

Research Article

Maternal Bisphenol A Exposure During Pregnancy and Its Association With Adipokines in Mexican-American Children

Vitaly Volberg,¹ Kim Harley,¹ Antonia M. Calafat,² Veronica Davé,¹ Jessica McFadden,¹ Brenda Eskenazi,¹ and Nina Holland^{1*}¹Center for Environmental Research and Children's Health (CERCH), Environmental Health Sciences, School of Public Health, University of California, Berkeley, California²Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, California

Bisphenol A (BPA) is a high volume production chemical that has been detected in 93% of the United States population. It is thought to have endocrine disrupting activity but human data are limited. In this study, we examined whether prenatal or concurrent urinary BPA concentrations are associated with key metabolism-related hormones, adiponectin and leptin (adipokines), in 9-year-old children. For this analysis, we used 188 mother-child pairs from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) prospective study. BPA was measured in urinary spot samples during early (12.6 ± 3.9 weeks gestation) and late (26.3 ± 2.5 weeks gestation) pregnancy and in 9-year-old children. We found that BPA concentrations during late pregnancy were associated with increased plasma

leptin in boys ($\beta = 0.06$, $P = 0.01$), controlling for maternal prepregnancy body mass index (BMI), pregnancy soda consumption, and smoking, years in US prior to pregnancy, maternal education, household poverty status, child BMI and child soda, fast food and sweet snack consumption at 9 years. Additionally, we found that BPA concentrations during early pregnancy are directly associated with plasma adiponectin levels in girls ($\beta = 3.71$, $P = 0.03$). However, we did not find any significant relationships between concurrent BPA concentrations and 9-year child adiponectin or leptin. Overall, our data suggest that prenatal BPA concentrations may influence adipokine levels in 9-year-old children. Environ. Mol. Mutagen. 54:621–628, 2013. © 2013 Wiley Periodicals, Inc.

Key words: leptin; adiponectin; prenatal; endocrine disruptor; sex-difference

INTRODUCTION

Bisphenol A (BPA) is used to make epoxy resins and polycarbonate plastics—present in household applications such as water bottles, food containers, canned food, dental fillings and household electronics [Vandenberg et al., 2007]. In the United States (US), BPA ranks among the highest volume production chemicals and National Health and Nutrition Examination Survey (NHANES) data show that urinary BPA is detectable in 93% of the US population [Calafat et al., 2008]. There is evidence that BPA can cross the placenta and is present in fetal circulation and amniotic fluid [Balakrishnan et al., 2010; Morck et al., 2010].

Increasing data argue that BPA may act as an “environmental obesogen”—a term used to describe chemicals that can alter energy balance, promoting adipogenesis and lipid accumulation [Grun and Blumberg, 2006]—and several reports suggest that BPA affects levels of important metabolism-related hormones, adiponectin and leptin—also

known as adipokines [Miyawaki et al., 2007; Hugo et al., 2008; Ben-Jonathan et al., 2009; Somme et al., 2009]. Adiponectin, a protein hormone secreted by adipose tissue, targets muscle and liver to increase uptake and catabolism of fatty acids and carbohydrates, promoting insulin sensitivity [Kadowaki and Yamauchi, 2005]. In children, lower adiponectin levels are associated with both metabolic syndrome and type 2 diabetes [Cruz et al., 2004; Shaibi et al., 2007]. Leptin, a hormone synthesized mainly by adipose tissue

*Correspondence to: Dr. Nina Holland, 733 University Hall, School of Public Health, UC Berkeley, CA 94720-7360, USA. E-mail: ninah@berkeley.edu

Received 23 April 2013; provisionally accepted 11 June 2013; and in final form 13 June 2013

DOI 10.1002/em.21803

Published online 1 August 2013 in Wiley Online Library (wileyonlinelibrary.com).

but also by the stomach, skeletal muscle and liver, acts on the hypothalamus to convey satiety and regulate long-term energy balance [Margetic et al., 2002; Mantzoros et al., 2011]. Plasma leptin levels correlate positively with adiposity, however, obese individuals commonly develop “leptin resistance” or tolerance—a hyperleptinemic state with a lack of leptin’s regulatory effects [Considine et al., 1996; Ong and Loos, 2006].

BPA exposure may be linked to changes in metabolic health in a sex-dependent manner. Using a mouse model, Miyawaki et al. [2007] showed that perinatal exposure to BPA resulted in sex-specific increases in body and adipose tissue weight and changes in leptin levels in the offspring [Miyawaki et al., 2007]. Additionally, Somm et al. [2009] showed that although perinatal exposure to BPA resulted in greater weight of both male and female Sprague-Dawley rats on postnatal day 1, this difference persisted only in females at postnatal day 21 [Somm et al., 2009]. In *in vitro* cell models, BPA has been shown to promote differentiation of 3T3-L1 fibroblast cells into adipocytes [Masuno et al., 2005] and inhibit adiponectin release from human adipocytes [Hugo et al., 2008].

