated BMI in adult women (14). Findings for the association between prenatal PCB exposure and childhood obesity have been less consistent (9). Only one previous study, which found a positive association, has examined the effect of prenatal HCB exposure on childhood obesity (15).

Although the mechanisms remain unclear, rapid weight gain in infancy has consistently been associated with a subsequent elevated risk of obesity in childhood and later in adulthood (16,17). Mendez et al. (10) were the first to show in the Spanish INMA-Sabadell birth cohort that prenatal DDE exposure may promote rapid weight gain in the first 6 months of life and subsequent overweight at 14 months. Maternal prepregnancy BMI status and less clearly infant sex were shown to influence these associations. As that study was too small to draw strong conclusions, particularly regarding interactions, the current study aims to evaluate these associations in a larger Spanish population, including the original cohort and two others, providing more precise and robust estimates for the effects of prenatal POP exposure on early postnatal growth. Breastfeeding may affect the risk of childhood obesity by improving energy intake regulation or by reducing the likelihood of excessive protein intake (16). Thus, we evaluated the role of breastfeeding as a potential effect modifier.

Methods

The population-based birth cohort study INMA ("Infancia y Medio Ambiente"- Environment and Childhood) recruited 2150 pregnant women in the Spanish regions of Sabadell ($N = 657$), Valencia ($N = 855$) and Gipuzkoa ($N = 638$) between 2003 and 2008 (18). Women were enrolled in the first trimester of pregnancy at public health care centers or hospitals. The inclusion criteria were age ≥ 16 years, intention to give birth in the reference hospital, no communication problems, singleton pregnancy, and no assisted conception (18). From those initially recruited, 1361 infants were born at term (≥ 37 weeks of gestation) and had complete data for POP exposure and rapid growth and thus were eligible for analysis. All participants provided informed consent. The study was approved by the hospital ethics committees of each participating region.

Information was collected through questionnaires administered by trained personnel in the first and third trimesters of pregnancy and later at ages 6 and 14 months. Questions referred to parental socio-demographic characteristics (age, education, occupation) and other maternal characteristics (including medical history, parity, medica-

tion, alcohol consumption in pregnancy, and active or passive smoking). Maternal diet was assessed in the first and third trimesters using a 101-item food frequency questionnaire validated for use in Spanish adults (19). Infant feeding practices (breast- and formula-feeding duration and solid food introduction) were reported in post-natal questionnaires. Exclusive breastfeeding duration was defined based on the timeframe during which infants received breast milk with or without supplementary nonmilk liquids such as water, without formula milk, or solid foods. Infant weight (nearest gram) and recumbent length (nearest 0.1 cm) at birth and around 14 months of age were measured by trained staff using standard protocols (20). Repeated weight measures from birth to 6 months of age were extracted from medical records. Infants were categorized as small for gestational age if their birth weight was <10 th percentile of a Spanish reference (21).

Postnatal Growth

For infants without weight measures available within ± 14 days of their exact 6-month anniversary ($N = 152$, 11% of the analysis population), we estimated their weight at 6 months of age using pre-existing sex-specific growth curves described in the literature (22). We compared six growth models (the Count, Kouchi, first- and second-order Reed, Jenss, and I-component of Karlberg models) (22). The best fit was obtained for the second-order Reed model for both sexes (data not shown). Age- and sex-specific z -scores for weight at birth and at 6 months of age were calculated using the World Health Organization referent (23). Rapid growth from birth to 6 months of age was then defined as a z -score weight gain >0.67 SDs (17); infants with a z -score weight gain ≤ 0.67 SDs were characterized as slow/average growers. Infant BMI (weight/length²) measured at 14 months was used to estimate age- and sex-specific BMI z -scores (23). Overweight at 14 months was defined as a BMI z -score ≥ 85 th percentile (23). Consistent results were obtained defining overweight at 14 months using weight-for-length z -scores (23) (data not shown).

POP Exposure

POP concentrations (DDT, DDE, HCB, β -hexachlorocyclohexane, and the PCB congeners 28, 52, 101, 118, 138, 153, and 180) were measured in maternal serum obtained between the 7th and 26th week of pregnancy. Samples were analyzed using gas chromatography with electron capture detection (GC-ECD) in the Gipuzkoa Basque Government Public Health Laboratory (samples from Sabadell and Gipuzkoa) and in the IDÆA-CSIC (Barcelona) (samples from Valencia), as described previously (24,25). The detection limits (LOD) were 0.071 ng/ml for all POPs measured in the Sabadell and Gipuzkoa subcohorts and between 0.010 and 0.071 ng/ml depending on the POP measured in the Valencia subcohort. With the aim of homogenizing data among subcohorts, POP concentrations with values below 0.071 ng/ml in Valencia were declared as non-detected (0–10% of analyzed samples). DDT, β -hexachlorocyclohexane, and the PCB congeners 28, 52, 101, and 118 were excluded from statistical analysis because the percentages of values below LOD were high ($>60\%$ of analyzed samples) in at least one of the subcohorts. A value equal to half the LOD was set to samples with concentrations below LOD. Because POPs are highly lipophilic, all concentrations were adjusted for maternal serum lipid content. Total PCB concentration was estimated by summation of the concentrations of the PCB congeners 138, 153, and 180 (Σ PCB hereafter). All POP

