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ORIGINAL ARTICLE

Prenatal phthalate exposure and 8-isoprostane among Mexican-American children with high prevalence of obesity

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Oxidative stress has been linked to many obesity-related conditions among children including cardiovascular disease, diabetes mellitus and hypertension. Exposure to environmental chemicals such as phthalates, ubiquitously found in humans, may also generate reactive oxygen species and subsequent oxidative stress. We examined longitudinal changes of 8-isoprostane urinary concentrations, a validated biomarker of oxidative stress, and associations with maternal prenatal urinary concentrations of phthalate metabolites for 258 children at 5, 9 and 14 years of age participating in a birth cohort residing in an agricultural area in California. Phthalates are endocrine disruptors, and *in utero* exposure has been also linked to altered lipid metabolism, as well as adverse birth and neurodevelopmental outcomes. We found that median creatinine-corrected 8-isoprostane concentrations remained constant across all age groups and did not differ by sex. Total cholesterol, systolic and diastolic blood pressure were positively associated with 8-isoprostane in 14-year-old children. No associations were observed between 8-isoprostane and body mass index (BMI), BMI *Z*-score or waist circumference at any age. Concentrations of three metabolites of high molecular weight phthalates measured at 13 weeks of gestation (monobenzyl, monocarboxyoctyl and monocarboxynonyl phthalates) were negatively associated with 8-isoprostane concentrations among 9-year olds. However, at 14 years of age, isoprostane concentrations were positively associated with two other metabolites (mono(2-ethylhexyl) and mono(2-ethyl-5-carboxypentyl) phthalates) measured in early pregnancy. Longitudinal data on 8-isoprostane in this pediatric population with a high prevalence of obesity provides new insight on certain potential cardiometabolic risks of prenatal exposure to phthalates.

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Key words: 8-isoprostane, obesity, oxidative stress, phthalates, pregnancy

Background

Oxidative stress (or the imbalance between the production of reactive oxygen species and the body's ability to readily detoxify these reactive intermediates and repair the resulting damage[1]) may provide an underlying pathway linking the observed associations between environmental exposures to phthalates and childhood obesity. Prenatal phthalate exposure has been associated with oxidative stress, measured by concentrations of isoprostane in urine samples of pregnant women,[2,3] and by several other biomarkers including 8-hydroxydeoxyguanosine and malondialdehyde.[4,5] 8-isoprostanes are prostaglandin-like compounds formed non-enzymatically by free radical catalyzed peroxidation of arachidonic acid.[6] Isoprostanes are formed *in vivo* in the phospholipid domain of cell membranes and circulating lipoproteins. Once they are cleaved by phospholipases, 8-isoprostanes are released extracellularly and are excreted in urine. According to pharmacokinetic and metabolic studies, the half-life of 8-isoprostanes in humans is ~16 min.[6,7] Previous studies have found that a single urine sample can reflect the levels of 8-isoprostane over a 1-year period [intra-class correlation coefficient for urinary 8-isoprostane is 0.69].[8] Isoprostanes have been used in numerous studies as a reliable biomarker of oxidative stress with low day-to-day variability in healthy subjects.[1,9–12] In addition, isoprostane levels have been associated with exposure to environmental pollutants, obesity and other diseases.[12–14] For instance, isoprostane concentrations have been found to be significantly elevated in obese adults.[15–20] However, limited data are available on isoprostane concentrations and body mass in children. In a cross-sectional study of 42 multi-ethnic obese children and adolescents, 8-isoprostane was significantly associated with body mass index (BMI), waist circumference and 24-h systolic blood pressure.[21] In a study of 72 Japanese children and adolescents, increased plasma isoprostane was associated with visceral fat, high molecular weight adiponectin and metabolic complications in obese children.[22]

Phthalates are ubiquitous in the environment; they are widely used as plasticizers, stabilizers and solubilizing agents in numerous consumer and industrial products such as cosmetics, medical devices, building materials, household furnishings and flexible plastics.[23] Detectable levels have been found in various environmental media including indoor air, household dust, food stuffs and drinking water. Di(2-ethylhexyl) phthalate (DEHP) is one of the most commonly used phthalate plasticizer in industrial products, while diethyl phthalate is frequently detected in consumer products such as shampoos,

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detergents and cosmetics.[24] As a result, humans are exposed through multiple routes including ingestion, inhalation, and dermal absorption. Phthalates can affect the hormonal systems and have been linked to numerous health outcomes related to endocrine disruption, such as obesity, asthma, preterm birth and neurobehavioral problems.[25–27]