Few data are available on associations between prenatal BPA exposure and children’s metabolic health. Chou et al. [2011] reported that mothers in the highest quartile of BPA blood concentrations at delivery had boys with increased odds of low adiponectin and high leptin at birth [Chou et al., 2011]. However, this study assessed BPA concentrations in participants’ blood—a measure known to have severe limitations due to high risk of contamination and blood collection occurred near time of birth where medical interventions in hospitals can dramatically increase BPA exposures [Calafat et al., 2009; Vandentorren et al., 2011; Duty et al., 2013]. Data from the National Health and Nutrition Examination Survey (NHANES) and other cross-sectional studies show that increased body mass index (BMI) is associated with greater urinary BPA concentrations [Trasande et al., 2012; Wang et al., 2012]. Nevertheless, whether higher BPA concentrations actually precede the onset of obesity remains uncertain [Becker et al., 2009; Lakind and Naiman 2011; Trasande et al., 2012; Wang et al., 2012].

In the first longitudinal study of BPA and obesity, we did not observe associations of prenatal BPA urinary concentrations and increased body weight in children participating in the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort. In fact, prenatal BPA urinary concentrations were negatively associated with BMI Z-score in girls [Harley et al., 2013]. Thus, whether and how prenatal or childhood BPA exposure leads to altered metabolic health remains unclear.

In this study, we assessed whether maternal urinary BPA concentrations during the first and second halves of pregnancy are associated with plasma adiponectin and

leptin levels in children when they reach 9 years of age. Additionally, we investigated whether concurrent BPA concentrations are associated with adipokine levels in children at 9 years. Given that many previous studies report different responses to BPA exposure by sex, we tested for interaction by sex in our models. Analyses were performed using data from the CHAMACOS study, a Mexican-American cohort with a high prevalence of childhood obesity.

MATERIALS AND METHODS

Subjects and Study Design

The CHAMACOS study is a longitudinal birth cohort designed to assess the health effects of environmental exposures on growth and development in children living in the Salinas Valley, CA [Eskenazi et al., 2004, 2005]. Mothers were enrolled during pregnancy between October 1999 and October 2000, with 537 mother-child pairs in the study at delivery and 327 pairs remaining at the 9-year visit. Eligible women were ≥ 18 years of age, < 20 weeks gestation at enrollment, English or Spanish speaking, eligible for low income health insurance (Medicaid), and planning to deliver at the county hospital. Women were interviewed at ~ 13 and ~ 26 weeks gestation and anthropometric measures of the children were obtained at birth, 6 months, and 1, 2, 3 $\frac{1}{2}$, 5, 7, and 9 years of age. Adiponectin and leptin were measured on a convenience subsample of 188 children having blood samples at 9 years and complete anthropometric and demographic data. Of these children, 131 had maternal urinary BPA measures for the first half of pregnancy, 179 for the second half and 172 had 9-year urinary BPA data. No differences were observed comparing maternal and child demographic and anthropometric measures included in Table 1 between this subsample and the overall CHAMACOS cohort. All study activities were approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley and the Centers for Disease Control and Prevention (CDC).

Questionnaire Data

Mothers were interviewed at the two prenatal visits using structured questionnaires administered in Spanish or English by bilingual, bicultural trained interviewers. We collected sociodemographic information, including maternal age at pregnancy, years of living in the US prior to pregnancy, education, household poverty category, and smoking during pregnancy. During the second prenatal interview, we used a previously validated food frequency questionnaire (FFQ) to assess dietary intake, including maternal soda consumption, during pregnancy [Block et al., 1990; Harley et al., 2005] and asked additional questions at the 9-year interview about child soda, fast food and sweet snack consumption at age 9. Maternal prepregnancy BMI was calculated using the mother’s self-reported prepregnancy weight and measured height. Infant birth weight, length, and gestational age were obtained from delivery medical records abstracted by a registered nurse. Children were categorized as “small for gestational age” if their birth weight was < 10 th percentile for gestational age based on their ethnicity, maternal parity, and infant sex [Overpeck et al., 1999]. Children were considered as “at term” if they were born at or after 37 weeks of gestation.

Anthropometric Measurements

Children’s weight and height were measured at the 9-year visit using a bioimpedance electronic scale (Tanita TBF-300A Body Composition Analyzer.) and wall-mounted stadiometer (Seca 222), respectively. Child height was measured in triplicate and the average of measurements was

TABLE 1. Demographic Characteristics of Mothers and Children from the CHAMACOS Sturii Salinas Valle CA (N = 188)

