Exposure to Bisphenol A and Phthalates during Pregnancy and Ultrasound Measures of Fetal Growth in the INMA-Sabadell Cohort

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BACKGROUND: Prenatal exposure to bisphenol A (BPA) and phthalates may affect fetal growth; however, previous findings are inconsistent and based on few studies.

OBJECTIVES: We assessed whether prenatal exposure to BPA and phthalates was associated with fetal growth in a Spanish birth cohort of 488 mother–child pairs.

METHODS: We measured BPA and eight phthalates [four di(2-ethylhexyl) phthalate metabolites (DEHPm), mono-benzyl phthalate (MBP), and three low-molecular-weight phthalate metabolites (LMWPm)] in two spot urine samples collected during the first and third trimester of pregnancy. We estimated growth curves for femur length (FL), head circumference (HC), abdominal circumference (AC), biparietal diameter (BPD), and estimated fetal weight (EFW) during pregnancy (weeks 12–20 and 20–34), and for birth weight, birth length, head circumference at birth, and placental weight.

RESULTS: Overall, results did not support associations of exposure to BPA or DEHPm during pregnancy with fetal growth parameters. Prenatal MBzP exposure was positively associated with FL at 20–34 weeks, resulting in an increase of 3.70% of the average FL (95% CI: 0.75, 6.63%) per doubling of MBzP concentration. MBzP was positively associated with birth weight among boys (48 g: 95% CI: 6, 90) but not in girls (~27 g: 95% CI: ~79, 25) (interaction p-value = 0.04). The LMWPm mono-n-buty phthalate (MNBP) was negatively associated with HC at 12–20 pregnancy weeks (~4.88% of HC average (95% CI: ~8.36, ~1.36%)].

CONCLUSIONS: This study, one of the first to combine repeat exposure biomarker measurements and multiple growth measures during pregnancy, finds little evidence of associations of BPA or phthalate exposures with fetal growth. Phthalate metabolites MBzP and MNBP were associated with some fetal growth parameters, but these findings require replication.

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Introduction

Bisphenol A (BPA) and phthalates are a class of synthetic chemicals produced and used in large quantities worldwide and present in many kinds of articles including plastics, cosmetics, carpets, building materials, toys, or cleaning products (Koch and Calafat 2009). In 1999, the European Union banned the use of some phthalates in the manufacture of toys and childcare articles, and in 2011, the use of BPA was banned in infant feeding bottles (European Commission 2005, 2011). In the United States, environmental and public health organizations have conducted numerous campaigns to reduce their use in consumer products and concentrations in the general population of some phthalates have started to decline (Zota et al. 2014). Diet is the predominant source of BPA and high-molecular-weight phthalates (HMWP) (Rudel et al. 2011; Wormuth et al. 2006), whereas personal care products are the

major source of the low-molecular-weight phthalates (LMWP) (Wormuth et al. 2006). Phthalates and BPA have a short biological half-life (i.e., few hours or days), but their ubiquity implies a constant but highly toxic load (i.e., few hours or days), but their ubiquity implies a constant but highly toxic load (Casas-Casas et al. 2012). BPA and phthalates and their metabolites have known endocrine-disrupting properties that may disrupt hormonal balance even at low doses of exposure (Casals-Casas and Desvergne 2011). BPA and phthalate metabolites can interact with the estrogen, androgen, thyroid hormone, glucocorticoid, and/or peroxisome proliferator–activated receptors (PPARs) that regulate important biological processes (PPARs) that regulate important biological processes for the control of adipogenesis, insulin levels, fluid retention, and bone metabolism (Ahmadian et al. 2013; Casals-Casas and Desvergne 2011). Some of these hypothesized effects, especially those mediated by the steroid hormone receptors, could be sex-specific. In animal
fewer measurements were available (Snijder et al. 2013). This emphasizes the necessity of using multiple measurements per subject to obtain a more reliable measurement of exposure levels. Furthermore, data from in vivo studies have revealed sex-dependent effects on body weight in rodents exposed perinatally to BPA (Rubin and Soto 2009), but few human studies have been able to evaluate sex-specific effects and findings are still controversial (Chou et al. 2011; Huang et al. 2014; Lee et al. 2014).

In this study we assessed whether prenatal exposure to BPA and phthalates may influence fetal growth and birth outcomes in a Spanish birth cohort of 488 mother–child pairs.

**Methods**

**Study population.** The INMA study (Infancia y Medio Ambiente; Childhood and Environment) is a population-based birth cohort study that recruited 657 pregnant women in the Spanish region of Sabadell between 2004 and 2006 (Guxens et al. 2012). Women were recruited at their first routine prenatal care visit (mean ± SD = 13.4 ± 1.7 weeks of gestation) in the primary care center if they fulfilled the inclusion criteria: age ≥ 16 years, intention to deliver in the reference hospital, singleton pregnancy, unassisted conception, and no communication problems (Guxens et al. 2012). The study was approved by the Ethics Committee of the reference hospital, and all participants gave their written informed consent.

