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Clinical Research Article

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Prenatal Exposure to Butyl Paraben Is Associated With Fat Percentage in 7-Year-Old Boys

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Abstract

Context: Parabens are used as preservatives in consumer products but are suspected of having endocrine-disrupting properties. A recent study reported an association between in utero exposure to butyl paraben and overweight in childhood, with a stronger trend in girls.

Objective: We therefore studied the association between parabens in maternal urine in third trimester and fat percentage in children aged 7 years.

Design, Setting, and Participants: We used data from the Odense Child Cohort, a mother-child cohort with enrollment from 2010 to 2012, in which the children are followed. Paraben concentration was assessed in maternal urine at median gestational week 28.7 and body composition measured as total, gynoid, and android fat percentages assessed by dual X-ray absorptiometry in their children at age 7 years.

Main Outcome Measurements: Total, gynoid, and android fat percentages and z-score for body mass index.

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Interventions: None.
Results: Paraben exposure was low. In multivariate linear regressions, detection of butylparaben in maternal urine was associated with an increase of 17% [95% confidence intervals (CI) 3.0%, 32%] in total body fat percentage and an increase of 23% (95% CI 5.1%, 43%) in android fat percentage in boys, compared to boys whose mother had no detectable butylparaben in urine. No significant associations between in utero exposure to methyl-, ethyl- or propyl parabens and body composition were found, and no significant associations were seen in girls.
Conclusion: Our findings suggest that parabens, which are believed to have low toxicity, may affect obesity development at vulnerable time periods during development.

Key Words: butyl paraben, endocrine disrupting chemicals, adipogenesis, android fat, prenatal programming, cohort study

Introduction

Parabens are commonly used as preservatives because of their bactericide and fungicide properties (1). Due to widespread exposure via cosmetics, pharmaceuticals, and other consumer goods, parabens are detectable in most people tested (2,3). Some parabens are suspected of having obesogenic and estrogenic as well as anti-androgenic effects in animals (4-6). Accordingly, the European Chemicals Agency has recently recognized butylparaben (BuP) as having endocrine-disrupting properties (7).

A recent mother-child cohort study (8) found a significant increase in odds ratio for overweight in 2- to 8-year-old children prenatally exposed to highest tertile of urine BuP compared to lower tertile. This effect was strongest in girls. The study also included mice and reported that fetal BuP exposure induced a higher food intake and weight gain in female mice offspring. The effect was attributed to an altered expression of leptin, which was accompanied by an epigenetic modification in the neuronal pro-opiomelanocortin (POMC) enhancer 1 leading to a reduced hypothalamic POMC expression in rodent models (8). POMC is a prohormone, which stimulates a wide range of physiological actions; for example, it induces satiety in the hypothalamus through alpha-melanocyte-stimulating hormone (8-10). Two other previous studies did, however, not report a significant associations between paraben exposure and obesity. In an American cohort study, the sum of urinary parabens at ages 6 to 8 were not associated with subsequent obesity (11), and an Indian cross-sectional study found no association between paraben exposure and obesity at ages 2 to 14 (12). These studies only measured methyl, ethyl, and propyl parabens and did not study prenatal exposure (11,12).

This prompted us to investigate whether maternal urinary parabens excretion during pregnancy was associated with an increase in fat percentages and z-score for body mass index (BMI) at 7 years of age in the offspring using dual X-ray absorptiometry (DXA) in 312 mother-child pairs participating in the Odense Child Cohort (OCC).

Method

Study design

The OCC is a prospective birth cohort in which 2365 children currently are being followed up (13). Between 2010 and 2012, pregnant women residing in Odense municipality were invited to participate in the cohort from early pregnancy. Women enrolled from gestational age 8 to 30. Information on smoking status, prepregnancy BMI, education, and health status was obtained through questionnaires filled in during pregnancy. Date of birth was obtained through pediatric and obstetric hospital records. The children are invited to participate in clinical examinations throughout their childhood and adolescence including recording of height and weight.

