Developmental programming: interaction between prenatal BPA exposure and postnatal adiposity on metabolic variables in female sheep

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Veiga-Lopez A, Moeller J, Sreedharan R, Singer K, Lumeng C, Ye W, Pease A, Padmanabhan V. Developmental programming: interaction between prenatal BPA exposure and postnatal adiposity on metabolic variables in female sheep. Am J Physiol Endocrinol Metab 310: E238–E247, 2016. First published December 8, 2015; doi:10.1152/ajpendo.00425.2015.—Among potential contributors for the increased incidence of metabolic diseases is the developmental exposure to endocrine-disrupting chemicals such as bisphenol A (BPA). BPA is an estrogenic chemical used in a variety of consumer products. Evidence points to interactions of BPA with the prevailing environment. The aim of this study was to assess the effects of prenatal exposure to BPA on postnatal metabolic outcomes, including insulin resistance, adipose tissue distribution, adipocyte morphometry, and expression of inflammatory markers in adipose tissue as well as to assess whether postnatal overfeeding would exacerbate these effects. Findings indicate that prenatal BPA exposure leads to insulin resistance in adulthood in the first breeder cohort (study 1), but not in the second cohort (study 2), which is suggestive of potential differences in genetic susceptibility. BPA exposure induced adipocyte hypertrophy in the visceral fat depot without an accompanying increase in visceral fat mass or increased CD68, a marker of macrophage infiltration, in the subcutaneous fat depot. Cohens effect size analysis found the ratio of visceral to subcutaneous fat depot in the prenatal BPA-treated overfed group to be higher compared with the control overfed group. Altogether, these results suggest that exposure to BPA during fetal life at levels found in humans can program metabolic outcomes that lead to insulin resistance, a forerunner of type 2 diabetes, with postnatal obesity failing to manifest any interaction with prenatal BPA relative to insulin resistance and adipocyte hypertrophy.

Bisphenol A; insulin resistance; adipocytes; inflammation; development origins and toxicology

In recent decades, the incidence of type 2 diabetes has increased at a staggering rate of 1.7 million new diagnoses per year (27). In parallel, the percentage of the population identified as overweight and obese continues to rise, with more than one-third of US adults being obese (29). The incidence of type 2 diabetes is higher in overweight and obese individuals, indicating that excess adiposity influences the onset and progression of type 2 diabetes. Increased attention has been focusing on the developmental origin of these metabolic diseases and how insults during fetal and postnatal development change their developmental trajectory and culminate in altered physiology and pathology (8).

Among potential contributors for the increased incidence of metabolic diseases is the exposure to endocrine-disrupting chemicals (EDCs) such as natural and man-made compounds during critical periods of organ development and differentiation. Human epidemiological studies and experimental animal studies indicate that EDCs have the ability to interfere with physiological functions. One such EDC is bisphenol A (BPA), a carbon-based compound with two hydroxyphenyl groups that is used in manufacturing plastic and epoxy resins. The ubiquitous nature of BPA is evident by its detection in dust, running water, drinking water, human urine, blood, tissues, and a wide variety of consumer products (48). Considerable evidence exists indicating that BPA has estrogenic, anti-androgenic, and thyrotrophic activities, interferes with insulin signaling, and disrupts biological processes (48).