**Prenatal BPA and phthalate exposure.** Maternal urine samples were collected at 12 ± 1.7 and 32 ± 1.4 weeks of gestation and stored in polypropylene (for BPA analysis) or polyethylene tubes (for phthalate metabolites analysis) at −20°C. Total BPA (free plus conjugated) was quantified by liquid chromatography–mass spectrometry in the Department of Analytical Chemistry laboratory–University of Cordoba (Spain) (Casas et al. 2013). Eight phthalate metabolites (free plus conjugated) were determined by liquid chromatography–mass spectrometry in the Bioanalysis Research Group at the Hospital del Mar Medical Research Institute (Spain) (Valvi et al. 2015b). Limits of detection (LODs) for each analyte are listed in Table 1. Creatinine was determined at the Jaffé method (kinetic with target measure-}

| **Table 1. Maternal urinary concentrations of creatinine, BPA, and phthalate metabolites.** |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Compounds** | **n** | **LOD (µg/L)** | **% < LOD (1st–3rd trimester)** | **GM (95% CI)** | **Minimum–maximum** | **ICC(95% CI)** |
| Creatinine (g/L) | | | | | | |
| 1st trimester | 488 | 0.82 (0.78, 0.86) | 0.1–2.7 | 0.22 (0.14, 0.31) | | |
| 3rd trimester | 488 | 0.88 (0.83, 0.92) | 0.1–3.0 | | | |
| BPA | 470 | 0.1 | 0–0.6 | 2.3 (2.1, 2.4) | 0.3–61.8 | 0.15 (0.06, 0.24) | |
| Phthalates | | | | | | |
| ΣDEHPm | 390 | 92.5 (86.9, 98.6) | 14.3–1428.9 | | | |
| MEHP | 390 | 9.6 (9.0, 10.3) | 1.3–202.0 | 0.20 (0.10, 0.29) | | |
| ΣMEHP | 390 | 5.3 (5.1, 5.8) | 32.7–835.7 | 0.19 (0.09, 0.29) | | |
| ΣMECPP | 390 | 36.2 (33.7, 38.9) | 4.6–476.1 | | | |
| MBP | 390 | 1.1 (1.0, 1.2) | 0.8–336.0 | 0.22 (0.12, 0.31) | | |
| ΣLMWPm | 390 | 428.7 (392.9, 467.8) | 43.1–5183.1 | | | |
| ME | 390 | 35.6 (32.4, 37.3) | 21.9–516.1 | 0.23 (0.14, 0.33) | | |
| MIBP | 390 | 28.9 (27.1, 31.0) | 4.0–387.6 | 0.22 (0.13, 0.32) | | |
| MnBP | 390 | 24.0 (22.1, 25.9) | 3.4–402.6 | 0.20 (0.10, 0.29) | | |
| Creatinine-adjusted (µg/g) | | | | | | |
| BPA | 470 | 0.1 | 0–0.6 | 2.6 (2.4, 2.8) | 0.3–69.4 | 0.14 (0.05, 0.22) | | |
| Phthalates | | | | | | |
| ΣDEHPm | 390 | 106.1 (99.8, 112.8) | 26.5–1670.0 | | | |
| MEHP | 390 | 11.3 (10.6, 12.1) | 1.8–266.9 | 0.18 (0.08, 0.28) | | |
| ΣMEHP | 390 | 29.0 (27.1, 31.0) | 1.5–503.4 | 0.06 (0.00, 0.18) | | |
| ΣMECPP | 390 | 41.4 (38.9, 44.1) | 7.7–718.9 | 0.19 (0.09, 0.29) | | |
| MBP | 390 | 1.2 (1.1, 1.3) | 1.5–405.1 | 0.23 (0.14, 0.33) | | |
| ΣLMWPm | 390 | 494.3 (456.2, 533.7) | 65.2–10030.2 | | | |
| ME | 390 | 389.1 (353.4, 424.8) | 34.0–9378.9 | 0.23 (0.13, 0.32) | | |
| MIBP | 390 | 33.0 (31.0, 35.1) | 5.1–334.2 | 0.19 (0.09, 0.29) | | |
| MnBP | 390 | 32.7 (30.4, 35.2) | 2.6–583.7 | 0.19 (0.09, 0.29) | | |

Abbreviations: BPA, bisphenol A; CI, confidence interval; DEHPm, di-2-ethylhexyl phthalate metabolites; GM, geometric mean; ICC, intraclass correlation coefficient; LMWPm, low molecular weight phthalate metabolites; LOD, limit of detection; MBP, mono-benzylic phthalate; MCPP, mono(2-ethyl-5-carboxy-pentaeryl) phthalate; MCPP, monooctyl-5-hydroxy-phthalate; MCPP, mono(2-ethyl-hexyl) phthalate; MCPP, mono(2-ethyl-5-oxo-hexyl) phthalate; MEP, mono-ethyl phthalate; MIBP, mono-isobutyl phthalate; MnBP, mono-n-butyl phthalate.