Several cross-sectional studies have reported associations between higher urinary phthalate metabolite concentrations and increased BMI.[28–33] However, only a few studies have examined effects of prenatal exposure to phthalates on body size of children, with mixed results.[34–38] For example, Valvi *et al.*[36] found associations of prenatal phthalate metabolite concentrations with higher BMI *Z*-scores in girls while Maresca *et al.*[37] showed that concentrations of phthalate metabolites (excluding those of DEHP) during pregnancy were inversely associated with BMI Z-scores, fat percentage, and waist circumference in boys. Recently, 8-isoprostane concentrations measured during pregnancy were found to mediate the association between phthalate metabolites and preterm birth.[39]

We previously reported in pregnant women participating in the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) study that urinary concentrations of two metabolites of high molecular weight (HMW) phthalates [monocarboxyoctyl phthalate (MCOP), and monocarboxynonyl phthalate (MCNP)] were associated with isoprostane concentrations at 13 weeks of gestation and all metabolites of low molecular weight (LMW) phthalates and some of the metabolites of HMW phthalates (mono (3-carboxypropyl) phthalate, MCPP; MCNP) were associated with isoprostane concentrations at 26 weeks of gestation.[2]

The goal of the present study was to characterize isoprostane changes in repeated samples in the CHAMACOS children at 5, 9 and 14 years of age and to explore potential association of *in utero* exposure to phthalates and isoprostane concentrations in children at these three ages. We also examined the relationship of children 8-isoprostane concentrations on their body mass and cardiometabolic characteristics.

Methods

Subjects and study design

CHAMACOS is a longitudinal birth cohort examining the effects of environmental exposures on neurodevelopment, growth and respiratory disease in pregnant women and their children living in Salinas Valley, California.[40] A total of 601 pregnant women were enrolled in the CHAMACOS study between October 1998 and 2000, with 536 live births. Mothers enrolled in the CHAMACOS cohort were primarily young (mean ± S.D., 26.3 ± 5.2 years), married, low-income, eligible for low income health insurance (Medi-Cal eligible), Mexican-born, and Spanish-speaking. Most were either farm workers themselves or lived with farm workers at the time of enrollment.

Pregnant women were interviewed at ~13 and 26 weeks of gestation, after delivery, and at the time of each child assessment. At the time of the pregnancy interviews (gestational weeks 13 and 26), maternal phthalate metabolite concentrations were measured in urine to capture early and late pregnancy exposure and to assess variations in phthalate exposure across pregnancy. Detection frequencies of all phthalate metabolites in CHAMACOS participants ranged from 90 to 100%, as described previously in more detail.[2,41] We measured 8-isoprostane concentrations in urine from a subset of 5- ($n = 257$), 9- ($n = 250$) and 14- ($n = 260$) year-old CHAMACOS children based on the availability of urine samples at all three time-points. Few of the children born to CHAMACOS mothers were preterm (8%) or had a low birthweight (4%). Their birthweight ranged from 1530 to 4885 g, with an average of 3441 g. This subset was not significantly different from the main CHAMACOS cohort in most demographic and exposure parameters (child sex, maternal country of birth, race, poverty index, education, pre-pregnancy BMI, smoking and alcohol use). However, mothers in this sample tended to be slightly older, lived in the United States longer, and more likely to be multiparous. Study protocols were approved by the University of California, Berkeley and the Centers for Disease Control and Prevention (CDC) Committees for Protection of Human Subjects. Written informed consent was obtained from all mothers, and verbal assent was obtained from children at 5 and 9 and written assent at 14 years of age.

Anthropometric and cardiometabolic data

Body weight was assessed using an electronic mother-baby scale (Tanita, Model 1582) at age 5 years and a bioimpedance electronic scale (Tanita, Model X) at age 9 and 14 years. Height without shoes was measured using a wall-mounted stadiometer and waist circumference was measured at the iliac crest with a measuring tape. Height and waist circumference measurements were performed in triplicate and then averaged. To calculate BMI, weight in kilograms was divided by height in meters squared. These values were compared to CDC reference data to generate BMI *Z*-scores standardized by sex and age. Using the age and sex-specific BMI cutoffs from the 2000 CDC child growth charts, children were categorized as normal and overweight (<85th and ⩾85th percentile, respectively). One participant with an extreme BMI (56.6 kg/m^2) was excluded from the analyses. Blood pressure measurements were obtained while the participant was at rest, using a Dinamap 9300 sphygmomanometer. Fasting blood samples were collected from children at 9 and 14 years of age, but were not available for 5-year-old children. Serum samples were analyzed at a local laboratory (Quest Diagnostics, San Jose, CA) for glucose and a standard lipid panel including triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol and cholesterol/HDL ratio.

