# Trimester-Specific Urinary Bisphenol A Concentrations and Blood Glucose Levels Among Pregnant Women From a Fertility Clinic

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**Context:** Women with a history of infertility are at increased risk of impaired glucose tolerance during pregnancy. Studies suggest higher urinary bisphenol A (BPA) concentrations are associated with diabetes in nonpregnant populations, but the association between BPA and glucose levels among pregnant women is unclear.

**Objective:** To assess trimester-specific urinary BPA concentrations in relation to blood glucose levels among subfertile women.

**Design:** Environment and Reproductive Health Study, an ongoing prospective cohort study.

**Setting:** A fertility center in a teaching hospital.

**Patients:** A total of 245 women contributed at least one urine sample during first and/or second trimesters, delivered a singleton or twin pregnancy, and had available blood glucose data (2005 to 2015).

**Main Outcome Measure:** Blood glucose levels after a nonfasting 50-g glucose challenge test at 24 to 28 weeks of gestation.

**Results:** The specific gravity-adjusted geometric mean urinary BPA concentrations during first and second trimesters were 1.39 and 1.27 μg/L, respectively. Second-trimester BPA concentrations were positively associated with blood glucose (*P*, trend = 0.01). Specifically, the adjusted mean glucose levels (95% confidence interval) for women in the highest quartile of second-trimester BPA concentrations was 119 (112, 126) mg/dL compared with 106 (100, 112) mg/dL for women in the lowest quartile. No associations were observed between first-trimester BPA concentrations and glucose levels.

**Conclusions:** BPA exposure during the second trimester may have adverse effect on blood glucose levels among subfertile women. As the findings represent the first report suggesting a potential etiologically relevant window for BPA and glucose in humans, further studies are needed. (***J Clin Endocrinol Metab*** **102: 1350–1357, 2017)**

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Abbreviations: BMI, body mass index; BPA, bisphenol A; GCT, glucose challenge test; GDM, gestational diabetes mellitus; IUI, intrauterine insemination; IVF, *in vitro* fertilization; NHANES, National Health and Nutrition Examination Survey; PCOS, polycystic ovarian syndrome; SD, standard deviation; SG, specific gravity.

Gestational diabetes mellitus (GDM), affecting ~7% of all pregnancies in the United States (1), is associated with an increased risk of adverse pregnancy (*e.g.,* maternal hypertensive disorder, cesarean delivery) and perinatal outcomes (*e.g.,* fetal macrosomia, neonatal hypoglycemia) (1). The burden of GDM may also extend beyond obstetric and neonatal periods, including a higher long-term metabolic risk for both mother and offspring (2). Certain populations, such as women undergoing assisted reproductive technologies, are at a higher risk of GDM compared with women conceived naturally, possibly due to underlying infertility problems or/and assisted reproductive technology procedures (3–5). Recently, growing evidence suggests that in addition to lifestyle factors such as obesity and recreational physical activity (6), endocrine disrupting chemicals such as bisphenol A (BPA) may also contribute to increased risk of elevated glucose levels, insulin resistance, and diabetes (7). However, little is known about whether BPA could affect glucose levels during pregnancy, particularly in high-risk groups such as women with a history of infertility.

BPA, a high production volume chemical widely used in the manufacturing of polycarbonate plastics and epoxy resins (8), may alter glucose levels in pregnancy through multiple pathways. In animal studies, the estrogenic effect of BPA has been shown to alter pancreatic $\beta$-cell function, with effects on increased insulin resistance and glucose intolerance (9). Other studies have found associations between BPA and diabetes-related outcomes, including inflammation and oxidative stress (10, 11). Of interest, pregnancy is known to be a naturally increasing insulin resistant state, with implications for sensitive windows of exposure. BPA exposure may accelerate deterioration of normal pancreatic $\beta$-cell function (12, 13), resulting in an increased risk of elevated glucose levels during pregnancy.

Despite the biological plausibility of BPA altering blood glucose levels during pregnancy, only 2 epidemiologic studies have evaluated this relationship. A case-control pilot study from the United States (14) and a prospective study in Canada (15) found no significant association between urinary BPA concentrations and risk of GDM. However, both studies evaluated clinical diagnosis of GDM and not continuous elevations in blood glucose levels that have been shown to be associated with adverse health outcomes without a clear threshold (16). In addition, urinary BPA concentrations in these studies (14, 15) were around 50% lower than those measured in the general US female population (8). Therefore, it may be difficult to extend these previous findings to other populations, particularly women at high risk of glucose intolerance in pregnancy. Furthermore, the timing of BPA exposure during different trimesters of pregnancy may have differing effects on glucose levels as maternal metabolism changes across pregnancy (17). Thus, the objective of this study was to examine the association of trimester-specific urinary BPA concentrations with blood glucose levels among women presenting to a fertility clinic.