BPA’s presence in human maternal samples, cord blood, amniotic fluid, and fetal blood samples (12, 48) highlights the risk of exposure during fetal development. Animal studies have demonstrated that exposure to BPA during this vulnerable yet critical window of development can lead to reproductive and metabolic defects in offspring (1, 34). The majority of studies testing BPA’s detrimental effects have been carried out in rodent models, and they indicate that prenatal exposure to BPA disrupts offspring growth trajectory (40), induces insulin resistance (1), and increases adiposity (25). Recently, there have been concerns regarding the widespread use of atricial rodent models for risk assessments of EDCs in humans (14). To translate findings that are precocial, results from rodents need to be validated in large-animal models that have a much more similar fetal developmental trajectory as humans. Sheep have been used extensively for understanding developmental origins of metabolic diseases (32, 55); in particular, studies using native steroids have identified days 30–90 of gestation as the critical window of susceptibility for development of adult metabolic perturbations like insulin resistance, altered adipocyte morphology, and hypertension (32). Exposure to excess testosterone (T), an estrogen precursor, during this window leads to a neuroendocrine, ovarian, and metabolic phenotype that parallels characteristics of women with polycystic ovary syndrome (32). Knowledge of the critical windows of reproductive and metabolic susceptibility in sheep provides an opportunity for testing the impact of exposure to BPA during development in this precocial animal model. Our studies indicate that prenatal BPA exposure induces oxidative stress (53), a forerunner of adult metabolic disease risk (7, 28) and reproductive defects (42), in the offspring.

In addition to altered prenatal developmental trajectories impacting the adult phenotype, there is evidence accumulating...
that such courses of development can be exacerbated by the postnatal environment, supporting a “two-hit hypothesis” (37) where a second insult occurs postnatally and can exacerbate a preexisting condition caused by the first insult earlier in life. For instance, reproductive defects in prenatal T-treated female sheep are exaggerated by postnatal overfeeding (31, 45). Similarly, postnatal exposure to estradiol increased the incidence of subluteal cycles in prenatal T-treated sheep (51). The two-hit hypothesis, as it relates to the impact of developmental exposure to EDCs on metabolic outcomes, has not been tested in a precocial large-animal model. Using sheep for this study, we tested the following hypotheses: 1) prenatal exposure to BPA during midgestation at a level relevant to human exposure leads to metabolic disruptions, namely insulin resistance, increased adiposity, altered adipocyte morphology, and adipose tissue inflammation; and 2) postnatal overfeeding would exacerbate the metabolic outcomes programmed by BPA.

**MATERIALS AND METHODS**

**Animals and Prenatal Treatments**

This study was conducted at the University of Michigan Sheep Research Facility (Ann Arbor, MI). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Michigan and are consistent with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals. Adult Suffolk breed sheep (2–5 yr old) were bred to generate control and prenatal BPA-treated animals. Breeder sheep used in the two studies described below were acquired from several local farmers. Details of husbandry and nutrition (22) and BPA treatment strategy (52) have been described previously. All animals were group housed and kept outdoors at the research facility (42°, 18’N) with access to shelter. Potential exposure to phytoestrogens from the diet or other environmental sources was not controlled for, but all groups, including the control group, were maintained under the same housing conditions throughout the experiment. Only females were studied. Males were removed prior to females reaching reproductive maturity to keep the females from getting pregnant.

**Study 1: Effects of Prenatal BPA on Insulin Sensitivity**

A dose response study was first conducted to study the impact of prenatal BPA exposure on insulin sensitivity. Gestational BPA treatment consisted of daily subcutaneous (SC) injections of one of three BPA doses, 0.05, 0.5, or 5 mg·kg⁻¹·day⁻¹ (B₀·₀₅, B₀·₅, and B₀·₅₀₅, respectively; purity ≥99%, cat. no. 239658; Aldrich Chemical, Milwaukee, WI), in corn oil from days 30 through 90 of gestation (term: ~147 days). Control (C) mothers received vehicle. Only one female offspring from each dam was utilized if twin pregnancies were involved. Lambs were weaned at ~8 wk of age. All females provided with a maintenance diet were fed 0.64 kg of corn, 0.64 kg hay·lamb⁻¹·day⁻¹, and 0.014 kg of supplement (36% crude protein) to achieve growth without excess fat deposition. Wooden feeders with a maintenance diet as described for the experiment. Only females were studied. Males were removed prior to females reaching reproductive maturity to keep the females from getting pregnant.