concentrations were log-transformed to obtain normal distributions and used both continuously and categorically (quartile cut-offs). Median cut-offs were further applied with the aim to test for potential chemical by chemical interactions on the effects of interest.

Statistical Analysis

We explored the shape of the relationships between POP concentrations and rapid growth and overweight using multivariable generalized additive models (GAMs). Multivariate generalized linear models for binary outcomes (with log link, Poisson distribution, and robust variance estimator) (26) were used to estimate relative risks (RRs) and 95% confidence intervals (CIs) for the associations between the POP exposures and the outcome variables. To evaluate whether findings were explained by the previously analyzed Sabadell subcohort (10), all main analyses were performed for the total study population (including the Sabadell subcohort) and for the two new subcohorts. Models always included study subcohort, infant sex, and exact age at examination at 6 months (for rapid growth) or at 14 months (for overweight). Potential confounders were selected based on previous literature related to predictors of POP exposure (6,8,27) and/or postnatal growth (1,10,15,28): gestational age, birth length, exclusive breastfeeding duration, maternal age at delivery, maternal country of origin, maternal and paternal education, maternal and paternal social class (based on occupation according to the International Standard Classification of Occupations-88 system; I and II: professionals and managers, III: other non-manual, IV and V: skilled, semiskilled, and unskilled manual), maternal and paternal prepregnancy BMI (both reported by the mother), maternal weight gain in pregnancy, maternal active smoking during pregnancy, parity, weeks of gestation at blood sampling, and maternal dietary intakes in the first or third trimester of pregnancy of total calories, total fat, proteins, carbohydrates, and fruits and vegetables. We have previously shown that fish intake in pregnancy is associated to maternal serum POP levels (27), thus fatty and lean fish consumption in pregnancy were also evaluated as confounders. We retained in the final models only covariates that modified the RRs for the POP exposure variables by $\geq 10\%$. For rapid growth, the final multivariable model included study subcohort, infant sex, exact age at 6-month examination, gestational age, exclusive breastfeeding duration, and several maternal characteristics: country of origin, social class, prepregnancy BMI status, age at delivery, and smoking during pregnancy. For overweight at 14 months, the final multivariable model included study subcohort, infant sex, exact age at 14-month examination, exclusive breastfeeding duration, and the following maternal characteristics: country of origin, education, prepregnancy BMI status, age at delivery, and smoking during pregnancy. Birth weight could be an intermediate in the effect of prenatal POP exposure on postnatal growth. Thus, we did not perform baseline adjustment for birth weight in the main models, but only in sensitivity analyses. Five percent of the eligible analysis population ($N = 1361$) had missing values in covariates (data not shown), thus, analyses for rapid growth included 1285 observations. Analysis for overweight included somewhat fewer observations because of missing values in this outcome variable ($N = 1198$).

We studied the associations of interest separately for each POP and, despite the moderate collinearity among the POPs, in a multipollutant model adjusted for all three POP exposures. Models were also stratified by infant sex, maternal prepregnancy BMI status, and exclusive breastfeeding duration in order to evaluate the homogene-

ity of effects between these subgroups of *a priori* interest. The statistical significance of interaction terms involving the exposures and these stratification variables was assessed by likelihood ratio tests. Sensitivity analyses included evaluating the impact of birth weight as a covariate in the multivariate models and, further, the exclusion of small for gestational age for weight infants and/or of infants with low birth weight (i.e., <2500 g) because they are prone to postnatal catch-up growth (29). Further, we repeated all analyses using the POP concentrations in ng/ml instead of the lipid-adjusted (in ng/g lipid) concentrations with and without further including maternal serum lipid concentrations as a separate covariate in the models.

All analyses were performed using the statistical package STATA 10.1 (Stata Corporation, College Station, TX, USA).