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8-Isoprostane and creatinine measurements

Spot urine samples collected from mothers during pregnancy and from children at 5, 9 and 14 years were stored at −80°C at the School of Public Health Biorepository in UC Berkeley until analysis. Concentrations were quantified using a urinary 8-isoprostane competitive enzyme-linked immunosorbent assay (ELISA) kit (Oxford Biomedical Research, Rochester Hills, MI, USA) as previously described.[2] Briefly, urine samples were pre-treated with β-glucuronidase (Oxford Biomedical Research) before running the ELISA. The limit of detection (LOD) for 8-isoprostane concentration was 0.08 ng/ml. Undetected oxidative stress measures were replaced with the LOD divided by the square root of 2. Additional quality assurance/quality control provisions included repeats of 5% of samples and blanks, and internal lab controls with good reproducibility of isoprostane (coefficient of variation <7%).

Creatinine levels in children's samples were analyzed using a urinary creatinine ELISA kit. Samples were randomized across plates and the coefficient of variation was less than 3%. All isoprostane concentrations were adjusted to account for urinary dilution by dividing isoprostane concentrations (ng/ml) by creatinine levels (mg/dl) with results reported in ng/mg creatinine.[2]

Maternal urinary phthalate metabolite measurements

A total of 11 phthalate metabolite concentrations were measured at the CDC in maternal urinary samples using online solid phase extraction coupled with isotope dilution high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry.[42] Briefly, these included metabolites of three LMW phthalates [monoethyl phthalate (MEP), mono-n-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP)], four metabolites of DEHP [mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)], and four metabolites of other HMW phthalates [monobenzyl phthalate (MBzP), MCPP, MCOP, MCNP]. Quality control procedures included laboratory blanks, calibration standards, and spiked controls with high and low concentrations. For concentrations below the LOD, the actual instrument reading value was used, if available, or a measurement below the LOD was imputed from a log-normal distribution using the 'fill-in' method described in Lubin *et al.* (2004), if not.[43] All phthalate metabolite concentrations were corrected for creatinine (μg/g creatinine) by dividing metabolite concentrations (μg/l) by creatinine levels (g/l).

Statistical analysis

Urinary 8-isoprostane and urinary phthalate metabolite concentrations were $\log_{10}$ transformed to normalize their distributions in all statistical analyses, with the exception of summary statistics describing their distributions by age and sex as reported in Tables 1 and 2. In addition, all descriptive statistics and correlation analyses used creatinine-corrected 8-isoprostane concentrations. Regression analyses, however, used uncorrected 8-isoprostane concentrations, instead including creatinine levels as a covariate in the model. Before analysis, two subjects, whose isoprostane concentrations at age 14 years were outliers (>3 S.D. from mean), were dropped. However, the results did not change appreciably when outliers for BMI or isoprostane levels were included in the analysis. Anthropometric and cardiometabolic parameters were $\log_{10}$

Table 1. *Population characteristics of CHAMACOS children at ages 5, 9 and 14 years*

Characteristic	Age 5 (n = 256) [mean (S.D.)]	Age 9 (n = 250) [mean (S.D.)]	Age 14 (n = 258) [mean (S.D.)]
Sex			
Male (%)	119 (46)	118 (47)	121 (47)
Female (%)	137 (54)	132 (53)	137 (53)
Weight (kg)	22.0 (5.2)	38.9 (11.5)	67.5 (18.2)
Height (cm)	110.3 (4.6)	135.6 (6.4)	161.6 (7.3)
Waist circumference	58.6 (7.6)	74.4 (12.1)	84.7 (16.4)
Waist/height ratio	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)
BMI (kg/m^2)	17.9 (3.0)	20.9 (4.7)	25.4 (6.5)
BMI Z-score	1.2 (1.1)	1.1 (1.0)	1.1 (1.0)
Weight status[a]			
Normal (<85th Percentile)	115 (45%)	108 (43%)	98 (43%)
Overweight (⩾85th percentile)	141 (55%)	143 (57%)	128 (57%)
8-Isoprostane (ng/mg creatinine)	4.6 (7.7)	4.2 (6.7)	6.0 (12.1)

BMI, body mass index.
[a]Total number of observations varies due to missing data.