**Overfeeding on Metabolic Variables**

As described above, gestational BPA treatment consisted of daily SC injections of BPA at a dose of 0.5 mg·kg⁻¹·day⁻¹, the middle dose used in the dose response study, which is within the range reported in humans (5, 12, 30). C mothers received the vehicle. At 14 wk of age, half of the C and BPA groups were assigned to one of two dietary groups, maintenance (C and BPA) and overfed diets (C + OF and BPA + OF), respectively. The number of female lambs were C: 10; BPA: 9; C + OF: 11; and BPA + OF: 12. The maintenance and overfed feeding regimens have been described previously (45). In brief, all females assigned to the maintenance diet were fed the same diet as described for study 1. The overfed group was fed 0.77 kg of corn, 0.014 kg of supplement, and 0.73 kg hay·lamb⁻¹·day⁻¹ initially and then hay ad libitum. The overfed group achieved a body weight 30% above the mean body weight of the maintenance group, as described previously (45). A glucose tolerance test was performed at ~15 mo of age during the anestrous season (July). At ~19 mo of age, adipose tissue distribution was assessed by computed tomography.

Glucose Tolerance Test

Because sheep are a ruminant species, longer fasting periods are required to achieve a reliable basal fasting glucose state. As such, they were fasted for 24 (prepubertal age) or 48 h (postpubertal age). After four fasting baseline samples were procured, glucose (300 mg/kg body wt) was given by jugular injections, and frequent blood samples were obtained until 120 min, as described previously (31). Basal insulin, glucose, and insulin/glucose ratio were calculated by averaging the values obtained prior to glucose administration. Insulin response was evaluated as described previously (31), and the following parameters were measured: acute insulin response after 2 and 5 min of glucose administration, cumulative insulin, cumulative glucose, cumulative insulin/glucose ratio, insulin area under the curve after 20 and 180 min from the glucose bolus administration, and insulin sensitivity index. Plasma glucose levels were measured by the glucose oxidase method (Pointe Scientific, Canton, MI). The inter- and intra-assay coefficients of variation (CVs) for glucose measured at 50 and 200 mg/dl were both <5.0% (n = 33 assays). Plasma insulin levels were measured using a radioimmunoassay kit (MP Biomedicals, Orangeburg, NY). The sensitivity of the insulin assay was 0.25 ± 0.04 μU/l (n = 14 assays; means ± SE). Mean intra-assay CVs based on three quality control pools measuring 48.4 ± 1.3, 120.4 ± 2.5, and 432.6 ± 7.2 mg/ml were 9.7, 7.2, and 6.5%, respectively. The interassay CVs for the same quality control pools averaged 8.8, 7.8, and 5.5%, respectively.
Adipose Tissue Distribution

At ~19 mo of age, adipose tissue distribution was assessed in a subset of females from study 2 (C: 6; BPA: 6; C + OF: 6; BPA + OF: 6) by computed tomography (CT) using a multislice CT scanner (16-slice Brightspeed; General Electric) at the Diagnostic Imaging Service at the Veterinary Teaching Hospital of Michigan State University, as described previously (50). Animals were first sedated using xylazine (0.2 mg/kg), and whole body CT scans were performed from the caudal skull to the ischium. Animals were placed on a CT calibration phantom to account for beam variability (Mindways). Slice thickness was 1.25 mm, and scan parameters were kept constant among all animals (120 kVp and 300–330 mA). Adipose tissue was assayed from the 10th thoracic vertebrae to the first lumbar vertebrae, highlighted, and computed if the tissue had an attenuation range from −50 to −150 Hounsfield units. Visceral and SC adipose tissue volume were quantified using imaging software (Analyze 6.0; AnalyzeDirect, Overland Park, KS).