Results

Study Population Characteristics

The prevalence of rapid growth in the first 6 months of life in the total study population was 24% ($N = 311$). Rapid growers were born on average 3.5 gestational days earlier than slow/average growers and had lower average weight and length at birth (mean difference 343 g and 0.9 cm, respectively) (Table 1). At ages 6 and 14 months, rapid growers were heavier and taller than slow/average growers. At 14 months of age, 49.4% of rapid growers were overweight, while slow/average growers were less likely to be overweight (23.2%). Rapid growers were more likely to be a first-born child, less likely to have been exclusively breastfed for >16 weeks and their mothers were on average somewhat younger at the time of delivery, of lower education, and more likely to have smoked during pregnancy compared to mothers of slow/average growers. Both parents of rapid growers were more likely to be of lower social class compared to parents of slow/average growers. Infants who were overweight at the age of 14 months ($N = 358$) were heavier at birth and at 6 months of age than infants with BMI z -scores below the 85th percentile (mean difference 130 and 799 g, respectively; Supporting Information Table S1). Mothers and fathers of overweight infants were more likely to have been overweight/obese before pregnancy and mothers were more likely to have a higher education and to have smoked during pregnancy (Supporting Information Table S1). After excluding the Sabadell subcohort we observed similar characteristics to be associated with rapid growth and overweight status in infancy (Table 1 and Supporting Information Table S1). Further, infants included in the total study population had similar characteristics to those excluded due to loss to follow-up or missing (data not shown).

DDE was the most highly detected POP in maternal serum followed by Σ PCB and HCB (Table 2). DDE and HCB average concentrations were higher in Valencia than in the Sabadell and Gipuzkoa subcohorts, while Σ PCB concentrations were highest in Gipuzkoa and Valencia. In the total study population, Spearman correlation coefficients (P -value) were 0.32 (<0.001) for DDE- Σ PCB, 0.36 (<0.001) for DDE-HCB, and 0.43 (<0.001) for Σ PCB-HCB.

Prenatal POP Concentrations and Postnatal Growth

Multivariable-adjusted GAMs (data not shown) indicated that relationships between all POPs and rapid growth and overweight were

TABLE 1 Population characteristics in the subgroups of slow/average and rapid growers in the total study population (including the Sabadell subcohort) and in the Gipuzkoa and Valencia subcohorts only

Covariates	Total study population (N = 1285)			Gipuzkoa and Valencia subcohorts only (N = 790)		
	Slow/average growers N = 974 Value ^a	Rapid growers N = 311 Value ^a	P-value ^b	Slow/average growers N = 606 Value ^a	Rapid growers N = 184 Value ^a	P-value ^b
Infant characteristics						
Female sex	49.9	46.9	0.36	50.2	47.3	0.49
Gestational age (weeks)	40.0 ± 1.1	39.5 ± 1.2	<0.01	40.0 ± 1.1	39.4 ± 1.2	<0.01
Birth weight (g)	3380 ± 395	3037 ± 405	<0.01	3404 ± 405	3017 ± 413	<0.01
Birth weight z-score (SD) - All	0.16 ± 0.81	-0.59 ± 0.90	<0.01	0.21 ± 0.82	-0.64 ± 0.93	<0.01
Females	0.14 ± 0.83	-0.70 ± 0.90	<0.01	0.18 ± 0.84	-0.73 ± 0.96	<0.01
Males	0.18 ± 0.78	-0.49 ± 0.88	<0.01	0.23 ± 0.80	-0.56 ± 0.90	<0.01
Birth length (cm)	49.9 ± 1.9	49.0 ± 2.0	<0.01	50.0 ± 1.9	48.9 ± 2.2	<0.01
Exact age at 6 months (months)	6.1 ± 0.1	6.1 ± 0.2	0.19	6.1 ± 0.1	6.1 ± 0.1	0.50
Weight at 6 months (g)	7489 ± 754	8358 ± 928	<0.01	7520 ± 762	8368 ± 976	<0.01
Weight z-score at 6 months (SD) - All	-0.21 ± 0.80	0.71 ± 0.88	<0.01	-0.17 ± 0.99	0.73 ± 0.93	<0.01
Females	-0.18 ± 0.79	0.57 ± 0.85	<0.01	-0.19 ± 0.79	0.57 ± 0.91	<0.01
Males	-0.24 ± 0.80	0.84 ± 0.89	<0.01	-0.16 ± 0.79	0.87 ± 0.94	<0.01
Length at 6 months (cm)	66.9 ± 2.3	67.6 ± 2.5	<0.01	67.0 ± 2.3	67.4 ± 2.5	0.02
Exact age at 14 months (months)	13.9 ± 1.2	13.9 ± 1.2	0.56	13.4 ± 1.3	13.2 ± 1.2	0.11
Weight at 14 months (kg)	10.0 ± 1.1	11.0 ± 1.3	<0.01	10.0 ± 1.1	10.8 ± 1.2	<0.01
Length at 14 months (cm)	76.7 ± 3.1	77.9 ± 3.1	<0.01	76.2 ± 3.1	77.3 ± 3.3	<0.01
BMI at 14 months (kg/m ²)	17.0 ± 1.6	18.0 ± 1.7	<0.01	17.1 ± 1.8	18.1 ± 1.7	<0.01
BMI z-score at 14 months (SD)	0.40 ± 1.04	1.07 ± 1.04	<0.01	0.45 ± 1.17	1.09 ± 1.11	<0.01
Overweight at 14 months - yes - All	23.2	49.4	<0.01	27.6	48.7	<0.01
Females	22.9	47.7	<0.01	28.8	47.8	<0.01
Males	23.4	51.0	<0.01	26.5	49.4	<0.01
Exclusive breastfeeding >16 weeks	50.4	40.2	<0.01	50.5	38.1	<0.01
Maternal characteristics						
Serum DDE ng/g (GM ± GSD)	128.7 ± 2.4	144.6 ± 2.5	0.04	131.0 ± 2.3	147.8 ± 2.7	0.10
Serum HCB ng/g (GM ± GSD)	40.7 ± 2.5	44.9 ± 2.6	0.10	42.7 ± 2.6	50.6 ± 2.7	0.04
Serum ∑PCB ng/g (GM ± GSD)	94.1 ± 2.0	90.4 ± 2.1	0.39	112.1 ± 2.0	107.8 ± 2.2	0.51
Parity—first-born child	54.3	62.9	0.01	53.0	63.6	0.01
Age at delivery (years)	32.0 ± 4.0	31.4 ± 4.2	0.03	32.2 ± 3.9	31.4 ± 4.0	0.01
Prepregnancy BMI ≥25 kg/m ²	25.5	29.6	0.15	24.3	30.4	0.10
Education						
Primary	22.5	30.3		21.8	27.8	
Secondary	40.4	41.3		38.8	41.8	
University	37.1	28.4	<0.01	39.4	30.4	0.06
Social class						
I+II	25.1	14.8		26.6	14.7	
III	28.2	28.0		26.4	26.1	
IV+V	46.7	57.2	<0.01	47.0	59.2	<0.01
Country of origin						
Spain	92.8	90.7		93.6	91.8	
Latin America/other	7.2	9.3	0.22	6.4	8.2	0.42
Smoking during pregnancy						
No	72.8	58.8		72.4	54.3	