## Materials and Methods

### Study population

The current study consisted of a subset of women enrolled in the Environment and Reproductive Health study, an ongoing prospective cohort designed to identify environmental and dietary determinants of fertility and pregnancy outcomes among couples presenting to Massachusetts General Hospital (MGH) Fertility Center (Boston, MA). Women were eligible if they were age 18 to 46 years at enrollment. For this analysis, women were included if they carried a singleton or twin pregnancy, contributed at least 1 urine sample during the first and/or second trimester, and had available data on blood glucose levels from a 50-g glucose challenge test (GCT) between March 2005 and May 2015. Of these, we excluded 1 woman who had a history of diabetes at baseline. For women who had more than 1 pregnancy during the study period (n = 14), we only included their first pregnancy in this analysis. Therefore, the current study consisted of 245 pregnant women, who prospectively provided a total of 417 urine samples (208 samples collected in the first trimester, and 209 samples collected in the second trimester) prior to the GCT. Of 245 women, 168 women provided a urine sample at each trimester. Informed consent was obtained and the study was approved by the Human Studies Institutional Review Boards of the MGH, Harvard T. H. Chan School of Public Health, and the Centers for Disease Control and Prevention.

### Blood sample collection and glucose data measurement

Blood glucose levels were obtained from a 1-hour nonfasting, 50-g GCT at ~24 to 28 weeks gestation (median: 27 weeks gestation) utilizing the first step of the Carpenter–Coustan method for the GDM screening test used at MGH (18). Results from glucose laboratory data were abstracted from medical records. For this analysis, we used continuous blood glucose levels from this 50-g GCT test, with women having glucose levels $\geq$140 mg/dL being further screened for GDM based on this screening method.

### Urine sample collection and BPA measurements

Women collected spot urine samples during their first and second trimesters of pregnancy in sterile polypropylene cups. Specific gravity (SG) (19) was measured using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD). The urine was divided into aliquots and frozen at –20°C, and stored at –80°C. Samples were shipped on dry ice overnight to the Centers for Disease Control and Prevention (Atlanta, GA).

The concentrations of free and conjugated BPA species (total BPA) were quantified using isotope dilution on-line solid phase extraction coupled with high-performance liquid chromatography–tandem mass spectrometry, as described elsewhere (20). Briefly, 100 $\mu$L of urine was treated with

β-glucuronidase/sulfatase (Helix pomatia, H1; Sigma Chemical Co, St. Louis, MO). After preconcentration on a C18 reversed-phase size-exclusion SPE column, BPA was separated from other urine matrix components using a pair of monolithic HPLC columns and detected by negative ion-atmospheric pressure chemical ionization–tandem mass spectrometry. The standard quality control procedures were previously described (20). The limit of detection was 0.4 μg/L.

### Covariates assessment

Information on demographic factors, personal and family medical history, and lifestyle factors were obtained from a brief nurse-administered questionnaire at enrollment and a detailed take-home questionnaire. Participants' weight and height were measured by the nurse. Body mass index (BMI) was calculated as weight (in kilograms) per height (in meters) squared. Clinical information, including a physician diagnosis of polycystic ovarian syndrome (PCOS), was abstracted from the patient's electronic medical record. Infertility diagnosis by a physician was assigned to each patient based on the Society for Assisted Reproductive Technology. Women in this population achieved pregnancies by *in vitro* fertilization (IVF), intrauterine insemination (IUI), or naturally without medical intervention prior to the start of treatment.