Table 2. *Urinary concentrations of 8-isoprostane (creatinine-adjusted) in 5, 9 and 14-year-old boys and girls (ng/mg creatinine).*

	n	Mean ± S.D.	P-value	Median	Minimum	Maximum
Age 5						
Boys	119	4.8 ± 8.4	0.86	3.0	0.6	67.7
Girls	137	4.5 ± 7.4		3.2	0.3	67.2
All	256	4.6 ± 7.9		3.1	0.3	67.7
Age 9						
Boys	118	4.5 ± 7.3	0.74	2.9	0.7	50.1
Girls	132	4.1 ± 6.1		2.8	0.8	51.1
All	250	4.2 ± 6.7		2.9	0.7	51.1
Age 14						
Boys	121	6.4 ± 13.3	0.38	2.8	0.2	107.1
Girls	137	5.7 ± 11.1		2.7	0.2	100.5
All	258	6.0 ± 12.2		2.6	0.2	107.1

P-values are for t-tests comparing isoprostane levels between boys and girls at each age.

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transformed to reduce the influence of outliers and to achieve homogeneity of variance.

To make pairwise comparisons of isoprostane concentrations between age groups and with maternal concentrations, Pearson's correlation coefficients were calculated. Differences in mean isoprostane concentrations by sex were determined by *t*-tests. To determine cross-sectional associations of isoprostane concentrations at ages 5, 9 and 14 years with physical parameters of obesity at the concurrent age, we calculated Pearson's correlation coefficients and performed linear regression models adjusting for sex and creatinine.

To model longitudinal changes of 8-isoprostane concentration by age, we performed generalized estimating equations (GEE) with an exchangeable correlation structure and controlled for creatinine, sex and BMI. We also performed mixed effects regression models, including a random effect for CHAMACOS participant, to confirm findings from the GEE models and to assess associations of child sex, age and concurrent BMI with isoprostane concentrations. Mixed effect models account for correlated measures of isoprostanes across the three time points.[44] Additional covariates, including gestational age and birthweight, were also considered for inclusion in the models based on some previous reports from other studies.[26,45,46] However, these variables were not significantly associated with phthalate or isoprostane levels in CHAMACOS participants. To examine associations of prenatal phthalate metabolite concentrations with isoprostane concentrations in children, separate linear regression models were constructed for each phthalate metabolite (11 individual metabolites and 3 sum measures) at each maternal visit during pregnancy (13 and 26 weeks of gestation) with isoprostane concentrations measured in children at ages 5, 9 and 14 years, controlling for sex and BMI. Linear regression models controlling for BMI, sex and creatinine were used to determine associations of isoprostane concentrations with cardiometabolic parameters (fasting glucose, triglycerides, LDL and HDL cholesterol, HDL cholesterol ratio, non-HDL cholesterol, total cholesterol, and systolic and diastolic blood pressure) at the concurrent age in 9 and 14-year-old children. All statistical analysis was performed in STATA (version 12.1; STATA Corp., College Station, TX). *P*-values <0.05 were considered significant.

Results

CHAMACOS study participant characteristics

Population characteristics of the CHAMACOS children at ages 5, 9 and 14 years are summarized in Table 1. Among participants in this study, there were similar numbers of boys and girls at each age. At 9 years of age, 36% of girls had entered puberty, compared with 10% of boys. All CHAMACOS participants, with the exception of 9% of males, reached puberty at 14 years of age. As expected, mean body composition measurements including weight, height, waist circumference and BMI increased with age. The prevalence of overweight in CHAMACOS children (⩾85th percentile) was 55, 57 and 57% at ages 5, 9 and 14 years, respectively; most of the children whose BMI was >85th percentile were obese (BMI *Z*-scores ⩾95th percentile).

Analysis of 8-isoprostane concentrations at different ages

The distribution of 8-isoprostane concentration varied by age, but the medians for 5, 9 and 14-year-old children were similar (3.1, 2.9 and 2.6 ng/mg creatinine, respectively, $P = 0.73$; Table 2). Isoprostane concentrations at 14 years had the greatest variability, ranging from 0.2 to 107.1 ng/mg creatinine when compared with 5- (0.25 to 67.7 ng/mg creatinine) and 9-year-old (0.7–51.1 ng/mg creatinine) children. There was no significant correlation between isoprostane concentrations at each of the three time points (all $r < 0.10$). The relatively large time gaps (4–5 years) between the isoprostane assessments may explain this finding. Although the mean 8-isoprostane concentrations were higher in boys than girls at ages 5, 9 and 14 years, the differences were not statistically significant (Table 2). We did not find any significant associations between age and urinary 8-isoprostane after adjusting for child sex and BMI. We also used mixed effects models, and the results did not change.