*Average of measurements at two time points in the first and third trimesters of pregnancy. The ICC is calculated by dividing the between-person variability by the sum of the between- and within-person variability. Values range from 0 (i.e., no reproducibility of the same measurement within a subject) to 1 (i.e., perfect reproducibility).
Bisphenol A, phthalates, and fetal growth

models. To determine the covariates included in the multivariate models, we applied directed acyclic graphs (DAGs) (Shrier and Platt 2008). Covariates were included in the DAGs if they were described to be associated with the exposure or the outcome in previous literature (Aguiñera et al. 2010; Casas et al. 2013; Snijder et al. 2013; Valvi et al. 2013), and such associations were shown in bivariate analyses of our data (see Supplemental Material, Table S2) ($p < 0.1$). Based on the DAGs, the final multivariate models were adjusted for maternal education (primary, secondary, university), smoking during pregnancy (never smoked, not during pregnancy, during pregnancy), and parity (nulliparous, multiparous). Departing from this multivariate model, we then conducted a forward stepwise selection procedure including other potential confounders and ancestors and testing if they changed the coefficient by > 10%; birth season, urinary cotinine levels during pregnancy, consumption of canned fish during pregnancy, time of urine collection, and use of household cleaning products. Birth season (winter, spring, summer, autumn) and urinary cotinine levels during pregnancy [non–secondhand tobacco smoke (SHS) exposure, $< 18$ ng/mL; SHS exposed, 18–50 ng/mL; active smoker, $> 50$ ng/mL] changed most of the coefficients by > 10% and hence they were included in all final models. Because fetal HC growth models were already adjusted for parity (see Supplemental Material, Table S1), this variable was not included in the fetal HC multivariate models. Finally, newborn’s HC models were also adjusted for type of delivery (vaginal, instrumental, cesarean) because passage through the birth canal may influence HC at birth. We are only presenting the adjusted models because they yielded results similar to the unadjusted ones.

Results for phthalates are presented for the sum (micrograms per liter) of the four DEHP [di(2-ethylhexyl) phthalate] metabolites ($\Sigma$DEHPm: MEHP [mono(2-ethyl-5-hydroxyhexyl) phthalate], MEHHP [mono(2-ethyl-5-hydroxyhexyl) phthalate], MEOHP [mono(2-ethyl-5-oxo-hexyl) phthalate], and MECPP [mono(2-ethyl-5-carboxypentyl) phthalate]).

Figure 1. Adjusted associations between maternal urinary BPA levels (μg/g creatinine) and fetal size and growth parameters in the overall population, in girls, and in boys: femur length (A), head circumference (B), abdominal circumference (C), and estimated fetal weight (D).

A. Femur length

- Overall ($n = 452$)
- Girls ($n = 220$)
- Boys ($n = 232$)

B. Head circumference

- Overall ($n = 455$)
- Girls ($n = 218$)
- Boys ($n = 237$)

C. Abdominal circumference

- Overall ($n = 452$)
- Girls ($n = 220$)
- Boys ($n = 232$)

D. Estimated fetal weight

- Overall ($n = 452$)
- Girls ($n = 220$)
- Boys ($n = 232$)

The adjusted associations are shown for each trimester of pregnancy (12–20 weeks and 20–34 weeks), and for boys and girls separately. Statistical differences between girls and boys were tested using a two-sample $t$-test. Multiple comparisons were adjusted using the Holm–Bonferroni method.
Results

Study population and exposure characteristics. Of the 675 pregnant women initially enrolled, 488 mother–child pairs had information on prenatal BPA (n = 470) and/or phthalate metabolites concentrations (n = 390) and fetal growth parameters or birth outcomes (see Supplemental Material, Figure S1). Women included in the study were more likely to be Spanish, well-educated, and from a higher socioeconomic position than INMA-Sabadell participants not included in the analysis (data not shown). Complete details of the characteristics of the study population are given in Supplemental Material, Table S2.

BPA and phthalate metabolites were detected in most of the urine samples (0–0.8% < LOD) (Table 1). The unadjusted geometric mean (GM) BPA concentration was 2.3 μg/L [95% confidence interval (CI): 2.1, 2.4]. Among phthalate metabolites, the low molecular weight phthalate MEHP presented the highest concentration (GM: 335.6 μg/L; 95% CI: 303.4, 371.3), whereas MEHHP and MBzP showed the lowest (GM: 9.6 μg/L; 95% CI: 9.0, 10.3; GM: 11.1 μg/L; 95% CI: 10.2, 12.1, respectively). The ICCs comparing samples collected in the first and third trimesters ranged from 0.06 to 0.23, with the highest ICC for MBzP and MEEP and the lowest for MEHHP and MEOHP (Table 1). Low correlations were found between the different exposure groups (BPA, ∑DEHPm, MBzP, and ∑LMWPm), with Pearson correlation coefficients ranging from 0.14 to 0.32 (see Supplemental Material, Table S3). A total of 1,452 ultrasound examinations were performed for the 488 pregnancies. Most women (n = 473) had one routine ultrasound examination in each trimester of pregnancy but 15 (3%) women had four to six examinations. The mean (± SD) gestational age, birth and placental weight were 39.7 ± 1.4 weeks, 3,319 ± 391 g, and 602 ± 110 g, respectively (data not shown).