TABLE 1. (continued).

Covariates	Total study population (N = 1285)			Gipuzkoa and Valencia subcohorts only (N = 790)		
	Slow/average growers N = 974 Value ^a	Rapid growers N = 311 Value ^a	P-value ^b	Slow/average growers N = 606 Value ^a	Rapid growers N = 184 Value ^a	P-value ^b
Yes—until first trimester	15.0	18.0		14.4	20.1	
Yes—until third trimester	12.2	23.2	<0.01	13.2	25.5	<0.01
Paternal characteristics						
BMI ≥ 25 kg/m ²	53.0	60.1	0.03	52.9	60.1	0.09
Social class						
I+II	23.9	14.5		22.4	13.2	
III	16.4	19.5		16.4	17.6	
IV+V	59.7	66.0	<0.01	61.2	69.2	0.02

^aValues are Mean ± SD or % if not indicated otherwise.

^bChi-square for categorical variables; t-test for continuous variables (all continuous variables included in the table are normally distributed).

likely to be linear (P -gain > 0.10) in each subcohort, in the total study population and in the different subgroups defined by infant sex, maternal overweight, and exclusive breastfeeding. The POP effects on rapid growth and overweight were homogeneous among the three subcohorts (P Q -test > 0.10 for all comparisons). In the total study population, prenatal DDE concentrations were associated with both rapid growth in the first 6 months and overweight at 14 months (Table 3). Infants in the top quartile of exposure had a 32% (95% CI = -3, 78) higher risk for rapid growth and 39% (95% CI = 7, 80) higher risk for overweight. The RRs (95% CI) per log ng/g-lipid increase in DDE were 1.13 (1.01, 1.26) for rapid growth and 1.15 (1.03, 1.28) for overweight. Prenatal HCB concentrations were also associated with both rapid growth and overweight (Table 3). Infants in the top quartile of HCB exposure had a 44% (95% CI = 4, 99) higher risk for rapid growth and a 45% (95% CI = 10, 92) higher risk for overweight compared to those in the

first quartile. The RRs (95% CIs) per log ng/g-lipid increase in HCB were 1.13 (1.00, 1.29) for rapid growth and 1.19 (1.05, 1.34) for overweight. Rapid growth or overweight were not associated with the Σ PCB (Table 3) or with concentrations of individual PCB congeners 138, 153, or 180 (data not shown). Excluding the Sabadell subcohort (Table 3) did not change effect estimates, but the smaller sample size ($N = 704$ for rapid growth and $N = 704$ for overweight) led to somewhat reduced statistical significance for associations of POPs with rapid growth; for overweight they remained statistically significant.