### Statistical analysis

Demographic characteristics of the study participants were reported using mean ± standard deviation (SD) or N (percentages). To adjust for urinary dilution, the following formula was used: Pc = P([1.015 – 1]/SG – 1), where Pc is the SG-adjusted BPA concentration (micrograms per liter), P is the measured BPA concentration (micrograms per liter), and 1.015 is the mean SG level in the study population (21). Nondetectable BPA concentrations were replaced with a value equal to the limit of detection divided by square root of 2 prior to SG adjustment (22). Due to the right skewed distribution, blood glucose levels and SG-adjusted BPA urinary concentrations were log transformed. Women's exposures to BPA were categorized into quartiles based on trimester-specific log SG-adjusted BPA concentrations. A multivariable linear model was used to assess the associations between urinary SG-adjusted BPA concentrations and log blood glucose levels. Results were back transformed to improve interpretability. Population marginal means were used to present population averages for each quartile adjusted for covariates in the model (23). Tests for trend were conducted across quartiles using the median of log BPA concentration in each quartile as a continuous variable in the regression models. Due to the small number of women requiring additional screening (n = 45 women requiring further GDM screening with a 3-hour oral glucose tolerance test and 10 women with diagnosed GDM based on the Carpenter–Coustan criteria (18), and a continuous relationship between glucose levels and adverse pregnancy outcomes (16), glucose levels from GCT were used as the main outcome.

We examined the following variables as potential confounders: age at GCT (years), prepregnancy overweight or obese ($< 25$ kg/m$^2$, $\geq 25$ kg/m$^2$), total physical activity (hours per week), race/ethnicity (white, nonwhite), smoking status at baseline (ever, never), education levels (some college degree or lesser, college graduate, or higher), family history of diabetes (yes, no), infertility diagnosis (female factor, male factor, or unexplained), number of fetuses (1, 2), types of treatment (IVF, IUI, or natural conception), urine sampling session (morning, afternoon), and time lag between urine and glucose measurements (days). Variables were selected for inclusion in the multivariable model based on the association with glucose levels in a univariate analysis ($P < 0.20$) or the evidence as risk factors of GDM from the literature review (6). The following covariates were selected in the final model: maternal age at GCT, prepregnancy overweight or obese, total physical activity, family history of diabetes, infertility diagnosis, number of fetus in a pregnancy, and urine sampling session. To evaluate the robustness of our findings, we conducted several sensitivity analyses by excluding women with physician diagnosis of PCOS or use of metformin, and, separately, excluding women with twin pregnancies. We also performed additional analyses by adjusting for dietary patterns scores (Western and Prudent dietary patterns), year of urine sample collection, in the final multivariable model. Moreover, we restricted the analysis to a subset of population who provided a urine sample at each trimester to compare the consistency of the findings. Finally, effect modification by maternal age ($< 37$ vs $\geq 37$ years), maternal BMI ($< 25$ kg/m$^2$ vs $\geq 25$ kg/m$^2$), treatment type (IVF vs IUI and natural conception), and year of urinary sample collection ($< 2010$ vs $\geq 2010$) were tested using cross-product terms in the multivariate models. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC). Two-sided significance levels less than 0.05 were considered as statistically significant.

## Results

Baseline characteristics of the participants were summarized in Table 1. Participants were primarily white (87%), never smoked (74%), and college graduate or higher (87%), with a mean age of 35.3 years at GCT. Most women's prepregnancy BMI was $<25$ kg/m$^2$ (69%). The primary infertility diagnosis at enrollment was unexplained (40%), followed by female factor (32%) and male factor (28%). Of 245 women, 23 women had a physician diagnosis of PCOS. The mean (standard deviation) blood glucose levels from the second-trimester 50-g GCT was 117 (27.3) mg/dL. BPA was detected in 83% and 78% of the samples collected at the early (median: 7 weeks of gestation) and midpregnancy (median: 21 weeks of gestation), respectively (Table 2). The geometric means of SG-adjusted BPA concentrations measured during first and second trimesters were 1.39 and 1.27 μg/L, respectively, concentrations slightly lower than for females in the 2011 to 2012 National Health and Nutrition Examination Survey (NHANES; 1.73 μg/L) (8). Baseline characteristics shown in Table 1 were not associated with urinary BPA concentrations, except that women in higher quartile of urinary BPA concentrations (regardless in the first or second trimester) were more likely to have collected urine in the afternoon (Table 1). Women included in the analysis did not systemically differ from women excluded due to lack of urine or glucose data in terms of characteristics presented in Table 1.