BPA and fetal growth and birth outcomes. Exposure to BPA during pregnancy was negatively associated with FL and EFW from 12 to 20 weeks resulting in a percent reduction in SD scores of −4.26% (95% CI: −7.91, −0.65%) and −3.84% (95% CI: −7.93, 0.30%), respectively, for each doubling of concentration of maternal urinary BPA (Figure 1). Negative associations of BPA with growth in FL and EFW from 12 to 20 weeks were evident in boys but not in girls (e.g., for growth in EFW: −5.74%; 95% CI: −11.08, −0.29%; and −0.98%; 95% CI: −7.35, 5.42% in boys and girls, respectively), but the differences between boys and girls were not significant (p interaction = 0.22 for FL, p = 0.15 for EFW) (Figure 1). Significant differences by sex in EFW at 12 weeks and 20 weeks were due to a significant positive association with size at 12 weeks in girls and a null association in boys (p interaction = 0.05), whereas at 20 weeks there was a weak positive association with EFW in girls and a stronger nonsignificant negative association in boys.

Table 2. Adjusted associations between maternal urinary BPA and phthalate metabolites levels (μg/g creatinine) and birth outcomes in the overall population, in girls, and in boys.

| Compounds | Overall | Girls | Boys |
|-----------|---------|-------|------|
| n | β (95% CI) | n | β (95% CI) | n | β (95% CI) | p Interaction |
| BPA | | | | | | | |
| Weight (g) | 448 | −16.24 (−54.10, 21.62) | 219 | −21.56 (−82.93, 39.92) | 229 | −11.01 (−57.38, 35.37) | 0.99 |
| Placental weight (g) | 398 | 0.53 (−10.79, 11.86) | 193 | −8.01 (−28.03, 12.01) | 205 | 6.98 (−6.47, 20.44) | 0.28 |
| Length (mm) | 441 | −0.37 (−2.00, 1.25) | 212 | −0.64 (−3.21, 1.93) | 229 | −0.27 (−2.31, 1.77) | 0.93 |
| Head circumference (mm) | 435 | 0.47 (−0.86, 1.60) | 208 | 0.38 (−1.47, 2.23) | 227 | 0.54 (−0.77, 1.85) | 0.77 |
| Gestational age (weeks) | 453 | 1.19 (−0.19, 2.59) | 220 | 1.65 (−0.56, 0.39) | 233 | 0.63 (−1.21, 2.46) | 0.54 |
| ∑DEHPm | | | | | | | |
| Weight (g) | 371 | 15.56 (−28.75, 59.97) | 179 | 5.92 (−60.59, 72.42) | 192 | 14.68 (−44.11, 73.48) | 0.82 |
| Placental weight (g) | 325 | 1.31 (−12.30, 14.93) | 154 | −14.25 (−36.73, 8.24) | 171 | 13.94 (−2.85, 30.72) | 0.04 |
| Length (mm) | 364 | 0.38 (−1.59, 2.35) | 172 | 0.78 (−2.06, 3.61) | 192 | −0.25 (−2.91, 2.40) | 0.84 |
| Head circumference (mm) | 359 | 0.16 (−1.15, 1.47) | 169 | 0.00 (−1.98, 1.87) | 190 | 0.17 (−1.46, 1.80) | 0.56 |
| Gestational age (weeks) | 375 | −0.13 (−1.72, 1.46) | 180 | 0.20 (−2.23, 2.63) | 195 | −0.48 (−2.65, 1.69) | 0.71 |
| MBzP | | | | | | | |
| Weight (g) | 371 | 14.11 (−19.08, 47.29) | 179 | −27.30 (−79.41, 24.80) | 192 | 47.78 (5.78, 89.78) | 0.04 |
| Placental weight (g) | 325 | 0.75 (−9.79, 11.30) | 154 | −20.57 (−39.22, −1.92) | 171 | 12.53 (0.27, 24.79) | 0.02 |
| Length (mm) | 364 | −0.42 (−1.90, 1.07) | 172 | −1.42 (−3.67, 0.84) | 192 | 0.43 (−1.49, 2.35) | 0.24 |
| Head circumference (mm) | 359 | −0.07 (−1.05, 0.92) | 168 | −0.92 (−2.47, 0.62) | 190 | 0.70 (−0.48, 1.88) | 0.07 |
| Gestational age (weeks) | 375 | −0.39 (−1.81, 0.83) | 180 | −0.43 (−2.34, 1.48) | 195 | −0.43 (−2.06, 1.20) | 0.97 |
| ∑LMWPm | | | | | | | |
| Weight (g) | 371 | −10.02 (−42.84, 22.80) | 179 | −11.55 (−62.08, 38.97) | 192 | 6.16 (−37.64, 49.95) | 0.47 |
| Placental weight (g) | 325 | 4.20 (−6.05, 14.46) | 154 | −1.85 (−19.31, 16.01) | 191 | 9.18 (−3.42, 21.78) | 0.18 |
| Length (mm) | 364 | −0.74 (−2.07, 0.22) | 172 | −0.24 (−2.38, 1.92) | 192 | −0.77 (−2.75, 1.20) | 0.97 |
| Head circumference (mm) | 359 | −0.54 (−1.51, 0.37) | 169 | −0.13 (−1.62, 1.38) | 190 | −0.62 (−1.85, 0.61) | 0.89 |
| Gestational age (weeks) | 375 | 0.27 (−0.94, 1.48) | 180 | 0.38 (−1.46, 2.24) | 195 | −0.29 (−1.96, 1.38) | 0.42 |