Multipollutant adjustment of the DDE and HCB effects for the other POPs resulted in slightly weaker associations that remained statistically for overweight but not for rapid growth (Supporting Information Table S2). There was no evidence of a chemical by chemical interaction on either rapid growth or overweight for any pair of

TABLE 2 Maternal serum POP concentrations (ng/g lipid) in pregnancy in the total study population and in the different study subcohorts

POP	Total study population (N = 1285)		Valencia (N = 409)	Gipuzkoa (N = 381)	Sabadell (N = 495)
	>LOD ^a (%)	GM ± GSD	GM ± GSD	GM ± GSD	GM ± GSD
DDE	99.4	132 ± 2.4	188 ± 2.4	94.3 ± 2.1	129 ± 2.3
HCB	90.8	41.7 ± 2.5	57.6 ± 2.9	34.0 ± 2.2	38.0 ± 2.2
PCB-138	86.1	22.8 ± 2.5	24.5 ± 3.6	30.9 ± 1.8	17.0 ± 1.9
PCB-153	97.7	38.8 ± 2.2	37.6 ± 2.7	52.6 ± 1.8	31.4 ± 1.9
PCB-180	91.4	27.9 ± 2.2	29.3 ± 2.4	38.5 ± 1.8	20.9 ± 1.9
Σ PCB ^b	99.1	93.2 ± 2.0	101 ± 2.3	123 ± 1.8	70.0 ± 1.8

GM, geometric mean; GSD, geometric standard deviation.

^aLOD = 0.071 ng/ml for all POPs.

^bThe sum of the PCB congeners shown in this table.

TABLE 3 Effects of POPs on early rapid growth and subsequent overweight in the total study population and in the Gipuzkoa and Valencia subcohorts only

POP concentrations (ng/g lipid)	Total study population				Gipuzkoa and Valencia subcohorts only			
	<i>N</i>	Rapid Growth 0–6 months ^a RR (95% CI)	<i>N</i>	Overweight at 14 months of age ^b RR (95% CI)	<i>N</i>	Rapid Growth 0–6 months ^a RR (95% CI)	<i>N</i>	Overweight at 14 months of age ^b RR (95% CI)
DDE								
≤73.6	300	1	282	1	173	1	150	1
>73.6-118.8	314	1.07 (0.80, 1.44)	300	1.14 (0.91, 1.45)	194	0.88 (0.59, 1.31)	182	1.17 (0.89, 1.53)
>118.8-203.1	336	1.24 (0.93, 1.66)	319	1.08 (0.85, 1.38)	201	1.04 (0.72, 1.51)	184	0.92 (0.68, 1.24)
>203.1	335	1.32 (0.97, 1.78)	297	1.39 (1.07, 1.80)	222	1.16 (0.80, 1.40)	188	1.38 (1.01, 1.89)
Per log ng/g lipid	1285	1.13 (1.01, 1.26)	1198	1.15 (1.03, 1.28)	790	1.11 (0.96, 1.28)	704	1.16 (1.01, 1.33)
HCB								
≤22.6	291	1	268	1	172	1	148	1
>22.6-41.7	302	1.01 (0.74, 1.37)	295	1.09 (0.84, 1.41)	172	0.96 (0.62, 1.49)	164	1.34 (0.99, 1.83)
>41.7-73.0	336	1.19 (0.88, 1.62)	321	1.28 (0.98, 1.66)	189	1.32 (0.88, 1.98)	173	1.32 (0.97, 1.82)
>73.0	356	1.44 (1.04, 1.99)	314	1.45 (1.10, 1.92)	257	1.54 (1.02, 2.31)	219	1.62 (1.16, 2.26)
Per log ng/g lipid	1285	1.13 (1.00, 1.29)	1198	1.19 (1.05, 1.34)	790	1.14 (0.97, 1.32)	704	1.20 (1.05, 1.37)
∑PCB								
≤65.4	321	1	305	1	122	1	108	1
>65.4-101.3	311	1.12 (0.84, 1.50)	299	0.84 (0.63, 1.10)	168	1.26 (0.81, 1.94)	156	0.82 (0.56, 1.20)
>101.3-144.6	328	1.15 (0.84, 1.57)	302	1.06 (0.80, 1.39)	225	1.45 (0.96, 2.18)	200	1.19 (0.85, 1.68)
>144.6	325	1.09 (0.76, 1.57)	292	1.11 (0.82, 1.51)	275	1.27 (0.82, 1.97)	240	1.06 (0.74, 1.52)
Per log ng/g lipid	1285	1.10 (0.93, 1.31)	1198	1.07 (0.90, 1.27)	790	1.09 (0.90, 1.32)	704	1.01 (0.83, 1.23)

^aRapid growth models are adjusted for study subcohort, infant sex, exact age at 6-month examination, gestational age, exclusive breastfeeding duration, maternal country of origin, maternal social class, maternal age at delivery, maternal prepregnancy BMI status, and maternal smoking during pregnancy.