Maternal isoprostane urinary concentrations at 13 and 26 weeks[2] were modestly correlated (all $r < 0.30$) with their children's concentrations at 5, 9 or 14 years (not shown). Maternal age and parity were not significantly associated with child isoprostane concentrations. Children whose mothers lived in the United States for >11 years (at the time of enrollment) had slightly lower $\log_{10}$ isoprostane concentrations at 9 years of age compared with children whose mothers had lived in the United States for <1 year [β (95% CI): −0.15(−0.27, −0.03)].

Examination of the relationship between isoprostane with body mass

After adjusting for sex, we did not observe significant associations between current isoprostane concentrations and children's BMI, BMI *Z*-score or waist circumference (Supplementary Table 1). Isoprostane concentrations did not differ significantly by weight categories (normal *v.* overweight/obese) within each age group. For example, at age 5 years, mean isoprostane concentrations were 0.49 ng/mg creatinine in normal weight children and 0.50 ng/mg creatinine in overweight/obese group ($P = 0.72$); at 9 and 14 years, isoprostane values were also similar between two BMI groups ($P = 0.06$ and 0.3, respectively).

Isoprostane and cardiometabolic parameters in 9- and 14-year-old children

Cardiometabolic parameters in 9 and 14-year-old children are summarized in Table 3. Among 9-year olds, no children had elevated over the reference range fasting glucose levels

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Table 3. *Cardiometabolic parameters and their cross sectional associations with 8-isoprostane urinary concentrations in CHAMACOS children at 9 and 14 years*

Metabolic parameters	Age 9 (n = 113) (mean ± S.D.)	Age 14 (n = 248) (mean ± S.D.)	9-year isoprostane [β (95% CI)]	P-value	14-year isoprostane [β (95% CI)]	P-value
Fasting glucose (mg/dl)	88.8 ± 6.0	82.7 ± 8.7[b]	−0.002 (−0.011, 0.007)	0.63	−0.001 (−0.008, 0.006)	0.70
Triglycerides (mg/dl)	86.1 ± 47.3	107.1 ± 68.0[b]	0.158 (−0.119, 0.434)	0.26	0.129 (−0.152, 0.411)	0.37
LDL cholesterol (mg/dl)	81.9 ± 22.5	79.1 ± 25.9[b]	0.001 (−0.002, 0.003)	0.58	0.001 (−0.001, 0.004)	0.26
HDL cholesterol (mg/dl)	51.6 ± 12.8	50.6 ± 13.3	0.001 (−0.005, 0.005)	0.87	0.004 (−0.001, 0.009)	0.10
HDL cholesterol ratio	3.1 ± 0.9	3.2 ± 1.9	0.176 (−0.309, 0.661)	0.47	0.112 (−0.363, 0.588)	0.64
Non-HDL cholesterol	99.2 ± 27.0	100.3 ± 30.9	0.001 (−0.001, 0.003)	0.52	0.001 (−0.001, 0.003)	0.15
Total cholesterol (mg/dl)	150.5 ± 26.4	150.9 ± 29.2	0.217 (−0.490, 0.923)	0.54	0.884 (0.202, 1.566)	**0.01**
Systolic blood pressure[a]	97.3 ± 10.9	110.1 ± 11.8[b]	−0.001 (−0.006, 0.003)	0.61	0.008 (0.002, 0.015)	**0.02**
Diastolic blood pressure[a]	53.9 ± 5.9	59.7 ± 6.9[b]	−0.002 (−0.008, 0.005)	0.65	0.011 (0.001, 0.02)	**0.02**

LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index.

β coefficients, 95% confidence intervals and P-values on the right side of the table represent results of each individual linear regression model of isoprostane concentrations (dependent variable) with cardiometabolic parameters (independent variable) at the concurrent age in 9 and 14-year-old children. All models controlled for BMI, sex and creatinine. Triglycerides, cholesterol HDL ratio and total cholesterol are log transformed.

Bolded values are significant, $P < 0.05$.

[a]Total number of observations varies due to missing data.

[b]Values are significantly different from 9 and 14 years, $P < 0.05$.

(⩾110 mg/dl), 25% had high triglyceride levels (⩾110 mg/dl), 15% had low HDL levels (⩽40 mg/dl), and 7.5% had high blood pressure (⩾90th percentile for age and sex). These numbers increased at age 14 with 0.4% of the subjects having high fasting glucose levels, 35% with high triglycerides, 23% with low HDL and 18% with high blood pressure. We found no significant associations of isoprostane with cardiometabolic parameters at 9 years of age. However, at 14 years, isoprostane urinary concentrations were positively and significantly associated with concurrent measures of total cholesterol (β: 95% CI = 0.88: 0.20–1.57, $P = 0.01$), systolic blood pressure (β: 95% CI = 0.01: 0.002–0.015, $P = 0.02$) and diastolic blood pressure (β: 95% CI = 0.011: 0.001–0.020, $P = 0.02$) (Table 3).