Abreviations: BPA, bisphenol A; CI, confidence interval; DEHPm, di(2-ethylhexyl) phthalate metabolites; MBzP, mono-benzyl phthalate; LMWPm, low-molecular-weight phthalate metabolites. Betas represent the estimated difference in each outcome associated per doubling of exposure levels (levels were log2-transformed). All models were adjusted for maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. Head circumference models also adjusted for type of delivery.
Bisphenol A, phthalates, and fetal growth (p interaction = 0.05) (Figure 1). There was also a significant difference by sex in the association of BPA with AC at 12 weeks, with a positive association among girls (6.41%; 95% CI: 1.16, 11.54%) and no association among boys (–0.75%; 95% CI: –5.35, 3.87%) (p interaction = 0.03) (Figure 1). Prenatal BPA exposure was not significantly associated with any of the birth outcomes studied, though there was a small and nonsignificant association with gestational age at birth (1.19 weeks per doubling of BPA concentration; 95% CI: –0.19, 2.58) (Table 2). None of the associations that were statistically significant for BPA in the main analysis (for FL and EFW growth from 12 to 20 weeks, and for AC and FL at 12 weeks in girls) were statistically significant in all the sensitivity analyses related to creatinine (i.e., adjusted for creatinine or after excluding samples with extreme creatinine values) (see Supplemental Material, Table S4). The inclusion of all four main exposures in one multi-pollutant model led to changes in coefficients between 10 and 80% compared with results with the single-pollutant model (see Supplemental Material, Table S5).

Phthalate metabolites and fetal growth and birth outcomes. Exposure to ΣDEHPm and its single metabolites during pregnancy was not significantly associated with any of the fetal growth (see Supplemental Material, Table S6) or birth outcomes assessed (Table 2; see also Supplemental Material, Table S7). The metabolite MnBP was associated with significantly lower HC growth from 12 to 20 weeks (–4.88%; 95% CI: –8.36, –1.36%) and EFW growth from 12 to 20 weeks (–4.32%; 95% CI: –8.33, –0.27%) (Figure 3; see also Supplemental Material, Table S8). MnBP was associated with greater AC and EFW growth from 20 to 34 weeks in boys (4.29%; 95% CI: 0.01, 8.53%) and 4.27%; 95% CI: –0.18, 8.68%, respectively) but not in girls (0.39%; 95% CI: –4.76, 5.54%) and 1.22%; 95% CI: –3.36, 5.78%, respectively), though differences were not significant (p interaction = 0.21 and 0.31, respectively) (Figure 3). MnBP was also associated with higher birth weight in boys (57 g; 95% CI: 3, 110) but not girls (11 g; 95% CI: –40, 62) (p interaction = 0.29) (see Supplemental Material, Table S7). Further, the metabolite MiBP was associated with lower birth weight in girls (–73 g; 95% CI: –137, –9) but not among boys (19 g; 95% CI: –35, 74) (p interaction = 0.08) (see Supplemental Material, Table S6). Associations of individual LMWP metabolites with other birth outcomes were not statistically significant overall or in girls or boys (see Supplemental Material, Table S7). Of the associations that were statistically significant in primary analyses, only the association between MnBP and HC growth from 12 to 20 weeks met our criteria for consistency (see Supplemental Material, Tables S4 and S5).

Discussion
In this population of pregnant women with a common exposure to BPA and phthalates, we found few associations between these compounds and fetal growth and birth outcomes. The HMWPm MBzP was significantly associated with greater FL growth from 20 to 34 weeks in the overall population and with significantly higher birth weight in boys, but not in girls. In contrast, the LMWPm MnBP was associated with lower HC growth from 12 to 20 weeks in the overall population, with no evidence of

Figure 2. Adjusted associations between maternal urinary MBzP levels (μg/g creatinine) and fetal size and growth parameters in the overall population, in girls, and in boys: femur length (A), head circumference (B), abdominal circumference (C), and estimated fetal weight (D). MBzP: mono-benzyl phthalate. Mean percent difference in standard deviation scores per doubling of MBzP levels (levels were log-transformed). Femur length model was adjusted for maternal age, maternal weight, maternal country of origin, maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. Head circumference model was adjusted for maternal age, maternal height/weight, paternal weight, and maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. Abdominal circumference model was adjusted for maternal age, maternal height/weight, paternal weight, maternal country of origin, maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. Estimated fetal weight model was adjusted for maternal age, maternal weight, paternal weight, maternal country of origin, maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. p interaction for sex ≤ 0.1.
Concentrations of BPA among pregnant women in our population were similar to populations in other studies where BPA and fetal growth outcomes were evaluated (Lee et al. 2008; Philippat et al. 2014; Snijder et al. 2013). In two of these studies, high prenatal BPA concentrations were associated with reduced fetal growth; but the sample size was relatively small \( n = 125 \) in Lee et al. (2008); \( n = 80 \) in Snijder et al. (2013)]. Also, they did not assess either sex-specific differences or the influence of other pollutants. In the EDEN cohort study, which included only boys, no associations were observed between BPA and fetal growth (Philippat et al. 2014). In our population, although we found statistically significant sex-specific associations between prenatal BPA exposure and FL, AC, and EFW, these associations did not meet our criteria for robustness in sensitivity analyses. We also did not find significant associations between BPA and any of the anthropometric measures at birth. Epidemiological studies assessing birth outcomes have also shown contradictory results, with some studies showing an increase in anthropometric measures (Lee et al. 2014; Philippat et al. 2012), and others finding a decrease (Chou et al. 2011; Miao et al. 2011), or null associations (Wolff et al. 2008). In animal studies discrepant results also exist regarding BPA effects on fetal weight gain and bone development (Agas et al. 2013; Kim et al. 2001; Somm et al. 2009). It is worth noting that prenatal BPA exposure was positively associated with waist circumference and BMI at 4 years in the same INMA-Sabadell birth cohort, but not at earlier ages (Valvi et al. 2013). Thus, whether prenatal exposure to BPA may start affecting fetal growth during pregnancy but with effects to be observed only at later ages needs to be further explored (Unuvar and Buyukgebiz 2012).