Adipocyte Morphometry

Adipose tissue from subset of females (C: 5; BPA: 5; C + OF: 8; BPA + OF: 6) was minced and suspended in 1× PBS supplemented with 4% bovine serum albumin and 5 mg/ml of collagenase A (Roche Diagnostics, Indianapolis, IN), as described previously (50). The samples were incubated in a dry shaker for 1 h at 40°C. After filtration, adipocyte suspension was then transferred to a siliconized glass slide, and adipocyte images were captured under bright-field illumination. All images were captured using the same magnification and camera settings. Uniform microspheres (diameter, 98.0 μm; Bangs Laboratories, Fishers, IN) were used as a reference. The mean cell diameter and area were determined by computerized image analysis (Image Pro Analyzer version 7.01; Media Cybernetics, Bethesda, MD).

Adipocyte Inflammatory Markers

SC and visceral adipose tissue from all females in study 2 (C: 11; BPA: 7; C + OF: 10; BPA + OF: 10) were flash-frozen in a methylbutane dry ice bath and stored at −80°C until further processing. RNA extraction was performed using the RNeasy kit (Qiagen). Quality and concentration of the RNA were assessed, measuring absorbance at 260 and 280 nm. After RNA was isolated from 100 mg of adipose tissue (visceral or subcutaneous), RT reactions were performed using the high-capacity cDNA reverse transcription kit. The relative expression of adiponectin and CD68 mRNA transcripts were measured using real-time PCR analysis (SYBR Green, ABI Prism 7200 Sequence Detection System; Applied Biosystems) and normalized against the housekeeping gene GAPDH. PCR primers used are listed in Table 1. The melt curves of the primers were examined to confirm that only one gene was amplified, as demonstrated by the single peak. Efficiency was confirmed by the similar slopes of the amplification curves for the gene of interest and the endogenous control. Relative expression was assessed by the comparative CT method correcting for amplification efficiency of the primers and performed in duplicate.

Table 1. List of primers

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer</th>
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<tbody>
<tr>
<td>GAPDH</td>
<td>GGT GGC GGC AAG AGG GTC ATC ATC</td>
</tr>
<tr>
<td>Forward</td>
<td>AGG TTT CTC CAG GGC GCA GGT CAG</td>
</tr>
<tr>
<td>Reverse</td>
<td>ATC AAA CTC TGG AAC CTC TCA TCT AC</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>GTC GGG AAG CAT TAC TCC AA</td>
</tr>
<tr>
<td>Forward</td>
<td>GTC CGT CTA CCA CGA CCA GT</td>
</tr>
<tr>
<td>Reverse</td>
<td>GTC GGG AAG CAT TAC TCC AA</td>
</tr>
</tbody>
</table>

Forward and reverse primers used for amplification with real-time PCR.

Statistical Analysis

The impact of prenatal BPA treatment and postnatal obesity on variables from glucose tolerance test, adipose tissue distribution, and mRNA expression was analyzed using ANOVA followed by Tukey post hoc test. Regression analyses were conducted to test the dose response effect on insulin sensitivity and the correlation between fat depot volumes and mRNA expression. Appropriate transformations were applied for all variables to account for heterogeneity of variances. When trends were evident, effects were also examined by effect size analysis (26), which allows comparison of the means between two treatments with respect to the magnitude of difference between them. The computed statistic, Cohen’s d value, 0.2, 0.5, and 0.8, can be considered as small, medium, and large effect sizes, respectively.

To assess the impact of prenatal BPA treatment on adipocyte size, an empirical cumulative distribution function was calculated for each measurement, and the difference between groups tested used a two-sample Kolmogorov-Smirnov test. Linear mixed-effect model was used to test whether prenatal BPA or overfeeding had an effect on adipocyte characteristics. In all analyses, the effect of number of offspring and sex of the offspring was accounted for. Significance was defined as P < 0.05. All results are presented as means ± SE. All analyses were carried out using SAS for Windows (release 9.1.3; SAS Institute, Cary, NC) and PASW Statistics for Windows release 18.0.1.