Characteristic	N	(%)
Child sex		
Boy	87	(46.3)
Girl	101	(53.7)
Maternal age at pregnancy		
18-24	82	(43.6)
25-29	59	(31.4)
30-34	30	(16.0)
35-45	17	(9.0)
Maternal years in US at pregnancy		
<1	37	(19.7)
1-10	103	(54.9)
>10	48	(25.4)
Maternal education at pregnancy		
≤6th Grade	86	(45.7)
7-12 Grade	64	(34.0)
≥High School	38	(20.2)
Maternal soda consumption during pregnancy		
< 1 drink/week	102	(54.3)
1-6 drinks/week	70	(37.2)
7+ drinks/week	16	(8.5)
Household poverty category at pregnancy		
≤Poverty threshold	117	(62.2)
>Poverty level but <200% poverty level	65	(34.6)
≥200% Poverty level	6	(3.2)
Maternal pre-pregnancy BMI		
Normal (18.5–24.9 kg/m ²)	68	(36.1)
Overweight (25–29.9 kg/m ²)	71	(37.8)
Obese (≥ 30 kg/m ²)	49	(26.1)
Child gestational age at birth		
34 - 36 Weeks	10	(5.3)
≥37 Weeks	178	(94.7)
Child birth size		
Small for gestational age (<10th percentile)	8	(4.3)
Appropriate for gestational age	161	(85.6)
Large for gestational age (>90th percentile)	19	(10.1)
Child soda consumption at 9 years		
< 1 time/week	78	(41.5)
1-6 times/week	90	(47.9)
7+ times/week	20	(10.6)
Child fast food consumption at 9 years		
< 1 time/week	82	(43.6)
1-2 times/week	99	(52.7)
3+ times/week	7	(3.7)
Child sweet snack consumption at 9 years		
< 1 time/day	166	(88.3)
1-2 times/day	19	(10.1)
3+ times/day	3	(1.6)
Child BMI^a at 9 years		
Normal (≤85th percentile)	83	(44.2)
Overweight (>85th, <95th percentile)	32	(17.0)
Obese (≥95th percentile)	73	(38.8)

^aChild's weight status was determined using age and sex adjusted body mass index cut offs for 85th and 95th percentiles from CDC child growth charts.

used. BMI was calculated as mass in kilograms divided by height in meters squared. Children were categorized as normal weight, overweight, or obese using the sex and age-specific BMI cut-offs (85th and 95th percentile, respectively) provided by the 2000 CDC child growth

data. Mothers were categorized as normal weight (18.5–24.9 kg m⁻²), overweight (25–29.9 kg m⁻²), or obese (≥30 kg m⁻²) using the standard CDC BMI cutoffs for adults.

Plasma Adiponectin and Leptin Measurements

Nonfasting blood samples were collected from the children at the 9-year visit and stored at –80°C. Adiponectin and leptin were measured in plasma using enzyme-linked immunoassay (ELISA) RayBio Human Adiponectin and Human Leptin kits (Norcross, GA) as previously described [Volberg et al., 2013]. The minimum detectable concentrations for adiponectin and leptin were 10 and 6 pg mL⁻¹, respectively. All samples were run in duplicate and the values were averaged. The intra- and interplate coefficients of variance (CV) were 4 and 12%, respectively, for adiponectin and 3 and 15%, respectively, for leptin.

Maternal and Child Urinary BPA Measurement

Urinary spot samples were collected in sterile, polypropylene urine cups during the first (12.6 ± 3.9 weeks gestation) and second (26.3 ± 2.5 weeks gestation) half of pregnancy and from children at 9 years of age (9.4 ± 0.4 years). Samples were stored at –80°C and shipped to the CDC (Atlanta, GA) for analysis using online solid-phase extraction coupled with isotope-dilution high-performance liquid chromatography tandem mass spectrometry with peak focusing [Ye et al., 2005]. Total urinary concentration of BPA (free and conjugated) was measured with a limit of detection (LOD) at 0.4 µg L⁻¹. For concentrations below the LOD, we used the instrument-reported values. When no signal was detected, we imputed values at random based on a log-normal probability distribution whose parameters were determined by maximum likelihood estimation [Lubin et al., 2004]. Blanks, low (~2.8 µg L⁻¹) and high (~10 µg L⁻¹) quality control materials were included in all runs. To account for urinary dilution, we corrected maternal BPA concentrations using specific gravity, assessed using a hand-held refractometer (National Instrument Company, Baltimore, MD). Child 9-year BPA concentrations were corrected using urinary creatinine determined by the Vitros CREA assay (Ortho Clinical Diagnostics, NJ).

Statistical Analyses

Child adiponectin levels were approximately normally distributed, but leptin levels were right-skewed. Thus, all leptin-based analyses use log₁₀-transformed values. Urinary BPA concentrations during early and late pregnancy and at 9 years were also right-skewed and were log₂-transformed to reduce the influence of outliers. Using multivariate linear regression, we examined separately effects of BPA exposure during first and second halves of pregnancy and at age 9 on 9-year child adiponectin and leptin. Our models included an interaction term between child sex and BPA concentrations and BPA effect coefficients for each sex were estimated using the “lincom” postregression command in Stata. Potential confounders were identified *a priori* using a directed acyclic graph and included maternal prepregnancy BMI, soda consumption and smoking during pregnancy, Years of residence in US, maternal education, household poverty status, and child soda, fast food and sweet snack consumption at 9 years. Many of these covariates were chosen based on previous literature showing that several risk factors for obesity, including consumption of soda, school lunches and meals prepared outside of the home, are also related to increased urinary BPA concentrations in the general US population of children [Lakind and Naiman, 2011]. Importantly, previous mechanistic evidence has shown that BPA may have direct effects on adipokines [Hugo et al., 2008; Ben-Jonathan et al., 2009]. Thus, models presented in this study control for child BMI to examine the BPA—adipokine association, independent of BMI's effect on adipokines. All statistical analyses were conducted using STATA 12 (College Station, TX) for Windows. We set statistical significance at $P < 0.05$ for main effects and $P < 0.10$ for interaction terms.