Phthalate metabolite levels in urine of pregnant women from our population were of similar magnitude to those reported in other studies assessing phthalates in relation to birth outcomes (Philippat et al. 2012; Suzuki et al. 2010; Wolff et al. 2008). Two other studies measured the parent and not the metabolite phthalate compounds in blood, so the comparison of levels with those of the present study is difficult (Huang et al. 2014; Zhang et al. 2009). Again, inconsistent results were found between studies, with one showing an increase in length and head circumference at birth (Wolff et al. 2008); some showing a decrease in birth weight, length, and head circumference among other growth parameters (Huang et al. 2014; Zhang et al. 2009); and others reporting no associations with any phthalates exposure (Philippat et al. 2012; Suzuki et al. 2010). To our knowledge, no study thus far has evaluated ultrasonound measurements in relation to phthalates exposure during pregnancy. We found that prenatal exposure to the HMW phthalate MBzP was positively associated with fetal FL growth from 20 to 34 weeks in the overall population and with higher birth weight in boys but not among girls. Our results are in line with toxicological and animal studies suggesting that exposure to MBzP or its parent compound benzyl butyl phthalate (BBP) may stimulate adipogenesis and increase osteoblast proliferation (Agas et al. 2013; Hurst and Waxman 2003). On the contrary, exposure to the LMW phthalate MnBP seemed to be associated with a decrease in fetal HC early in pregnancy. Huang et al. (2014) observed a reduction in HC and in other fetal parameters at birth associated with exposure to dibutyl phthalate (DBP), the precursor of MnBP. Zhang et al. (2009) assessed birth weight and found an increased risk of low birth weight associated with DBP exposure; however, both of these studies measured phthalates exposure in blood samples, which may not produce reliable estimates of exposure. In rodents, decreased growth was also observed after exposure to DBP (Marsman 1993). In the INMA-Sabadell birth cohort, boys presented reduced weight gain and reduced risk of overweight from birth until 7 years of age linked to a high HMWPm exposure during pregnancy; no associations were found for LMWPm (Valvi et al. 2015a). Children with higher weight at birth compared with children with lower birth weights may tend to grow more slowly during the first years of life, and they could be at lower risk of obesity later in childhood and adult life (Labayen et al. 2012). More studies are needed to disentangle the potential effects of exposure to phthalates during pregnancy on fetus and child growth.

This study has some limitations. First, although we used the average of two BPA and

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**Figure 3.** Adjusted associations between maternal urinary MnBP levels (\( \mu g / creatinine \)) and fetal and size growth parameters in the overall population, in girls, and in boys: femur length (A), head circumference (B), abdominal circumference (C), and estimated fetal weight (D). MnBP, mono-n-butyl phthalate. Mean percent difference in standard deviation scores per doubling of MnBP levels (levels were log-transformed). Femur length model was adjusted for maternal age, maternal height, paternal weight, maternal country of origin, maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. Head circumference model was adjusted for maternal age, maternal height/weight, paternal weight, maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy. Abdominal circumference model was adjusted for maternal age, maternal height/weight, paternal height/weight, paternal weight, maternal country of origin, maternal education, smoking during pregnancy, parity, birth season, and urinary cotinine levels during pregnancy.

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phthalate measurements during pregnancy, the high within-person and within- and between-day variability of these compounds means that exposure misclassification cannot be ruled out. Such misclassification is likely to be random with respect to our outcomes, and is thus most likely to have led to an attenuation of associations (Pollack et al. 2013). Second, we were able to include BPA and phthalate exposures in one model, and we showed that those more strongly associated to fetal growth and birth outcomes were MBzP and MnBP. Third, because of the small number of ultrasound exams obtained from week 35 onward, we could not assess the influence of BPA and phthalates on fetal growth during late pregnancy (i.e., weeks 35–38), when most of the constitutional variation in fetal parameters occur (Hindmarsh et al. 2002). Fourth, INMA-Sabadell cohort participants included in the analysis were more likely to be Spanish, more educated, and from a higher socioeconomic class than those excluded from the analysis. Lower education and lower socioeconomic class are linked to higher urinary concentrations of BPA and phthalates in our population (Casas et al. 2013; Valvi et al. 2015b) and to higher risk of adverse pregnancy outcomes (de Graaf et al. 2013); thus, the most highly exposed and most susceptible women could have been excluded from analysis. Finally, we performed quite a large number of comparisons between exposure and outcomes, which may have led to spurious findings.