**Table 1. Baseline Characteristics Among 245 Pregnant Women in the EARTH Study**

| | First Trimester (n = 208)[a] | | | Second Trimester (n = 209)[b] | | |
|---|---|---|---|---|---|---|
| **Characteristic** | **Q1** | **Q4** | ***P* Value[c]** | **Q1** | **Q4** | ***P* Value[c]** |
| N | 53 | 52 | | 52 | 52 | |
| Age at pregnancy (y) | | | | | | |
| Mean ± SD | 35.2 ± 3.5 | 35.5 ± 3.6 | 0.69 | 35.7 ± 3.2 | 35.4 ± 3.3 | 0.67 |
| Range | 27–43 | 29–42 | | 29–42 | 30–42 | |
| Prepregnancy BMI (kg/m$^2$) | | | | | | |
| BMI <25 | 41 (77.4) | 36 (69.2) | 0.32 | 35 (67.3) | 35 (67.3) | 0.99 |
| BMI ≥25 | 12 (22.6) | 16 (30.8) | | 17 (32.7) | 17 (32.7) | |
| Family history of DM | 8 (15) | 9 (17) | 0.13 | 6 (12) | 5 (10) | 0.85 |
| Total physical activity (h/wk) | 7.5 (9.6) | 7.6 (6.0) | 0.47 | 8.3 (7.2) | 5.6 (4.1) | 0.39 |
| Smoking status at baseline | | | 0.07 | | | 0.93 |
| Never smoked | 43 (81.1) | 34 (65.4) | | 40 (76.9) | 38 (73.1) | |
| Former smoker | 10 (18.9) | 16 (30.8) | | 11 (21.2) | 13 (25.0) | |
| Current smoker | 0 (0) | 2 (3.9) | | 1 (1.9) | 1 (1.9) | |
| Race | | | 0.37 | | | 0.23 |
| White | 43 (81.1) | 45 (86.5) | | 46 (88.5) | 47 (90.4) | |
| Black/African American | 1 (1.9) | 1 (1.9) | | 1 (1.9) | 0 (0) | |
| Asian | 4 (7.6) | 6 (11.5) | | 4 (7.7) | 3 (5.8) | |
| Other | 5 (9.4) | 0 (0) | | 1 (1.9) | 2 (3.9) | |
| Education | | | 0.84 | | | 0.31 |
| High school graduate or less | 7 (13.2) | 4 (7.7) | | 4 (7.7) | 6 (11.5) | |
| Some college | 2 (3.8) | 1 (1.9) | | 4 (7.7) | 0 (0) | |
| College graduate or higher | 44 (83.0) | 47(90.4) | | 44 (84.6) | 46 (88.5) | |
| Infertility diagnosis | | | 0.82 | | | 0.12 |
| Male factor | 17 (32.1) | 17 (32.7) | | 18 (34.6) | 12 (23.1) | |
| Unexplained | 20 (37.7) | 16 (30.8) | | 20 (38.5) | 25 (48.1) | |
| Female factor | 16 (30.2) | 19 (36.5) | | 14 (26.9) | 15 (28.9) | |
| Physician diagnosis of PCOS | 2 (4) | 4 (8) | 0.21 | 3 (6) | 5 (10) | 0.43 |
| Treatment | | | 0.84 | | | 0.15 |
| IVF | 34 (64.2) | 32 (61.5) | | 32 (61.5) | 30 (57.7) | |
| IUI | 9 (17.0) | 10 (19.2) | | 7 (13.5) | 11 (21.2) | |
| Natural | 10 (18.9) | 10 (19.2) | | 13 (25.0) | 11 (21.2) | |
| Fetus number in one pregnancy | | | 0.62 | | | 0.68 |
| 1 | 46 (86.8) | 42 (80.8) | | 39 (75.0) | 40 (76.9) | |
| 2 | 7 (13.2) | 10 (19.2) | | 13 (25.0) | 12 (23.1) | |
| Time differences between BPA and glucose measurements (day) | | | | | | |
| Mean ± SD | 131 (16) | 130 (16) | 0.91 | 40 (20) | 40 (23) | 0.93 |
| Urine sampling session | | | 0.08 | | | 0.03 |
| Morning | 11 (21) | 3 (9) | | 29 (56) | 18 (35) | |
| Afternoon | 42 (79) | 48 (28) | | 23 (44) | 33 (65) | |
| Evening | 0 (0) | 1 (2) | | 0 (0) | 0 (0) | |

Data are presented as mean ± SD or n (%) unless otherwise specified.

Abbreviations: DM, diabetes mellitus; EARTH, Environmental and Reproductive Health; Q1, quartile 1; Q4, quartile 4.