Relationship between urinary prenatal phthalate metabolite concentrations and isoprostane concentrations

There were no significant associations between phthalate metabolite concentrations at 13 weeks of gestation and isoprostane concentrations in 5-year-old children, after adjusting for sex and BMI (Table 4). Associations between prenatal maternal phthalate metabolite concentrations and isoprostane concentrations in 9-year olds were also not statistically significant with the exception of three metabolites of HMW phthalates – MBzP, MCOP and MCNP, which were negatively associated with isoprostane (Table 4). Isoprostane concentrations in 14-year-old children were positively associated with concentrations of maternal MEHP, MECPP and ∑DEHP metabolites during pregnancy [β: 95% CI = 0.10 (0.001, 0.20), 0.14 (0.001, 0.29), 0.14 (0.001, 0.28), respectively] and borderline significantly associated with MEHHP and MEOHP concentrations [β: 95% CI = 0.12 (−0.01, 0.25), 0.10 (−0.01, 0.22), respectively]. None of the prenatal phthalate metabolite concentrations at 26 weeks of gestation were associated with children's isoprostane concentrations at either 5, 9 or 14 years of age.

We used PARAMED, a STATA package, to run mediation analysis testing the hypothesis that oxidative stress mediates the effect of phthalate exposure on cardiometabolic parameters. We investigated the effect of MEOHP and MEHHP on 14-year diastolic blood pressure, as mediated by 14-year isoprostane levels. These parameters were chosen as the only significant results after tests for associations between phthalate metabolite levels in maternal urine samples and cardiometabolic parameters in 14-year-old children. Although we observed a marginally significant natural indirect effect for MEOHP and MEHHP ($P = 0.08$ and 0.06, respectively), they were no longer significant after accounting for child sex and BMI at 14 years of age.

Discussion

In the CHAMACOS longitudinal birth cohort study, we found no differences in isoprostane concentrations by children's sex or age (ages 5, 9 or 14 years). Maternal concentrations of DEHP metabolites (MEHP, MECPP, ∑DEHP) at 13 weeks of gestation (but not at 26 weeks) were positively associated with isoprostane concentrations in the 14-year-old children and these isoprostane concentrations were positively associated with concurrent measures of total cholesterol and blood pressure, but not associated with BMI, BMI Z-score, and waist circumference (at 5, 9 and 14 years). In contrast, other high molecular weight metabolites (MBzP, MCOP, MCNP) at

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Table 4. *Associations of prenatal maternal urinary phthalate metabolite concentrations at 13 weeks with isoprostane urinary concentrations in their children at 5, 9 and 14 years*

	5 years (n = 254)		9 years (n = 259)		14 years (n = 223)	
Exposure at 13 weeks	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
MEP	−0.01 (−0.09, 0.06)	0.71	−0.03 (−0.10, 0.03)	0.29	−0.03 (−0.13, 0.06)	0.48
MBP	−0.03 (−0.11, 0.05)	0.48	−0.06 (−0.13, 0.01)	0.10	−0.01 (−0.12, 0.10)	0.89
MiBP	−0.01 (−0.08, 0.06)	0.76	−0.03 (−0.10, 0.03)	0.32	0.03 (−0.07, 0.13)	0.60
Σ LMW	−0.02 (−0.10, 0.06)	0.64	−0.04 (−0.11, 0.03)	0.25	−0.03 (−0.13, 0.07)	0.55
MEHP	−0.06 (−0.13, 0.02)	0.13	0.01 (−0.05, 0.08)	0.69	0.10 (0.001, 0.20)	**0.04**
MEHHP	−0.04 (−0.14, 0.05)	0.37	−0.01 (−0.10, 0.07)	0.78	0.12 (−0.01, 0.25)	0.07
MEOHP	−0.05 (−0.13, 0.04)	0.31	−0.02 (−0.09, 0.06)	0.67	0.10 (−0.01, 0.22)	0.08
MECPP	−0.06 (−0.16, 0.05)	0.31	0.02 (−0.08, 0.12)	0.71	0.14 (0.001, 0.29)	**0.05**
Σ DEHP	−0.06 (−0.16, 0.05)	0.27	0.001 (−0.09, 0.09)	0.99	0.14 (0.001, 0.28)	**0.05**
MBzP	−0.02 (−0.11, 0.07)	0.71	−0.09 (−0.17, −0.01)	**0.03**	−0.06 (−0.17, 0.06)	0.34
MCPP	−0.01 (−0.07, 0.06)	0.79	−0.03 (−0.09, 0.03)	0.28	−0.03 (−0.11, 0.05)	0.50
MCOP	0.01 (−0.08, 0.11)	0.78	−0.09 (−0.18, −0.01)	**0.03**	0.04 (−0.10, 0.17)	0.60
MCNP	−0.02 (−0.12, 0.07)	0.58	−0.09 (−0.17, −0.01)	**0.03**	0.02 (−0.10, 0.14)	0.77
Σ HMW	−0.04 (−0.16, 0.08)	0.50	−0.03 (−0.13, 0.07)	0.58	0.12 (−0.03, 0.28)	0.12