Paraben assessment

At approximately gestational week 28 (median 28.7 weeks, range 26.4-30.4) fasting spot urine samples were collected from the pregnant women before 09.30 AM and stored in a −80°C freezer at the Open Patient data Explorative Network in Odense. Methyl- (MeP), ethyl- (EtP), propyl- (PrP), n-butyl- (n-BuP), and benzylparaben (BzP) concentrations were measured in 536 Caucasian women by liquid chromatography-mass spectrometry as previously described in detail (3,14). In brief, samples were analyzed in 17 batches each including control material, prepared in a pool of urine spiked in low and high concentration levels. The mean recovery was >95% for all parabens in both spike levels and the relative standard deviation ranged from 8% to 20% and from 7% to 10% for the low and high spike levels, respectively. All sample concentrations were osmolarity adjusted accordingly (15).

Assessment of body composition

On the day of clinical examination at age 7-years a whole-body DXA scan was performed (Lunar Prodigy, GE Healthcare, Madison, WI, USA) running ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI, USA). Fat mass (g) and body mass (g) were calculated by the software as well as a wide array of subdivisions including android (abdominal fat including visceral and subcutaneous fat) and gynoid (hip, breast, and extremity) fat mass. The fat percentages were calculated by the DXA software by dividing by fat mass with the total mass (16). Z-score for BMI was calculated using Danish standard curves adjusted for age (available from http://vækstkurver.dk).

Statistical analysis

The data were stratified according to gender and the osmolality-adjusted urinary MeP, EtP, n-PrP, and n-BuP were divided into tertiles and osmolality-adjusted n-BuP being divided into above and below the limit of detection (LOD), as the concentrations in the majority (67%) of the samples were below LOD. n-BuP was only detected in 33% of samples (n = 104). As BzP, iso-PrP, and iso-BuP were only detectable in, respectively, 9%, 5%, and 0% of the samples these parabens were not analyzed further. Data on total fat and android fat percentages were not normally distributed and accordingly transformed by the use of natural logarithm. Data on parabens were not normally distributed and were divided into tertiles, with the exception of n-BuP, which was divided into above and below LOD. Parabens were not inserted in the model as continuous variables as between 14% and 50% of the parabens were below LOD. Multivariable linear regressions were performed to analyze associations between third-trimester maternal urinary paraben excretion and body composition in the 7-year-old offspring adjusting for parity; additionally, the models were adjusted for maternal BMI before pregnancy, which did not affect the results. The analyses were stratified for gender. *P*-values for trend were calculated assuming linearity between quantiles of urinary paraben excretion and body composition. Effect estimates from log-transformed data were subsequently back-transformed and expressed as a percentage change. Regression model diagnostics were evaluated by inspection of residual plots and homogeneity of variances. Data were presented as beta coefficients and 95% confidence intervals (95% CI), and *P*-values below 0.05 are considered significant. Data were evaluated using RStudio version 1.3.959 [Studio Team (2020). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA, USA, http://www.rstudio.com/] running R version 4.0.0.

Results

A total of 312 mother-child pairs had available maternal urinary paraben measurements and DXA scans of the children at 7 years of age. Nulliparous women had higher paraben concentrations than multiparous women (data not shown). Apart from that maternal paraben exposure was not associated with maternal or child characteristics including child BMI at 18 months.

Detectable n-BuP concentrations in maternal urine during pregnancy was significantly associated with a 17% (95% CI 3.0%, 32%) increase in total fat percentages and a 23% (95% CI 5.1%, 43%) increase in android fat percentages in boys compared to boys whose mothers had n-BuP below LOD after adjustment for parity. In girls, prenatal n-BuP exposure was associated with lower BMI z-scores, total fat, android, and gynoid fat percentages although not significant (Table 1). No significant associations between maternal MeP, EtP, and n-PrP exposure and body fat percentages or BMI at age 7 years in the offspring were found (Table 1).