[a]Of 245 women, 208 women provided first-trimester urine samples.

[b]Of 245 women, 209 women provided second-trimester urine samples.

[c]From Kruskal–Wallis test for continuous variable and fisher exact tests for categorical variables.

Second-trimester BPA concentrations were positively associated with second-trimester blood glucose levels (*P*, trend = 0.01). Specifically, the adjusted mean glucose (95% confidence interval) for women in the highest quartile of second-trimester urinary BPA concentrations was 119 (112 to 126) mg/dL compared with 106 (100 to 112) mg/dL for women in the lowest quartile (Table 3). Excluding women with a history of taking metformin at baseline (n = 8), women with a diagnosis of PCOS (n = 21), or those with twin pregnancies (n = 43) did not change the findings (*P*, trends = 0.04, 0.04, and 0.03, respectively). Results remained unchanged after additional adjustment for dietary patterns, year of urine sample collection, or time lag between BPA and glucose measurement. The positive association was similar, albeit weaker, when we assessed risk of GDM based on 100-g oral glucose tolerance test results, with adjusted odds ratios (95% confidence interval) of 2.29 (0.18 to 29.7) for women in the fourth quartile compared with women in the first quartile. On the other hand, no substantial

**Table 2. Distributions of Trimester-Specific Urinary BPA Concentrations (Micrograms per Liter) in Pregnant Women From the EARTH Study**

| | N of Samples | Detection Frequency | Geometric Mean (95% CI) | Percentile | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Min | 25th | 50th | 75th | 90th | 95th | Max |
| Urine samples collected in the first trimester | | | | | | | | | | |
| BPA | 208 | 83% | 1.15 (0.99 to 1.34) | <LOD | 0.50 | 1.20 | 2.70 | 4.40 | 7.10 | 12.0 |
| SG-adjusted BPA | | | 1.39 (1.23 to 1.57) | <LOD | 0.83 | 1.33 | 2.48 | 3.69 | 5.82 | 17.0 |
| Urine samples collected in the second trimester | | | | | | | | | | |
| BPA | 209 | 78% | 0.94 (0.81 to 1.09) | <LOD | 0.40 | 0.90 | 1.90 | 3.70 | 5.30 | 93.6 |
| SG-adjusted BPA | | | 1.27 (1.12 to 1.45) | <LOD | 0.72 | 1.17 | 2.25 | 3.75 | 5.69 | 63.82 |

All concentrations below LOD were assigned a value equal to the LOD divided by square root of 2 to calculate the geometric means of urinary BPA.

Abbreviations: CI, confidence interval; EARTH, Environmental and Reproductive Health; LOD, limit of detection (0.4 μg/L); max, maximum; min, minimum.

associations were observed between first-trimester urinary BPA concentrations and blood glucose levels, regardless of adjustment for covariates (Table 3). When we restricted the analysis to the subset of women who provided 1 urine sample in each trimester, results were consistent (Supplemental Table 1). However, there was no association of average BPA concentrations from the first and second trimesters with glucose levels (Supplemental Table 1).

Lastly, there was no evidence of heterogeneity for the association between trimester-specific urinary BPA concentrations and blood glucose levels by maternal age, BMI, mode of conception, or year of urinary sample collection ($P$, interaction $> 0.10$; data not shown).

## Discussion

In this population of women at high-risk of glucose intolerance who were attending a fertility center (3,4), we found urinary BPA concentrations in the second trimester to be positively associated with blood glucose levels subsequently measured later on in the second trimester of pregnancy. On the other hand, there was no association between first-trimester urinary BPA concentration and blood glucose levels. Together, these results suggest that timing of BPA exposure, a nonpersistent chemical, may be important with respect to glucose levels during the more insulin-resistant time period of later pregnancy.