MEP, monoethyl phthalate; MBP, mono-n-butyl phthalate; MiBP, mono-isobutyl phthalate; LMW, low molecular weight; MEHP, mono (2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; DEHP, di-2-ethylhexyl phthalate; MBzP, monobenzyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MCOP, monocarboxyoctyl phthalate; MCNP, monocarboxynonyl phthalate; HMW, high molecular weight.

Separate linear regression models were performed for each phthalate metabolite (11 individual metabolites and 3 sum measures) at 13 weeks of gestation with isoprostane concentrations measured in children at ages 5, 9 and 14 years, controlling for sex and body mass index.

Bolded values are significant, $P < 0.05$.

13 weeks were negatively associated with isoprostane concentrations at 9 years.

In a prospective study of children at risk for type 1 diabetes, isoprostane concentrations were found to be highest in infancy, decreased nonlinearly until the children reached 6 years of age and then increased until 7 years of age.[47] Our results showing no association with age may have differed because our study population was older and more overweight than the other study population. We did not find that isoprostane concentrations were associated with concurrent measures of BMI, BMI Z-score or waist circumference. These findings were unexpected based on available cross-sectional reports showing a positive relationship of isoprostane concentrations and BMI, waist circumference and percent body fat in adolescent children.[18,21,48] One possible explanation for differences in study results is that in our relatively overweight/obese population there is a compensatory mechanism involved in the maintenance of energy homeostasis related to the children's body mass.[49–52] Fat oxidation plays an important role in metabolic adaptation because it helps regulate the physiological response to weight gain or loss. Prospective studies indicate that lower baseline levels of fat oxidation (lower half) predict weight gain and individuals are also more likely to sustain weight gain if their metabolic adaptation is weak.[50,51,53]

Previous studies have found positive relationships between biomarkers of oxidative stress, specifically 8-isoprostane, with important metabolic parameters in both children and adults.[54,55] Our results suggest that isoprostane concentrations were significantly and positively correlated with total cholesterol, systolic and diastolic blood pressure in 14-year children. This is in agreement with similar findings of higher urinary isoprostane concentrations in children with elevated 24-h ambulatory blood pressure.[21] Similarly, another study found that levels of peroxy radicals in urine of obese children and adolescents were positively correlated with total cholesterol, systolic and diastolic blood pressure.[56] These findings suggest that an oxidative stress measure is associated with increased blood pressure and total cholesterol; however, it is not clear whether changes in blood pressure and cholesterol precede changes in oxidative stress or vice versa.

Previously, we observed positive relationships between phthalate metabolite urinary concentrations and isoprostane concentrations in pregnant women in our cohort.[2] In the present study, we did not find any significant associations of isoprostane concentrations in the 5-year-old children from the same cohort with either early or late prenatal phthalate exposure nor their average. However, we observed negative associations with MBzP, MCOP and MCNP, all metabolites of HMW phthalates, measured in maternal urine at 13 weeks of gestation with isoprostane concentrations in older CHAMACOS children at age 9 years. In addition, DEHP metabolite concentrations at 13 weeks (MEHP, MECPP, MEHHP and MEOHP) were either significantly or borderline significantly positively associated with isoprostane in 14-year-old children.

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As no associations between isoprostane concentrations in each of these three childhood time points were observed for prenatal phthalate metabolite concentrations at 26 weeks, our results suggests that 13 weeks of gestation may coincide with a critical window of *in utero* phthalate exposure that may affect childhood growth and development later in life.