The major strength of this paper relies on its prospective design and the use of repeated measurements of fetal biometry. Also, we had repeated measurements of BPA and phthalates at the first and third trimester of pregnancy in almost 500 pregnant women, making this, to our knowledge, the largest and most extensive study on this topic. Finally, we had data available for a large number of potential confounders.

Conclusions

This study is one of the first to combine repeat exposure biomarker measurements and multiple growth measures during pregnancy. We did not find consistent or strong evidence of associations between BPA or phthalate exposures and fetal growth, though the phthalate metabolites MBzP and MnBP were associated with some fetal growth parameters. These findings require replication. Production of some phthalates and BPA has already been banned in some countries and replaced by other chemicals such as the BPA analogs BPE or BPF that can also have endocrine-disrupting activity (Rossmann et al. 2014). Investigating the effects of phthalates and BPA is relevant for improving current knowledge of health effects in children and provides further guidelines for an effective regulatory policy, given that they have similar structure and mechanisms of action.

References

Agas D, Sabbieti MG, Marchetti L. 2013. Endocrine disruptors and bone metabolism. Arch Toxicol 87:735–751.

Aguilera I, Garcia-Esteban R, Iñiguez C, Nieuwenhuijsen MJ, Rodriguez A, Paez M, et al. 2010. Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA-Sabadell cohort. Environ Health Perspect 118:705–711; doi:10.1289/ehp.0901228.

Ahmadian M, Suh JM, Han N, Liddle C, Atkins AR, Downes M, et al. 2013. PPAr signaling and metabolism: the good, the bad and the future. Nat Med 19:557–566.

Aitio A. 1996. Liability assurance. In: Biological Monitoring of Chemical Exposure in the Workplace. Guidelines, Volume I (Mikhuev MI, ed). Geneva:World Health Organization, 20–51.

Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ Health Perspect 120:739–745; doi:10.1289/ehp.1101459.

Casals-Casas C, Desvergne B. 2011. Endocrine disruptors: from endocrine to metabolic disruption. Annu Rev Physiol 73:135–162.

Casas M, Valvi D, Luque N, Ballestero-Gomez A, Carins AE, Fernandez MF, et al. 2013. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int 56:10–18.

Chou WC, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY. 2011. Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ Health 10:94; doi:10.1186/1476-069X-10-94.

de Graaf JP, Steegers EA, Bonsel GJ. 2013. Inequalities in perinatal and maternal health. Curr Opin Obstet Gynecol 25:98–108.

European Commission. 2005. Directive 2005/84/EC of the European Parliament and of the Council of 14 December 2005 amending for the 22nd Time Directive 76/769/EEC on the Approximation of the Laws, Regulations and Administrative Provisions of the Member States relating to Restrictions on the Marketing and Use of Certain Dangerous Substances and Preparations (Phthalates in Toys and Childcare Articles). Off J EU L 344(27.12.2005):40–43.

European Commission. 2011. Commission Directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as Regards the Restriction of Use of Bisphenol A in Plastic Infant Feeding Bottles. Off J EU L 261/29.2011:11–14.

Gurrin LC, Blake KV, Evans SF, Newnham JP. 2001. Statistical measures of foetal growth using linear mixed models applied to the foetal origins hypothesis. Stat Med 20:3391–3409.

Guxens M, Ballester F, Espada M, Fernández MF, Grimalt J, Ibañezu J, et al. 2012. Cohort profile: the INMA–Infancia y Medio Ambiente (Environment and Childhood) Project. Int J Epidemiol 41:930–940.

Haddock PF, Harrist RB, Sharram RS, Deter RL, Park SK. 1985. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. Am J Obstet Gynecol 151:332–337.

Hindmarsh JC, Geary MP, Redeck CH, Kingdom JC, Cole TJ. 2002. Intrauterine growth and its relationship to size and shape at birth. Pediatr Res 52:263–268.

Huang Y, Li J, Garcia JM, Lin H, Wang Y, Yan P, et al. 2014. Prenatal levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women. PLoS One 9:e87430; doi:10.1371/journal.pone.0087430.

Hurst CH, Waxman DJ. 2003. Activation of PPARα and PPARγ by environmental phthalate monoesters. Toxicol Sci 74:297–308.

Iñiguez C, Esplugues A, Sunyer J, Basterrechea M, Fernández-Somoano A, Costa O, et al. 2015. Prenatal exposure to NO2 and ultrasound measures of fetal growth in the Spanish INMA cohort. Environ Health Perspect 123:242–245; doi:10.1289/ehp.1409423.

Kim JC, Shin HC, Cha SW, Koh WS, Chung MK, Han SS. 2001. Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy. Life Sci 69:2611–2625.