Our understanding of the effect of BPA on glucose homeostasis mainly comes from experimental animal models. Based on *in vivo* and *in vitro* studies, BPA was found to activate estrogen receptors or act through estrogen receptor–independent signaling pathways that regulate glucose homeostasis and insulin secretion (9, 24). Animal studies demonstrated that after 4 days of BPA administration, adult mice developed chronic hyperinsulinemia, followed by insulin resistance (9). BPA can also increase oxidative stress (10), suppress adiponectin,

**Table 3. Quartile of Trimester-Specific Urinary BPA (Micrograms per Liter) and Blood Glucose Levels Among Pregnant Women in the EARTH Study**

| Second-Trimester Adjusted Blood Glucose Levels[a] (95% CI) in Milligrams per Deciliter From GCT | | | | | |
|---|---|---|---|---|---|
| Quartile (Range) of SG-Adjusted BPA Concentration | BPA Measured in the First Trimester (n = 208) | | Quartile (Range) of SG-Adjusted BPA Concentration | BPA Measured in the Second Trimester (n = 209) | |
| | Unadjusted | Adjusted[b] | | Unadjusted | Adjusted[b] |
| Q1 (<LOD to 0.78) | 119 (111 to 126) | 121 (113 to 129) | Q1 (<LOD to 0.66) | 107 (100 to 113) | 106 (100 to 112) |
| Q2 (0.78 to 1.24) | 111 (104 to 118) | 115 (107 to 122) | Q2 (0.67 to 1.05) | 112 (106 to 119) | 115 (108 to 121) |
| Q3 (1.24 to 2.28) | 115 (108 to 122) | 116 (109 to 124) | Q3 (1.09 to 2.10) | 116 (109 to 123) | 114 (107 to 121) |
| Q4 (2.35 to 15.9) | 112 (105 to 119) | 114 (106 to 122) | Q4 (2.24 to 59.6) | 118 (111 to 126)[c] | 119 (112 to 126)[c] |
| $P$ trend[b] | 0.30 | 0.19 | P-trend[b] | 0.02 | 0.01 |

Abbreviations: CI, confident interval; EARTH, Environmental and Reproductive Health; LOD, limit of detection; Q, quartile.

[a] Adjusted models controlled for maternal age (years), prepregnancy overweight or obese (<25 kg/m$^2$, ≥25 kg/m$^2$), total physical activity (h/wk), family history of diabetes, race (white, nonwhite), infertility diagnosis (male factor, female factor, unexplained), urine sampling session (morning collection, afternoon collection), and fetus number in a pregnancy (1, 2).

[b] Tests for linear trend were performed using the median urinary BPA concentration in each quartile as a continuous variable in the model.

[c] $P$ value for comparison against Q1 <0.05.

and stimulate inflammatory adipokines (11), all of which enhance the risk of diabetes (25).

Evidence from human studies of BPA in relation to diabetes is mixed and mostly limited to cross sectional study designs (26). Positive associations between urinary BPA and self-reported diabetes were reported in NHANES 2003 to 2004 (27) and a pooled analysis from 2003 to 2006 (28). However Silver *et al.* (29) later used HbA1C and self-reported diabetes as the outcomes in the analysis of data from 3 NHANES cycles, pointing out that a statistically significant result was driven by data in 2003 to 2004, but not in 2005 to 2006 or 2007 to 2008. In one prospective study, Sun *et al.* (30) found that urinary BPA concentration was associated with the higher risk of type 2 diabetes among middle-aged but not older women in a nested case-control study. One possibility for conflicting results could be that timing of BPA exposure might be critical for the risk of developing insulin resistance. This may be particularly relevant to BPA, a nonpersistent chemical.

Recently, researchers proposed that pregnancy might be a sensitive period for BPA exposure (31). During pregnancy, changes in blood estrogen, placental lactogen and placental growth hormones lead to the reorientation of maternal metabolism, which favors glucose sparing for the fetus (32–34). Because estrogen receptors, together with estrogen and other hormones, play an important role on the maintenance of insulin and glucose homeostasis (32), additional exposure to an environmental estrogen such as BPA during this sensitive period may disturb the normal physiologic estrogen levels and possibly result in increased insulin resistance. A series of studies by Alonso-Magdalena *et al.* showed that pregnant mice administered a low dose of BPA (10 μg/kg) from days 9 to 16 of gestation displayed a tendency for insulin resistance during pregnancy (13), and induced glucose intolerance later in life (12). Interestingly, in agreement with our findings, exposure timing on gestation days 9 to 16 in the pregnant mice studies is equivalent to the second trimester of human pregnancy (35). The observed association in the second trimester may represent a biologically susceptible window when human placental lactogen and placental growth hormone levels start to increase due to the full formation of the placenta at this time period (17).