TABLE 2. Leptin and Adiponectin Levels in 9-Year-Old CHAMCOS Children

Adipokine	N	Mean (SD)	T-test ^a by sex
			P-value
Leptin^b (ng/mL)			
Boys	87	7.1 (1.0)	0.08
Girls	101	9.3(1.2)	
Total	188	8.2(1.1)	
Adiponectin (μg/mL)			
Boys	87	42.1 (19.7)	0.75
Girls	101	43.0 (17.6)	
Total	188	42.6 (18.6)	

^aT-test for difference by sex.^bGeometric means and standard deviations presented for leptin.

RESULTS

Child and Maternal Characteristics

Of the 188 children in this study, 87 (46%) were boys and 101 (54%) were girls (Table 1). At time of pregnancy, mothers tended to be young (mean 26.3 ± 5.2 years), to have resided in the US for 10 years or less (75%), and to not have completed high school (80%). Almost half of mothers reported consuming one or more sodas per week (46%). During the pregnancy, the majority of families (62%) were living at or below the federal threshold for poverty (US Census Bureau, 2000). Most mothers were overweight (38%) or obese (26%) according to their prepregnancy BMI. Children were mainly delivered at term (≥ 37 weeks, $N = 178$, 95%). Approximately 5% of the children were born preterm and 4% were small for gestational age. Mothers reported that the majority of children consumed soda and fast food at least weekly (58 and 57%, respectively) and 22% consumed sweet snacks daily. At 9 years, 17% of children were overweight and 39% were obese.

Child Adiponectin and Leptin Plasma Levels

At 9 years of age, average child adiponectin plasma levels were $42.6 \pm 18.6 \mu\text{g mL}^{-1}$ (Table 2). No statistically significant difference was found between levels in boys and girls. Adiponectin levels were significantly and inversely correlated with 9-year child BMI ($r = -0.38$, $P < 0.001$). Average child plasma leptin levels were $8.2 \pm 1.1 \text{ ng mL}^{-1}$. Boys ($7.1 \pm 1.0 \text{ ng mL}^{-1}$) tended to have slightly lower leptin levels compared to girls ($9.3 \pm 1.2 \text{ ng mL}^{-1}$; $P = 0.08$). Leptin levels were significantly and positively correlated with 9-year child BMI ($r = 0.82$, $P < 0.001$).

Maternal and Concurrent Child Urinary BPA Levels

Table 3 shows urinary BPA concentrations (both uncorrected and corrected for urinary dilution) for mothers during pregnancy and for their children at 9 years, stratified

TABLE 3. Early and Late Pregnancy and 9-Year Child BPA Concentrations

Timing	N	%>LOD ^c	Uncorrected	Corrected for
			(μg/L)	urinary dilution
			GM (GSD)	(μg/L ^a , μg/g ^b)
Early pregnancy^a				
Boys	66	81	0.9 (0.9)	1.1 (0.8)
Girls	65	78	0.9 (1.1)	1.2 (1.0)
Total	131	79	0.9 (1.0)	1.1 (0.9)
Late pregnancy^a				
Boys	81	81	1.0 (0.9)	1.2 (0.8)
Girls	98	85	1.1 (0.9)	1.2 (0.8)
Total	179	83	1.1 (0.9)	1.2 (0.8)
9 year child^{b,d}				
Boys	77	96	1.7 (1.0)	1.6 (0.8)
Girls	95	87	1.5 (1.0)	1.7 (0.8)
Total	172	91	1.6 (1.0)	1.7 (0.8)

^aSpecific gravity corrected.^bCreatinine corrected (μg/g-creatinine).^cLOD = 0.4 μg/L.^dT-test by sex P -value = 0.74.

by child sex. Geometric mean (GM) concentrations presented here (0.9 and 1.1 $\mu\text{g L}^{-1}$ for early and late pregnancy, respectively) are lower than those previously reported for pregnant women from NHANES 2003–2004 (GM = $2.53 \mu\text{g L}^{-1}$) [Woodruff et al., 2011]. CHAMCOS 9-year-old children tended to have lower urinary BPA concentrations (GM = $1.6 \mu\text{g L}^{-1}$) than those reported for NHANES children aged 6–11 (GM = $3.6 \mu\text{g L}^{-1}$) [Calafat et al., 2008]. Of note, a recent report indicated even higher urinary BPA levels in similar age Danish children (Average age 8.5 years, Mean = $6 \mu\text{g g}^{-1}$ -creatinine) and their mothers (Mean = $4 \mu\text{g g}^{-1}$ -creatinine) [Frederiksen et al., 2013]. BPA concentrations measured during the first and second halves of pregnancy were weakly but significantly correlated ($r = 0.15$, $P = 0.01$). No statistically significant difference was found in 9-year BPA concentrations between boys and girls ($P = 0.74$).