RESULTS

Study 1: Effects of Prenatal BPA on Insulin Sensitivity

Prepubertal. Responses of C, Blow, Bmed, and Bhigh groups of animals at 6 wk of age to the intravenous glucose tolerance test are shown in Fig. 1. ANOVA showed effects of BPA on fasting glucose approaching significance (P = 0.059), with fasting glucose being significantly higher in the Blow group compared with the C group (P < 0.05). Effect size comparing control and BPA-treated groups revealed a large effect (d Cohen was 1.3, 1.1, 1.0 for the Blow, Bmed, and Bhigh groups, respectively). Although the insulin response to the glucose challenge was numerically higher in all BPA groups compared with the C group for most of the outcomes studied (see Fig. 1), ANOVA revealed no statistical significance. Effect size analyses found medium to large size differences relative to reduction in insulin sensitivity index for the three prenatal BPA treatment groups. Regression analysis showed no significant dose response effect.

Postpubertal. The response to the intravenous glucose tolerance test in the Blow, Bmed, and Bhigh groups at 13 mo of age is shown in Fig. 2. The insulin response to the glucose challenge was different among all groups, and this was indicated by an increased mean cumulative insulin response at 20 (P < 0.05) and 180 min (P = 0.054) and an increased mean cumulative insulin/glucose ratio response at 20 (P < 0.05) and 180 min (P < 0.05). Post hoc analyses revealed that the Bmed group was significantly different from the C group for cumulative insulin and cumulative insulin/glucose ratio response at 20 and 180 min (P < 0.05). The Blow and Bhigh groups were not significantly different, although they followed a trend similar to that of the Bmed group. Acute insulin response at 5 min also tended to be higher in all BPA groups (P = 0.080, respectively) relative to the C group. Post hoc analyses revealed that only the acute insulin response at 5 min was significantly higher in all BPA groups compared with the C group (P = 0.039, respectively).
different in the Bmed group compared with the C group ($P < 0.05$). Cohen’s effect size analyses found medium to large reductions in insulin sensitivity index for all three prenatal BPA treatments. Regression analysis showed no significant dose response effect.

**Study 2: Interaction Between Prenatal BPA and Postnatal Overfeeding on Metabolic Variables**

**Insulin sensitivity.** Responses of the C, BPA, C + OF, and BPA + OF groups of animals at 15 mo of age to the intravenous glucose tolerance test are shown in Fig. 3. Independent of prenatal BPA exposure, fasting insulin was higher in overfed groups compared with maintenance-fed groups ($P < 0.05$). No significant differences in insulin response (acute response, area under the curve, or cumulative response over 180 min and insulin sensitivity index) or glucose clearance were observed among treatment groups at this age.

**Visceral adiposity.** Body weights and adipose tissue distribution of the C, BPA, C + OF, and BPA + OF groups by CT scan are shown in Fig. 4. As expected, total body weight at the time of the scan was higher in the overfed groups compared with the maintenance groups (Fig. 4B). Overfeeding increased ($P < 0.05$) total, subcutaneous, and visceral fat in the C + OF and BPA + OF groups compared with their maintenance-fed counterparts without any BPA-specific effect. The ratio of visceral to subcutaneous fat was higher in the BPA + OF group compared with the C + OF group [BPA + OF: 1.42 ± 0.07 vs. C + OF: 1.14 ± 0.04, with Cohen’s $d$-test revealing a strong effect size between the BPA + OF and C + OF groups (Cohen’s $d$-test: $1.97$)].

**Adipose tissue morphometry.** Cell size distribution in prenatal BPA-treated females was right shifted toward a larger area ($P < 0.01$) and diameter ($P < 0.01$) in the visceral adipose tissue (Fig. 5). Overfeeding also increased mean adipocyte area ($P < 0.01$) and diameter ($P < 0.01$), consistent with obesity-driven adipocyte hypertrophy, but overfeeding did not exaggerate the effects of prenatal BPA treatment on adipocyte size.