The observed associations between phthalate metabolite concentrations at 13 weeks of gestation and isoprostane concentrations at 9 and 14 years represent novel findings. The negative associations of early pregnancy HMW metabolites with 9-year isoprostanes, as well as the positive associations of DEHP metabolites with 14-year isoprostanes, may indicate potential remodeling of associations during hormonal shifts that occur as youth enter puberty at varying time points. It may also be related to a difference in the effects of these two phthalates during *in utero* exposure on the developmental processes during childhood and adolescence. For instance, endocrine disrupting chemicals (EDC), including some phthalates, have been linked to puberty onset[57–59] and prenatal EDC exposure has been associated with changes in hormone levels in boys from the CHAMACOS cohort.[60] We found that maternal blood concentrations of brominated diphenyl ether (BDE)-100 and −153 during pregnancy were associated with an increase of the leutenizing hormone levels, and BDE-153 was associated with an increase in testosterone in 12-year-old CHAMACOS boys; while dichlorodiphenyltrichloroethane (DDT), after accounting for Tanner stage, was associated with decreases in the hormone levels. In animal studies, high levels of phthalates have been associated with decreased production of both estrogen and testosterone.[23] In a Japanese youth cohort, grade in school (used as a surrogate of age) was negatively associated with markers of oxidation, potentially mediated by sex hormones.[61] Although we have not yet examined the relationships between prenatal phthalate exposure and hormone levels in the CHAMACOS youth, we hypothesize that phthalate exposure sets off a cascade of effects, including hormone and oxidation shifts, which could explain the contrasting relationships between different phthalates *in utero*, and isoprostanes at 9 and 14 years. It is possible that endocrine disruption caused by phthalate exposure with potential shifts in the hypothalamic–pituitary–gonadal axis could also alter oxidative stress biomarkers.

Although we found significant associations between maternal urinary concentrations of phthalate metabolites at 13 weeks of gestation and isoprostane concentrations in 9 and 14-year-old children, additional research to validate the current findings and examine whether childhood phthalate metabolite concentrations (as yet not available for our cohort) are also associated with isoprostane concentrations would be of interest.

To explore the nature of biological relationships between isoprostanes, phthalates and the cardiometabolic parameters assessed in the current study, we rely on several lines of evidence. Using mediation analysis, we observed a marginal indirect effect for two of the 11 phthalate metabolites on cardiometabolic parameters in 14-year-old children. However, it was abated after accounting for child sex and BMI. It is known that phthalate exposure *in utero* can potentially disrupt developmental processes that may lead to an increased risk of cardiometabolic diseases later in life.[36] Phthalates are agonists for peroxisome proliferator-activated receptors, which play a key role in adipogenesis and lipid accumulation.[62] In addition, a relationship between obesity, oxidative stress and an increased risk of cardiometabolic disease is demonstrated in adults and children.[63,64] Phthalate metabolites have been also shown to be associated with markers of inflammation and oxidative stress.[4,65]

We note several limitations of the current study. Given the high prevalence of overweight and obesity in this Mexican-American cohort, its low socioeconomic status and exposure to other factors typical for an agricultural setting, our results may not be generalizable to other ethnic groups or populations where overweight/obesity rates are lower. The overall percentage of overweight and obese children in our cohort was consistently above 55% at all ages from 5 to 14 years, which is higher than the National Health and Nutrition Examination Survey prevalence of obesity (overweight and obese combined) in Hispanic youth (38.9%) or all U.S. youth aged 2–19 years (31.8%).[66] Our study used indirect measurements for generalized adiposity and central adiposity, which may be less accurate, compared with other direct measurements, such as dual-energy X-ray absorptiometry images. In addition, maternal phthalate metabolites and isoprostane concentrations were measured using spot urine samples. These concentrations may be subject to temporal changes and high within-person variability over time and thus may not accurately reflect average exposures over pregnancy, leading to non-differential misclassification and bias toward the null. However, stable measurements of phthalate metabolites in spot urine samples were reported over relatively long periods of time.[26,67–69]

In conclusion, oxidative stress levels, assessed by average urinary concentrations of 8-isoprostane, in a cohort of mainly obese or overweight Mexican-American children did not appreciably change over ages 5, 9 and 14 years. These concentrations did not differ by sex, BMI, BMI *Z*-score or waist circumference, however the relationship of 8-isoprostane with three cardiometabolic parameters was statistically significant in 14-year-old children. Total cholesterol, systolic and diastolic blood pressure were positively associated with isoprostane concentrations in 14-year-old children, supporting the hypothesis that oxidative stress may be related to certain cardiometabolic risk factors. The significant relationships between prenatal phthalate metabolite concentrations at 13 weeks (but not at 26 weeks) of gestation with isoprostane concentrations in children at 9 and 14 years suggests that 13 weeks may be a critical period when the fetus is more vulnerable to phthalate exposure.

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Conflicts of Interest

None.

Supplementary material:

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