Prenatal and Concurrent Urinary BPA Concentration and 9-Year Child Plasma Adipokines

Table 4 shows associations between prenatal and concurrent urinary BPA measures and child adiponectin and leptin plasma levels at 9 years, controlling for maternal pre-pregnancy BMI, pregnancy soda consumption and smoking, years in US prior to pregnancy, maternal education, household poverty status, child 9-year BMI, and child soda, fast food and sweet snack consumption at 9 years. We observed significant effect modification by sex for associations between late pregnancy BPA and leptin (interaction $P = 0.01$) and between early pregnancy BPA and adiponectin (interaction $P = 0.1$).

TABLE 4. Prenatal and Concurrent Associations Between log₂ BPA and Adiponectin and Leptin in 9-Year-Old Children

	N	Boys			Girls			Sex-interaction
		β	(95% CI)	P-value	β	(95% CI)	P-value	P-value
Leptin								
Early pregnancy ^a	131	0.03	(-0.03, 0.09)	0.27	0.02	(-0.03, 0.06)	0.49	0.67
Late pregnancy ^a	179	0.06	(0.01, 0.11)	0.01	-0.03	(-0.07, 0.02)	0.26	0.01
9 year child ^b	172	-0.04	(-0.23, 0.14)	0.64	-0.11	(-0.27, 0.04)	0.15	0.58
Adiponectin								
Early pregnancy ^a	131	-0.67	(-4.77, 3.43)	0.75	3.71	(0.38, 7.04) ^c	0.03	0.10
Late pregnancy ^a	179	2.42	(-0.94, 5.78)	0.16	1.72	(-1.33, 4.77)	0.27	0.76
9 year child ^b	172	2.69	(-9.65, 15.04)	0.67	9.44	(-0.76, 19.6)	0.07	0.41

Adjusted for: maternal pre-pregnancy BMI, Years in the US, poverty status, maternal education, smoking during pregnancy, soda consumption during pregnancy, child 9-year BMI, and child soda, fast food and sweet snack consumption at 9 years.

^aSpecific gravity adjusted.

^bCreatinine adjusted.

P-value < 0.05.

Focusing on the sex-specific associations, we found that among boys, late pregnancy urinary BPA concentrations were positively associated with 9-year leptin levels ($\beta = 0.06$, $P = 0.01$). Additionally, among girls, we found that early pregnancy BPA concentrations were positively associated with 9-year adiponectin levels ($\beta = 3.71$, $P = 0.03$). Concurrent measures of urinary BPA were not associated with adipokine levels in 9-year-old children after controlling for BMI.

DISCUSSION

In this cohort of Mexican-American children, we observed that the BPA-adipokine association may vary by child sex. We found that late pregnancy urinary BPA concentrations were positively associated with leptin levels in 9-year-old boys, adjusting for covariates including child BMI. Additionally, early pregnancy BPA concentrations were positively related to adiponectin levels in 9-year-old girls. While the mechanism for this sexual dimorphism remains unclear, it may be related to BPA's ability to interfere with activity of endogenous estrogens (17 β -estradiol), disrupting normal binding at either non-classical membrane or nuclear estrogen receptors [Gould et al., 1998; Kuiper et al., 1998; Alonso-Magdalena et al., 2005; Alonso-Magdalena et al., 2006; Wetherill et al., 2007]. Previous studies indicate that biosynthesis and function of 17 β -estradiol, along with tissue distribution of estrogen receptors may vary by sex [Simpson et al., 1999; Nilsson et al., 2001; Gillies and McArthur, 2010]. We speculate that this may account for some of the sex-specific responses observed with respect to BPA exposure.

Our finding of a positive association between prenatal BPA and leptin in boys is consistent with several animal studies. Wei et al. [2011] showed increased leptin levels in male rats and Miyawaki et al. [2007] found increased

body and adipose tissue weight in male mice as a result of perinatal BPA exposures [Miyawaki et al., 2007; Wei et al., 2011]. Although we did not observe an association of prenatal BPA with BMI in boys in this cohort [Harley et al., 2013], we now report an association with increased leptin independent of BMI. BMI may not be as sensitive an endpoint and it is plausible that BPA may directly alter fetal leptin secretion from adipocytes given that it has been shown to do so with respect to adiponectin [Hugo et al., 2008]. This, in turn, may affect leptin secretion postnatally, however, additional mechanistic data are needed to examine the BPA-leptin relationship.