^bOverweight models are adjusted for study subcohort, infant sex, exact age at 14-month examination, exclusive breastfeeding duration, maternal country of origin, maternal education, maternal age at delivery, maternal prepregnancy BMI status, and maternal smoking during pregnancy.

POPs tested (P interaction > 0.10 for all comparisons, data not shown). The association between DDE and rapid growth was stronger in male than female infants (RR [95% CI] per log ng/g-lipid increase = 1.18 [1.03, 1.35] and 1.05 [0.86, 1.28], respectively, [P -interaction = 0.04]) (Table 4). The effect of DDE on overweight was stronger in infants who were exclusively breastfed for ≤16 weeks compared to those exclusively breastfed for a longer period (RR [95% CI] per log ng/g-lipid increase = 1.26 [1.11, 1.43] and 1.02 [0.86, 1.21], respectively, [P -interaction = 0.04]). The association between HCB and rapid growth was statistically significant in infants of mothers with maternal prepregnancy BMI < 25 kg/m² but not in those whose mothers had higher prepregnancy BMIs (P -interaction = 0.05). There was no clear evidence for other interaction effects (Table 4).

Sensitivity Analyses

Rapid growers were at a higher risk for overweight at 14 months of age (RR [95% CI] = 2.28 [1.91, 2.78] in the total study population). Inclusion of the rapid growth covariate in the models for overweight slightly decreased the effect estimates between DDE or HCB and overweight, but did not influence their statistical significance (data not shown). Risk estimates were not meaningfully changed by the

inclusion of birth weight in the models nor by the exclusion of small for gestational age for weight infants (N = 126) or of infants with low birth weight (i.e., <2500 g, N = 110) (data not shown). Further, effect estimates did not change in the analyses using the POP concentrations in ng/ml with and without further adjusting the models for the maternal serum lipid concentration covariate (data not shown).

Discussion

We found that prenatal exposure to DDE and HCB is associated with rapid growth in the first 6 months of life and subsequent overweight at 14 months of age. Prenatal PCB exposure did not influence postnatal growth. This study, the largest conducted on this topic, provides evidence that the effects of prenatal exposure to persistent environmental chemicals on childhood obesity may be observed very early in infancy. Rapid weight gain even in the first few months of life has been shown to increase the risk of obesity later in childhood (16,17). Childhood overweight has been shown to track into adult obesity; however, some studies suggest that overweight in earlier ages than 2 years of life might not be predictive of adult BMI (28). Thus, evaluating the persistence of the effects on overweight at later ages should be a priority for future research.

TABLE 4 Effects of POPs on early rapid growth and subsequent overweight in the subgroups defined by infant sex, maternal prepregnancy BMI status, and exclusive breastfeeding duration