Koch HM, Calafat AM. 2009. Human body burdens of chemicals used in plastic manufacture. Philos Trans R Soc Lond B Biol Sci 364:2063–2078.

Labayen I, Ortega FB, Ruiz JR, Sjostrom M. 2012. Birth weight and subsequent adiposity gain in Swedish children and adolescents: a 6-year follow-up study. Obesity (Silver Spring) 20:276–281.

Lee B, Ha E, Park H, Kim B, Sae O, Chang M, et al. 2008. Exposure to bisphenol A in pregnant women and early fetal growth [Abstract]. Epidemiology 19:S365.

Lee BE, Park H, Hong YC, Ha M, Kim Y, Chang N, et al. 2014. Prenatal bisphenol A and birth outcomes: MOCHEN (Mothers and Children’s Environmental Health) study. Int J Hyg Environ Health 217:328–334.

Mamelle N, Boniol M, Rivière O, Joly MO, Mellier G, Maria B, et al. 2006. Identification of newborns with Fetal Growth Restriction (FGR) in weight and/or birth weight of offspring. Reprod Toxicol 32:64–68.

Marrsman D. 1995. NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84–74–2) administered in feed to F344/N Rats and B6C3F1, mice. Toxic Rep Ser 40:310.1-5.

Miao M, Yuan W, Zhu G, He X, Li DK. 2011. In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reproductive Toxicology 32:64–68.

Philippat C, Button J, Calafat AM, Ye X, Charles MA, Slama R, et al. 2014. Prenatal exposure to phthalates and growth in boys. Epidemiology 25:625–635.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. 2012. Exposure to phthalates and phenols during pregnancy and offspringsize at birth. Environ Health Perspect 120:464–470; doi:10.1289/ehp.1103824.

Pollack AZ, Perkins NJ, Mumford SL, Ye A, Schisterman EF. 2013. Correlated biomarker measurement error: an important threat to inference in environmental epidemiology. Am J Epidemiol 177:84–92.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. Vienna, Austria:R Foundation for Statistical Computing. Available: http://www.R-project.org (accessed 7 September 2014).

Rigby RA, Stanisopoulos DM. 2004. Smooth centile curves for skew and kurtotic data modelled using the Box–Cox power exponential distribution. Stat Med 23:3053–3076.

Rosenmai AK, Dybdahl M, Pedersen M, Alice van Vught-Lussenburg BM, Wedebye EB, Taxvig C, et al. 2014. Are structural analogues to bisphenol A safe alternates? Toxicol Sci 139:35–47.

Rubin BS, Murray MK, Damassa DA, King JC,
Soto AM. 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect 109:675–680.

Rubin BS, Soto AM. 2009. Bisphenol A: perinatal exposure and body weight. Mol Cell Endocrinol 304:55–62.

Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, et al. 2011. Food packaging and bisphenol A and bis(2-ethylhexyl) phthalate exposure: findings from a dietary intervention. Environ Health Perspect 119:914–920; doi:10.1289/ehp.1003170.

Shrier I, Platt RW. 2008. Reducing bias through directed acyclic graphs. BMC Med Res Methodol 8:70; doi:10.1186/1471-2288-8-70.

Snijder CA, Heederik D, Pierik FH, Hofman A, Jaddoe VW, Koch HM, et al. 2013. Fetal growth and prenatal exposure to bisphenol A: the Generation R study. Environ Health Perspect 121:393–398; doi:10.1289/ehp.1205296.

Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, et al. 2009. Perinatal exposure to bisphenol A alters early adipogenesis in the rat. Environ Health Perspect 117:1549–1555; doi:10.1289/ehp.11342.

Suzuki Y, Niwa M, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H. 2010. Prenatal exposure to phthalate esters and PAHs and birth outcomes. Environ Int 36:699–704.

Tanaka T. 2005. Reproductive and neurobehavioural effects of bis(2-ethylhexyl) phthalate (DEHP) in a cross-mating toxicity study of mice. Food Chem Toxicol 43:581–589.

Unüvar T, Büyükgöz A. 2012. Fetal and neonatal endocrine disruptors. J Clin Res Pediatr Endocrinol 4:51–60.

Valdi D, Casas M, Mendez MA, Ballesteros-Gómez A, Luque N, Rubio S, et al. 2013. Prenatal bisphenol A urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology 24:791–799.

Valdi D, Casas M, Romaguera D, Monfort N, Ventura R, Martinez D, et al. 2015a. Prenatal phthalate exposure and childhood growth and blood pressure: evidence from the Spanish INMA-Sabadell Birth Cohort Study.

Wolff MS, Engel SM, Berkwitz GS, Ye X, Silva MJ, Zhu C, et al. 2008. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 116:1092–1097; doi:10.1289/ehp.11007.

Wormuth M, Scheringer M, Vollenweider M, Hungerbühler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal 26:803–824.

Zhang Y, Lin L, Cao Y, Chen B, Zheng L, Ge RS. 2009. Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. J Pediatr 155:500–504.

Zota AR, Calafat AM, Woodruff TJ. 2014. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001–2010. Environ Health Perspect 122:235–241; doi:10.1289/ehp.1306681.