Previous data on CHAMACOS children suggest that prenatal BPA concentrations are negatively associated with BMI z-score in girls only [Harley et al., 2013]. Here, adjusting for child BMI, we found that prenatal BPA was positively associated with adiponectin in girls. It is important to note that while many previous studies have shown perinatal BPA exposure to be positively associated with offspring obesity [Rubin et al., 2001; Miyawaki et al., 2007; Somm et al., 2009; Hiyama et al., 2011], and, in an *in vitro* model, with adiponectin suppression [Hugo et al., 2008], others have found no or reverse associations [Honma et al., 2002; Negishi et al., 2003; Ryan et al., 2010; Nakamura et al., 2012]. A recent study by Anderson et al. [2013] indicated that perinatally exposed male and female mice exhibited increased energy expenditure and activity and, further, females had marginally lower body weight and improved adiponectin and leptin levels throughout their lives [Anderson et al., 2013]. Overall, the reasons for differences in reported associations in animal and human studies remain unclear. Both timing and dose of BPA exposure, as well as diet, do vary for the animal studies mentioned and this may contribute to the inconsistencies in results.

Only one other study has provided data on prenatal BPA and adiponectin and leptin in children. Chou et al. [2011] examined associations between prenatal BPA and

adiponectin and leptin in children at birth [Chou et al., 2011]. They reported a positive association between BPA blood concentrations during pregnancy and leptin in boys at birth. They also found an inverse relationship between prenatal BPA and adiponectin in girls, which we did not. Reasons for this discrepancy remain unclear, but it is important to note that the Chou study measured BPA in maternal plasma collected at birth. There are several limitations to measurement of BPA in blood compared to urine, including higher proportion of non-detects and greater risk of cross-contamination yielding incorrect results [Calafat, 2011]. Additionally, studies have shown that exposures to medical devices in the hospital near the time of birth can dramatically increase BPA levels [Calafat et al., 2009; Vandentorren et al., 2011; Duty et al., 2013].

We did not find significant associations between concurrent BPA exposure and child adipokines at 9 years, controlling for BMI. To our knowledge, no such data on adiponectin and leptin are available from other reports. Previously, we found positive associations between concurrent urinary BPA concentrations and child BMI [Harley et al., 2013]. Several other studies have also reported a direct association between concurrent BPA and BMI in children [Trasande et al., 2012; Wang et al., 2012]. Using NHANES 2003–2008, Trasande et al. (2012) found a positive association between urinary BPA concentrations and BMI in children aged 6–19 [Trasande et al., 2012]. Such data are limited by their cross-sectional nature and cannot address whether BPA exposure preceded development of obesity or is merely a reflection of it. Overall, our findings suggest that prenatal, and not concurrent, BPA exposure is more relevant in determining child adipokine levels. Future work should focus on further exploring potential metabolic health consequences of BPA exposure during this critical developmental period.

One of the limitations of this study is the short-term nature of the BPA exposure measurement. Urinary BPA concentrations vary widely throughout the day and the spot urine samples used in this analysis reflect only recent exposure. Ye et al. (2011) observed large within-day variance for spot collections (70%) [Ye et al., 2011]. However, we expect this misclassification to be nondifferential, biasing our results towards the null. Additionally results may not be generalizable to other populations as this study was conducted on a cohort of largely first generation, immigrant and relatively low socioeconomic status Mexican-American families from an agricultural community. A major strength of this study is its prospective nature, providing data on associations between prenatal BPA exposure and child adipokine levels 9 years later. Further, these results are directly relevant to the CHAMACOS population, given its high prevalence of obesity.

Future directions include examining mechanisms of sex differences in response to BPA exposure. The exact biological pathway in which BPA can affect leptin and

adiponectin levels remains unknown. BPA has been shown to interact with estrogen receptors [Gould et al., 1998; Kuiper et al., 1998; Alonso-Magdalena et al., 2005; Alonso-Magdalena et al., 2006; Wetherill et al., 2007] and may also affect key adipogenic transcriptional factors including CCAAT/enhancer binding proteins (C/EBP β) and peroxisome proliferator-activated receptor γ (PPAR γ) [Phrakonkham et al., 2008]. Downstream effects of this may result in altered adipokine levels. Further, it has been shown that estrogen and leptin both target common neuronal sites and both estrogen and leptin receptors are co-localized in several brain regions [Gao and Horvath, 2008]. Given this close relationship, it is possible that BPA-mediated alterations in estrogen signaling affect leptin levels. However, additional work is needed to clarify this pathway.

ACKNOWLEDGMENTS

The authors acknowledge X. Ye, X. Zhou, T. Jia, and R. Hennings for technical assistance in measuring the urinary concentrations of BPA and R. Aguilar for support with statistical analyses.

AUTHOR CONTRIBUTIONS

VV, VD, JM and AC were involved in data collection. VV, KH, BE, NH and AC were involved in study design and manuscript writing and editing.