POP and infant subgroup	Total study population			
	Rapid growth 0–6 months ^a		Overweight at 14 months ^b	
	N	RR (95% CI)	N	RR (95% CI)
DDE (per log ng/g lipid)				
All	1285	1.13 (1.01, 1.26)	1198	1.15 (1.03, 1.28)
Girls	632	1.05 (0.86, 1.28)	572	1.18 (0.99, 1.40)
Boys	653	1.18 (1.03, 1.35)	626	1.14 (0.99, 1.31)
<i>P</i> -sex interaction		0.04		0.39
Maternal BMI <25 kg/m ²	945	1.17 (1.01, 1.34)	881	1.18 (1.03, 1.35)
Maternal BMI ≥25 kg/m ²	340	1.02 (0.84, 1.23)	317	1.12 (0.94, 1.34)
<i>P</i> -maternal BMI status interaction		0.70		0.73
Exclusive breastfeeding ≤16 weeks	669	1.18 (1.01, 1.38)	621	1.26 (1.11, 1.43)
Exclusive breastfeeding >16 weeks	616	1.09 (0.91, 1.30)	575	1.02 (0.86, 1.21)
<i>P</i> -exclusive breastfeeding interaction		0.61		0.04
HCB (per log ng/g lipid)				
All	1285	1.13 (1.00, 1.29)	1198	1.19 (1.05, 1.34)
Girls	632	1.07 (0.88, 1.30)	572	1.27 (1.07, 1.52)
Boys	653	1.16 (0.97, 1.39)	626	1.11 (0.94, 1.32)
<i>P</i> -sex interaction		0.90		0.40
Maternal BMI <25 kg/m ²	945	1.19 (1.02, 1.39)	881	1.20 (1.05, 1.38)
Maternal BMI ≥25 kg/m ²	340	0.91 (0.70, 1.17)	317	1.17 (0.94, 1.47)
<i>P</i> -maternal BMI status interaction		0.05		0.15
Exclusive breastfeeding ≤16 weeks	669	1.12 (0.95, 1.32)	621	1.26 (1.07, 1.48)
Exclusive breastfeeding >16 weeks	616	1.11 (0.89, 1.37)	575	1.14 (0.95, 1.37)
<i>P</i> -exclusive breastfeeding interaction		0.33		0.90
ΣPCB (per log ng/g lipid)				
All	1285	1.10 (0.93, 1.31)	1198	1.07 (0.90, 1.27)
Girls	632	0.99 (0.76, 1.29)	572	1.13 (0.84, 1.51)
Boys	653	1.16 (0.93, 1.46)	626	1.01 (0.81, 1.26)
<i>P</i> -sex interaction		0.44		0.25
Maternal BMI <25 kg/m ²	945	1.08 (0.88, 1.33)	881	1.18 (0.95, 1.48)
Maternal BMI ≥25 kg/m ²	340	1.09 (0.80, 1.47)	317	0.90 (0.70, 1.14)
<i>P</i> -maternal BMI status interaction		0.94		0.10
Exclusive breastfeeding ≤16 weeks	669	1.04 (0.82, 1.31)	621	1.16 (0.90, 1.48)
Exclusive breastfeeding >16 weeks	616	1.17 (0.89, 1.52)	575	1.00 (0.79, 1.25)
<i>P</i> -exclusive breastfeeding interaction		0.30		0.60

^aRapid growth models are adjusted for study subcohort, infant sex, exact age at 6-month examination, gestational age, exclusive breastfeeding duration, maternal country of origin, maternal social class, maternal age at delivery, maternal prepregnancy BMI status, and maternal smoking during pregnancy.

^bOverweight models are adjusted for study subcohort, infant sex, exact age at 14-month examination, exclusive breastfeeding duration, maternal country of origin, maternal education, maternal age at delivery, maternal prepregnancy BMI status, and maternal smoking during pregnancy.

This study confirms the association between prenatal DDE exposure and early postnatal growth reported previously in the Sabadell subcohort (10) in two other INMA subcohorts and further suggests that prenatal HCB exposure is also related to rapid growth and overweight in infancy. Low-level prenatal DDE exposure has also been linked to elevated BMI in infancy ($N = 138$) (11), later in childhood at the age of 6.5 years ($N = 344$) (12) and in adult life in women ($N = 213$) (14). Two recent studies in highly exposed Mexican populations reported null associations between prenatal DDE exposure

and BMI at 12 months ($N = 253$) (30) and 18 months of age ($N = 788$ males) (31). Adverse health effects of endocrine-disrupting chemicals, such as POPs, could follow non-monotonic dose responses with increased risks at lower concentrations and null or inverse risks at higher concentrations (32), thus the substantial differences in the exposure levels may explain the disparity in findings across these studies. Differences in other characteristics, such as race, maternal age at delivery (mean age difference approximately 10 years), breastfeeding duration, or lifestyle, may further explain

differences in findings. Consistent with our current findings, we previously examined the effects of prenatal HCB exposure in the Spanish Menorca cohort and found a positive association with obesity at the age of 6.5 years (15). We found no evidence of effects of PCBs on postnatal growth. A few other studies have linked prenatal PCB exposure to elevated BMI in later childhood (age 5–7 years) or in puberty, while null associations have been reported in younger children (9–13). This might suggest that PCB effects on obesity, if indeed there are any, may become apparent at later ages. The potential contribution of POP exposure to the obesity epidemic suggested by this and other studies may appear unexpected given the decline of POP concentrations during the last few decades, while the prevalence of obesity has increased. However, predicting the potential effect of POP exposure on temporal trends in obesity is difficult for several reasons, most notably the complexity posed by the hypothesis that the health effects of endocrine-disrupting chemicals may follow non-monotonic dose responses (32). Additionally, the influence of temporal trends in POP exposure on obesity trends would not likely outweigh the impact of the main childhood obesity risk factors (diet, physical activity, maternal obesity). Moreover, some of these other factors may modify the effects of POP exposure (e.g., high fat intakes (33)) making changes over time in the contribution of POP exposure to obesity hard to predict.