As far as we are aware, only two epidemiologic studies examined the association between BPA exposure and GDM. In agreement with our findings, a Canadian birth cohort study (n = 1274), using a single urine sample collected at the 6∼14 weeks of gestation between 2008 and 2011, found no association between urinary BPA concentrations and risk of GDM (15). Yet, the study did not have urinary data measured at the second trimester, making it not comparable to our main findings that early second-trimester concentrations are positively associated with later second-trimester blood glucose levels. A small pilot case control study comparing urinary BPA concentrations (measured in the second trimester between 2009 and 2010) of 22 cases of GDM diagnosed by Carpenter–Coustan criteria and 72 controls without GDM found that urinary BPA levels were not associated risk of GDM (14). Of note, in this pilot study, urinary BPA concentrations were at least 50% lower than the representative sample of the US population. The authors concluded that larger studies were needed as well as studies that examined different windows of pregnancy. In both reports, glucose tolerance in pregnancy was approached categorically as GDM vs no GDM, whereas we assessed glucose tolerance as a continuous variable in the current study. In addition, our study participants consisted of women presenting to a fertility clinic, who may be more susceptible to manifesting glucose effects of BPA exposure because they are otherwise at higher risk of developing GDM (3–5).

Although the current study expands on previous research by examining timing of BPA exposure, it has several limitations. First, urine samples were collected at the clinic visit and were thus convenience samples that may be influenced by fasting status at time of collection. Second, BPA concentrations measured at first and second trimesters were based on single spot urine samples and misclassification of exposure is possible. Third, although we used the clinically relevant results from standard GDM screening test, nonfasting glucose levels may be influenced by the timing of last meal. However, we lacked of necessary data to account for this in the present analysis. Nonetheless, it is important to highlight that the sources of error mentioned earlier are most likely to be nondifferential, which would probably attenuate the observed association. In addition, due to small sample size, we were underpowered to evaluate the risk of GDM in the analysis. Nonetheless, data suggest that lesser degrees of hyperglycemia in pregnancy below the clinical threshold for GDM diagnosis also increase the risk of adverse pregnancy outcomes in a continuous manner (16, 36). Also, we cannot rule of the possibility of residual confounding due to exposure to other chemicals that may affect glucose levels and be correlated with urinary BPA concentrations. Further confirmation studies that account for exposure to chemical mixtures are needed. Another limitation was the potential for clinical variation in the use of diagnostic criteria for PCOS by individual physicians in the fertility clinic. Nonetheless, further accounting for a physician diagnosis of PCOS had only minimal impact on estimated associations; thus, PCOS was unlikely to account for the observed association of

BPA with glucose levels. Lastly, due to our fertility clinic–based study population, the results may not be generalizable to women conceiving naturally. Nonetheless, demographic characteristics of the study participants are comparable with those presenting to fertility clinics nationwide (37), suggesting that the findings may be generalizable to other women seeking care at fertility clinics.

Our study has several strengths. First, we used a prospective study design with multiple urinary BPA concentration measurements during both the first and second trimesters of pregnancy, allowing us to evaluate potential sensitive time windows in pregnancy that may be more susceptible to BPA exposure. Second, our study benefitted from having a wide range of urinary BPA concentrations, which were comparable to those reported in the nationally representative data (8). Third, we were able to adjust for a variety of lifestyle factors and reproductive history, finding consistent results despite adjustment.

To our knowledge, this is the first report of its kind to examine the relationship of urinary BPA concentrations with blood glucose levels among women presenting to a fertility clinic. Our study showed that first-trimester (~7 weeks of gestation) BPA concentrations were unrelated to blood glucose levels, possibly because placenta development was not completed until the end of the first trimester of pregnancy (10 to 12 weeks of gestation) (38). However, we observed an average of 13 mg/dL differences in glucose levels between women in the highest and lowest quartile of second-trimester BPA concentrations. Despite this difference being relatively small, the Hyperglycemia and Adverse Pregnancy Outcome, a multicenter randomized controlled trial, showed that maternal glucose levels were associated with adverse pregnancy outcomes (including risks of large for gestational age, primary cesarean section, clinical neonatal hypoglycemia, and cord-blood serum C peptide > 90th percentile) in a continuous manner with no obvious thresholds (16), suggesting that all degrees of hyperglycemia may increase risk of adverse pregnancy and perinatal outcomes. Nonetheless, due to exploratory nature of the study and the inherent limitations we acknowledged earlier, the findings should be interpreted with caution. Future studies, especially using multiple urine measures across pregnancy and fasting glucose levels, are needed to improve our understanding of this potential relationship.

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