REFERENCES

- Alonso-Magdalena P, Laribi O, Ropero AB, Fuentes E, Ripoll C, Soria B, Nadal A. 2005. Low doses of bisphenol A and diethylstilbestrol impair Ca²⁺ signals in pancreatic alpha-cells through a nonclassical membrane estrogen receptor within intact islets of Langerhans. *Environ Health Perspect* 113:969–977.
- Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. 2006. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect* 114:106–112.
- Anderson OS, Peterson KE, Sanchez BN, Zhang Z, Mancuso P, Dolinoy DC. 2013. Perinatal bisphenol A exposure promotes hyperactivity, lean body composition, and hormonal responses across the murine life course. *FASEB J*.
- Balakrishnan B, Henare K, Thorstensen EB, Ponnampalam AP, Mitchell MD. 2010. Transfer of bisphenol A across the human placenta. *Am J Obstet Gynecol* 202:393 e391–397.
- Becker K, Goen T, Seiwert M, Conrad A, Pick-Fuss H, Muller J, Wittassek M, Schulz C, Kolossa-Gehring M. 2009. GerES IV: Phthalate metabolites and bisphenol A in urine of German children. *Int J Hyg Environ Health* 212:685–692.
- Ben-Jonathan N, Hugo ER, Brandebourg TD. 2009. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. *Mol Cell Endocrinol* 304:49–54.
- Block G, Woods M, Potosky A, Clifford C. 1990. Validation of a self-administered diet history questionnaire using multiple diet records. *J Clin Epidemiol* 43:1327–1335.

- Calafat AM. 2011. Background Paper on BPA Biomonitoring and Biomarker Studies; FAO/WHO Expert Meeting on Bisphenol A (BPA) Ottawa, Canada; 2010.
- Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. 2008. Exposure of the US population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environ Health Perspect* 116:39-44.
- Calafat AM, Weuve J, Ye X, Jia LT, Hu H, Ringer S, Huttner K, Hauser R. 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. *Environ Health Perspect* 117:639-644.
- Chou WC, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY. 2011. Bio-monitoring 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.
- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292-295.
- Cruz M, Garcia-Macedo R, Garcia-Valerio Y, Gutierrez M, Medina-Navarro R, Duran G, Wachter N, Kumate J. 2004. Low adiponection levels predict type 2 diabetes in Mexican children. *Diabetes Care* 27:1451-1453.
- Duty SM, Mendonca K, Hauser R, Calafat AM, Ye X, Meeker JD, Ackerman R, Cullinane J, Faller J, Ringer S. 2013. Potential sources of bisphenol a in the neonatal intensive care unit. *Pediatrics* 131:483-489.
- Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Perspect* 112:1116-1124.
- Eskenazi B, Gladstone EA, Berkowitz GS, Drew CH, Faustman EM, Holland NT, Lanphear B, Meisel SJ, Perera FP, Rauh VA, Sweeney A, Wyatt RM, Yolton K. 2005. Methodologic and logistic issues in conducting longitudinal birth cohort studies: Lessons learned from the Centers for Children's Environmental Health and Disease Prevention Research. *Environ Health Perspect* 113:1419-1429.
- Frederiksen H, Nielsen JK, Morck TA, Hansen PW, Jensen JF, Nielsen O, Andersson AM, Knudsen LE. 2013. Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs. *Int J Hyg Environ Health*. DOI: 10.1016/j.ijheh.2013.02.006.
- Gao Q, Horvath TL. 2008. Cross-talk between estrogen and leptin signaling in the hypothalamus. *Am J Physiol Endocrinol Metab* 294:E817-E826.
- Gillies GE, McArthur S. 2010. Estrogen actions in the brain and the basis for differential action in men and women: A case for sex-specific medicines. *Pharmacol Rev* 62:155-198.
- Gould JC, Leonard LS, Maness SC, Wagner BL, Conner K, Zacharewski T, Safe S, McDonnell DP, Gaido KW. 1998. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. *Mol Cell Endocrinol* 142:203-214.
- Grun F, Blumberg B. 2006. Environmental obesogens: Organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology* 147(6 Suppl):S50-S55.
- Harley K, Eskenazi B, Block G. 2005. The association of time in the US and diet during pregnancy in low-income women of Mexican descent. *Paediatr Perinat Epidemiol* 19:125-134.
- Harley KG, Aguilar Schall R, Chevrier J, Tyler K, Aguirre H, Bradman A, Holland NT, Lustig RH, Calafat AM, Eskenazi B. 2013. Prenatal and postnatal bisphenol A exposure and body mass index in childhood in the CHAMACOS cohort. *Environ Health Perspect*.
- Hiyama M, Choi EK, Wakitani S, Tachibana T, Khan H, Kusakabe KT, Kiso Y. 2011. Bisphenol-A (BPA) affects reproductive formation across generations in mice. *J Vet Med Sci* 73:1211-1215.
- Honma S, Suzuki A, Buchanan DL, Katsu Y, Watanabe H, Iguchi T. 2002. Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod Toxicol* 16:117-122.
- Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N. 2008. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. *Environ Health Perspect* 116:1642-1647.
- Kadowaki T, Yamauchi T. 2005. Adiponectin and adiponectin receptors. *Endocr Rev* 26:439-451.
- Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 139:4252-4263.
- Lakind JS, Naiman DQ. 2011. Daily intake of bisphenol A and potential sources of exposure: 2005-2006 National Health and Nutrition Examination Survey. *J Expo Sci Environ Epidemiol* 21:272-279.
- Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L, Hartge P. 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect* 112:1691-1696.
- Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris A. 2011. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* 301: E567-E584.
- Margetic S, Gazzola C, Pegg GG, Hill RA. 2002. Leptin: A review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 26:1407-1433.
- Masuno H, Iwanami J, Kidani T, Sakayama K, Honda K. 2005. Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway. *Toxicol Sci* 84:319-327.
- Miyawaki J, Sakayama K, Kato H, Yamamoto H, Masuno H. 2007. Perinatal and postnatal exposure to bisphenol a increases adipose tissue mass and serum cholesterol level in mice. *J Atheroscler Thromb* 14:245-252.
- Morck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L, Knudsen LE. 2010. Placental transport and in vitro effects of Bisphenol A. *Reprod Toxicol* 30: 131-137.
- Nakamura K, Itoh K, Dai H, Han L, Wang X, Kato S, Sugimoto T, Fushiki S. 2012. Prenatal and lactational exposure to low-doses of bisphenol A alters adult mice behavior. *Brain Dev* 34:57-63.
- Negishi T, Kawasaki K, Takatori A, Ishii Y, Kyuwa S, Kuroda Y, Yoshikawa Y. 2003. Effects of perinatal exposure to bisphenol A on the behavior of offspring in F344 rats. *Environ Toxicol Pharmacol* 14:99-108.
- Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. 2001. Mechanisms of estrogen action. *Physiol Rev* 81:1535-1565.
- Ong KK, Loos RJ. 2006. Rapid infancy weight gain and subsequent obesity: Systematic reviews and hopeful suggestions. *Acta Paediatr* 95:904-908.
- Overpeck MD, Hediger ML, Zhang J, Trumble AC, Klebanoff MA. 1999. Birth weight for gestational age of Mexican American infants born in the United States. *Obstet Gynecol* 93:943-947.
- Phrakonkham P, Viengchareun S, Belloir C, Lombes M, Artur Y, Canivenc-Lavier MC. 2008. Dietary xenoestrogens differentially impair 3T3-L1 preadipocyte differentiation and persistently affect leptin synthesis. *J Steroid Biochem Mol Biol* 110:95-103.
- 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.