Discussion

We found that prenatal n-BuP exposure was associated with a significant increase in total fat mass and android fat mass in 7-year-old boys, whereas no association was found in girls. Maternal exposure to MeP, EtP, or n-PrP was not associated with fat distribution at age 7 years in the offspring. The paraben exposure in this cohort was low but similar to another Danish study (17). Our results support the possible adipogenic effects of n-BuP suggested by Leppert et al (8), who reported that prenatal exposure to n-BuP was associated with higher BMI within the first 8 years of life, with a stronger trend in girls. The concentrations of all analyzed parabens and specifically n-BuP [median (quartiles) 0.00 ng/mL (0.00, 0.86)] were significantly lower in our cohort compared to the cohort by Leppert et al (8), where n-BuP [median (quartiles)] was 0.41 (0.10, 2.10) and 1.24 (0.30, 4.55) for women using cosmetic leave-on products without and with parabens, respectively. However, even in our low exposed cohort, we were still able to detect a significant association between n-BuP exposure with adverse trends in body composition.

We measured BuP in utero, which may be the most vulnerable exposure window, whereas 2 previous studies assessed paraben exposure in childhood, and BuP was not measured. They found no clear association between paraben exposure in childhood and obesity (11,12). All previous studies (8,11,12) used BMI for establishing overweight; however, BMI may not be a suitable proxy for body fat in children (18). With the use of whole-body

Table 1. Multiple linear regression of parity-adjusted BMI z-scores, fat percentage, android fat percentage, and gynoid fat percentage calculated by DXA-scans in girls and boys 7 years of age according to maternal osmolarity-adjusted urinary paraben concentrations in tertiles or a detectable concentration

Osmolality-adjusted paraben	Fat percentage % change (95% CI)	BMI (z-score) ß-coefficient (95% CI)	Android fat percentage % change (95% CI)	Gynoid fat percentage ß-coefficient (95% CI)
Girls				
Adjusted MeP				
1st tertile	Reference	Reference	Reference	Reference
2nd tertile	−0.03 (−0.16, 0.1)	−0.09 (−0.46, 0.28)	−0.04 (−0.22, 0.14)	−0.34 (−3.04, 2.35)
3rd tertile	−0.09 (−0.23, 0.05)	−0.16 (−0.54, 0.22)	−0.12 (−0.30, 0.07)	−1.96 (−4.72, 0.80)
P-value for trend	0.19	0.41	0.22	0.16
Adjusted EtP				
1st tertile	Reference	Reference	Reference	Reference
2nd tertile	−0.19 (−2.74, 2.43)	−0.10 (−0.46, 0.27)	−1.17 (−4.65, 2.43)	−0.98 (−3.66, 1.69)
3rd tertile	−0.74 (−3.31, 1.89)	−0.10 (−0.47, 0.27)	−1.34 (−4.86, 2.29)	−0.68 (−2.10, 3.17)
P-value for trend	0.74	0.59	0.55	0.65
Adjusted n-PrP				
1st tertile	Reference	Reference	Reference	Reference
2nd tertile	−0.06 (−2.61, 2.56)	−0.1 (−0.47, 0.27)	0.63 (−2.92, 4.32)	0.05 (−2.63, 2.73)
3rd tertile	−0.68 (−3.28, 1.97)	−0.15 (−0.46, 0.17)	−0.61 (−4.18, 3.10)	−1.11 (−4.72, 0.8)
P-value for trend	0.61	0.45	0.79	0.43
Adjusted n-BuP				
<LOD	Reference	Reference	Reference	Reference
>LOD	−7.07 (−18.28, 4.14)	−0.24 (−0.55, 0.07)	−6.81 (−21.26, 8.38)	−1.25 (−3.55, 1.05)
P-value for trend	0.18	0.13	0.34	0.29
Boys				
Adjusted MeP				
1st tertile	Reference	Reference	Reference	Reference
2nd tertile	0.02 (−0.12, 0.17)	0.01 (−0.39, 0.42)	0.06 (−0.12, 0.23)	−0.72 (−3.34, 1.9)
3rd tertile	−0.05 (−0.19, 0.09)	−0.23 (−0.63, 0.18)	−0.03 (−0.2, 0.15)	−1.43 (−4.05, 1.19)
P-value for trend	0.5	0.28	0.78	0.29
Adjusted EtP				
1st tertile	Reference	Reference	Reference	Reference
2nd tertile	0.40 (−2.31, 3.19)	−0.06 (−0.47, 0.35)	0.18 (−2.03, 2.44)	−0.35 (−2.98, 2.28)
3rd tertile	0.41 (−2.30, 3.20)	−0.05 (−0.36, 0.45)	−0.16 (−2.29, 2.02)	−0.09 (−0.34, 0.17)
P-value for trend	0.99	0.82	0.75	0.48
Adjusted PrP				
1st tertile	Reference	Reference	Reference	Reference
2nd tertile	1.24 (−0.99, 3.53)	0.00 (−0.4, 0.41)	1.58 (−1.13, 4.37)	0.86 (−1.77, 3.48)
3rd tertile	0.15 (−2.06, 2.41)	−0.04 (−0.45, 0.37)	0.70 (−1.98, 3.46)	0.01 (−.02, 0.02)
P-value for trend	0.76	0.86	0.29	0.93
Adjusted BuP				
<LOD	Reference	Reference	Reference	Reference
>LOD	16.70 (3.14, 32.27)	0.25 (−0.1, 0.61)	22.86 (5.66, 42.90)	1.63 (−0.68, 3.93)
P-value	0.02	0.16	0.01	0.17