The underlying mechanisms explaining the POP effects on adipogenesis and lipid metabolism during the sensitive period of development are largely unexplored (9). However, recent studies have shown mixtures of POPs to increase weight and visceral fat gain in animals, as well as fat uptake and storage in cultured adipocytes (33). Moreover, other widely studied endocrine-disrupting chemicals, such as the synthetic estrogen diethylstilbestrol and the non-persistent organic chemicals, bisphenol A, and phthalates, have been shown to perturb nuclear hormone receptor signaling in preadipocytes and mature adipocytes and through epigenetic modifications to alter adipogenic gene expression promoting adipocyte differentiation or/and fatty acid storage (34). The peroxisome-proliferator-activated, retinoid X, estrogen-related, thyroid and glucocorticoid receptors are some of the nuclear receptors suggested to mediate the effects of environmental obesogens (35). DDE is an estrogen receptor agonist and an androgen receptor antagonist; HCB has been suggested to be an androgen receptor and an estrogen-related receptor antagonist (36) while PCB congeners may exhibit estrogenic, antiestrogenic, and/or anti-androgenic effects (37) and may alter thyroid hormone secretion and metabolism (38). DDE was also recently shown to increase adiponectin and resistin gene transcription and to increase fatty acid accumulation in mature NIH2T3-L1 adipocytes leading to adipocyte hypertrophy (39). The specific mechanisms that explain possible obesogenic effects of DDE and HCB require further exploration.

Associations between DDE and rapid growth, and similarly, but not statistically significantly, between HCB and rapid growth, were stronger in males. This pattern may be attributable to sex differences in the biosynthesis, secretion, and/or responsiveness of hormones that play an important role in adipogenesis, as do estrogens and androgens, as well as to differences in the regulatory pathways underlying sexual development of the adipose tissue potentially perturbed by POPs. A positive association between prenatal DDE exposure and pubertal weight-adjusted-for-height in boys but not in girls has also been reported previously (13). Maternal prepregnancy BMI >25 kg/m² was associated with a decreased risk for rapid growth linked to prenatal HCB exposure and to DDE. Maternal overweight is an important predictor of infant rapid growth and overweight in

this population independently of prenatal POP exposure levels (data not shown). This association may be due to the genetic predisposition and/or epigenetic alterations in metabolic programming caused by environmental factors, such as maternal diet (40). A better understanding of the genetic susceptibility and the epigenetic alterations linked to intrauterine chemical exposures and/or maternal weight status is needed to explain these findings. Prolonged exclusive breastfeeding was associated with an attenuated association between DDE and overweight. This suggests that breastfeeding may protect against the obesogenic effects of DDE despite postnatal exposure to POPs through breast-milk. Exclusively breast-fed infants may be less prone to excessive intakes of energy and protein than infants fed formula or solid foods because of breast milk composition and a greater reliance on internal feeding cues than maternal control (16). The reasons for which exclusive breastfeeding duration appeared to influence the effects of DDE but not HCB are uncertain. We cannot also rule out the possibility that some of the results suggesting effect modification may be due to chance as they relate to smaller population subgroups.

Major strengths of this study are the larger sample size compared to previous studies and the prospective design that reduces the risk of reverse causality. The fact that DDE and HCB were shown to be associated with both rapid growth and subsequent overweight—measured in different age windows in infancy—strengthens the confidence in our findings. Further, the multi-pollutant adjusted model that, because of the moderate collinearity between POPs, is more prone to an over-adjustment bias compared to the single pollutant models, did not substantially change effect estimates. However, our study has also some limitations. Weight and BMI are only indirect measures of total body fat as they may reflect both excesses in lean and fat mass, thus analyses using direct measurements of fat mass and body fat distribution to assess these relationships, is an important consideration for future studies. Moreover, the exposure, dilution, and elimination of lipophilic chemicals, may differ in rapid growers and overweight infants compared to infants with normal growth velocity and normal weights (4), thus postnatal POP exposure may have confounded the observed associations. However, we have shown previously in the Menorca cohort that postnatal POP concentrations measured at child serum at 4.5 years of age did not attenuate the associations between prenatal POP exposure and childhood overweight (12). We cannot also rule out the possibility that prenatal and/or postnatal exposure to other unmeasured chemicals correlated to POPs may have confounded the associations under study.

This study suggests that prenatal exposure to persistent environmental chemicals such as DDE and HCB can influence rapid growth and overweight very early in life. Further studies are needed to confirm these findings in different settings and to evaluate their persistence at older ages. Obesity prevention may be particularly efficient if started in early life, and therefore, the potential capacity of early life environmental exposures to influence metabolic programming should be considered in future preventive strategies for childhood obesity. ○

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