- Ryan KK, Haller AM, Sorrell JE, Woods SC, Jandacek RJ, Seeley RJ. 2010. Perinatal exposure to bisphenol-a and the development of metabolic syndrome in CD-1 mice. *Endocrinology* 151: 2603–2612.
- Shaibi GQ, Cruz ML, Weigensberg MJ, Toledo-Corral CM, Lane CJ, Kelly LA, Davis JN, Koebnick C, Ventura EE, Roberts CK, Goran MI. 2007. Adiponectin independently predicts metabolic syndrome in overweight Latino youth. *J Clin Endocrinol Metab* 92:1809–1813.
- Simpson E, Rubin G, Clyne C, Robertson K, O'Donnell L, Davis S, Jones M. 1999. Local estrogen biosynthesis in males and females. *Endocr Relat Cancer* 6:131–137.
- Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, Huppi PS. 2009. Perinatal exposure to bisphenol A alters early adipogenesis in the rat. *Environ Health Perspect* 117:1549–1555.
- Trasande L, Attina TM, Blustein J. 2012. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. *JAMA* 308:1113–1121.
- Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. 2007. Human exposure to bisphenol A (BPA). *Reprod Toxicol* 24: 139–177.
- Vandentorren S, Zeman F, Morin L, Sarter H, Bidondo ML, Oleko A, Leridon H. 2011. Bisphenol-A and phthalates contamination of urine samples by catheters in the Elfe pilot study: Implications for large-scale biomonitoring studies. *Environ Res* 111: 761–764.
- Volberg V, Harley KG, Aguilar RS, Rosas LG, Huen K, Yousefi P, Dave V, Phan N, Lustig RH, Eskenazi B, Holland N. 2013. Associations between perinatal factors and adiponectin and leptin in 9-year-old Mexican-American children. *Pediatr Obes*.
- Wang T, Li M, Chen B, Xu M, Xu Y, Huang Y, Lu J, Chen Y, Wang W, Li X, Liu Y, Bi Y, Lai S, Ning G. 2012. Urinary bisphenol A (BPA) concentration associates with obesity and insulin resistance. *J Clin Endocrinol Metab* 97:E223–E227.
- Wei J, Lin Y, Li Y, Ying C, Chen J, Song L, Zhou Z, Lv Z, Xia W, Chen X, Xu S. 2011. Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. *Endocrinology* 152:3049–3061.
- Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM. 2007. In vitro molecular mechanisms of bisphenol A action. *Reprod Toxicol* 24:178–198.
- Woodruff TJ, Zota AR, Schwartz JM. 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ Health Perspect* 119:878–885.
- Ye X, Kuklennyik Z, Needham LL, Calafat AM. 2005. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal Chem* 77:5407–5413.
- Ye X, Wong LY, Bishop AM, Calafat AM. 2011. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. *Environ Health Perspect* 119: 983–988.

Accepted by—
D. Dolinoy