Presented as ß-coefficient and 95% confidence interval (95% CI). Total fat and android fat percentage are transformed by use of the natural logarithm and back-transformed showing percentage change in body fat and android fat percentage. All MeP, EtP, n-PrP, n-BuP, PrP, and BuP values are adjusted for parity. *P*-values are calculated assuming a linearity between tertile of exposure and outcome.

DXA scans, we had the possibility to more accurately estimate fat mass and to assess regional fat distribution [eg, android (abdominal) fat and gynoid fat]. Leppert et al (8) used BMI and found the strongest odds ratio for obesity in prenatally exposed girls, whereas we found a stronger association in boys, which may be due to the fact that we used a more sensitive and valid measurement for obesity—DXA-verified fat percentages. The differences may also be due to the fact that boys are more susceptible to exposure to endocrine-disrupting chemicals, especially in utero (19).

BuP may change appetite regulation through altered expression of leptin (8,20), which in turn leads to reduced hypothalamic expression of POMC causing reduced physical activity, which may also account for obesity (21). Our findings of sex differences are in accordance with the hypothesis that boys are more susceptible to prenatal exposure to endocrine-disrupting chemicals (19) and that there is a sex difference in the function of POMC neurons altering energy intake and insulin sensitivity (21), findings that need confirmations in epigenetic studies.

Our study has several strengths: it was large, population-based and prospective, and participants had neither prior knowledge of their paraben exposure nor of the DXA scan results, and both were measured objectively and blinded. However, some limitations need mentioning. The women included in the OCC were older, more often nulliparous when compared to the background population (13); in addition, a random subset of 312 mother-child pairs was included; however, since the women had no knowledge of their paraben exposure or the DXA scan results of their child at enrollment, these factors are unlikely to have affected their participation. Another limitation is the fact that we did not obtain information about weight gain during pregnancy, which is highly associated with childhood adiposity. Parabens are quickly metabolized with a urinary excretion half-life of fewer than 24 h (22). A single spot-urine sample collected around gestational week 28 may therefore not reflect fetal or childhood exposure. We did not measure childhood exposure and can therefore not exclude that maternal and childhood paraben exposure are correlated, which has been reported in another Danish study (17). However, since paraben exposure is mainly through personal care and cosmetic products, we believe that exposure during vulnerable periods in development may be of larger importance for future fat distribution than early childhood exposure. It would, however, be interesting to measure parabens in children's urine.

Our findings are of public health importance, as previous studies on fat distribution in children and adolescents have shown, that android fat is significantly and independently associated with less favorable plasma lipids and blood pressure, and android fat is a more important risk factor for cardiovascular disease than overall adiposity (23).

In conclusion, even in a low-exposed cohort, we found an increase of total and android fat percentages in 7-year old boys with increasing prenatal n-BuP exposure. No association between maternal exposure to other parabens and DXA scan fat measures at age 7 was found in either sex. BuP has recently been recognized as having endocrine-disrupting properties (7), and our findings of an obesogenic effect emphasize the importance of even low exposure to parabens during vulnerable time periods for future obesity. Further studies are urgently warranted.

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Additional Information

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Disclosures: The authors have nothing to disclose.

Data availability: Restrictions apply to some or all the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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