**Adipose tissue inflammation.** The results of mRNA expression in both the visceral and SC adipose depots are shown in Fig. 6. In the SC adipose tissue depot, prenatal BPA increased CD68 expression relative to the C group ($P = 0.02$). The impact of postnatal overfeeding on adiponectin was restricted to the BPA group (BPA vs. BPA + OF, $P = 0.02$). In the visceral adipose tissue depot of both overfed groups (C + OF and BPA + OF), adiponectin mRNA expression was reduced ($P < 0.05$) and CD68 increased ($P < 0.05$) relative to matching maintenance-fed groups. There was no prenatal BPA-specific effect. Regression analyses revealed a significant inverse relationship between adiponectin expression and SC ($r^2 = -0.474$, $P = 0.01$) and visceral fat volumes ($r^2 = -0.498$, $P < 0.005$) (Fig. 6).

**DISCUSSION**

Using sheep, a precocial large-animal model, and targeting pregnancy as the critical window of exposure, the present study demonstrates a risk of metabolic derangements during postna-
In the current study, prenatal BPA-treated females were hyperglycemic only at low BPA doses (indicated by elevated fasting glucose levels) prior to puberty. Considering that chronic hyperglycemia and associated poor insulin release are often reflections of advanced type 2 diabetes (16), relating fasting hyperglycemia in the absence of a diminished insulin release to a defective insulin response in prepubertal sheep proves to be difficult. However, although not statistically significant, the general trend of an increased insulin response (acute and cumulative) coupled with a reduced insulin sensitivity index suggests a compromised insulin sensitivity.

The finding of prenatal BPA-induced insulin resistance manifesting postpubertally in sheep (this study) is consistent with the delayed onset of insulin resistance in mice (1). In male mice, prenatal BPA-induced insulin resistance is evident at 6 mo but not at 3 mo of age. Long-term studies are limited in large-animal models. Age-dependent changes in insulin homeostasis have also been reported in other animal models. In a magnitude comparable with peripubertal controls, insulin sensitivity in prenatal T-treated sheep is reduced during prepubertal development, reaching Tanner stage 5 (6, 13, 18). The importance of this transient reduction in pubertal insulin sensitivity is unclear, although other metabolic, endocrine, and body fat distribution changes occur at this time. Importantly, in our study, the significant effect of prenatal BPA was observed in the Bmed dose (but not the Bhigh dose) and comparable with the exposure level observed in humans (5, 30). The effect of prenatal BPA on insulin resistance was also dose dependent in mice with the lower (BPA maternal exposure: 10 μg·kg−1·day−1) but not the higher (100 μg·kg−1·day−1) dose, showing an effect in males (1), whereas in the present study, the effects were seen in female sheep (males not studied). A nonmonotonic dose-response relationship is a common feature of BPA toxicity (48).

Effects of Prenatal BPA and Postnatal Overfeeding on Insulin Sensitivity

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Exposure to 0.5 mg·kg⁻¹·day⁻¹ BPA did not lead to insulin resistance in study 2 (second cohort; Fig. 3) when examined at 15 mo of age (2 mo later than the BPA dose response study discussed above; Fig. 2). The breed, animal setting, breeders’ body condition, and exposure dose were the same across cohorts of animals. Whether this is a compensatory mechanism to overcome developing insulin resistance is unclear. There is precedence for this; previous studies with prenatal T-treated

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Fig. 3. Study 2. Circulating levels of glucose (top left), insulin (top right), and insulin/glucose ratio (bottom right) before and after administration of a 300 mg/kg glucose bolus via an indwelling catheter in C, control overfed (C + OF), BPA-treated (BPA), and BPA-treated overfed (BPA + OF) females at −15 mo of age. Bottom left: histogram of mean fasting insulin. *Significantly different (P < 0.05); no other glucose tolerance outcome measures were significantly different. □ C group; □ Blow group; gray squares, Bmed group; ■ Bhigh group.

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Fig. 4. Study 2. A: representative computed tomography (CT) scans from C, C + OF, BPA, and BPA + OF animals. Visceral and subcutaneous (SC) adipose tissue depots are represented in purple and blue, respectively. B: histograms of body weight (BW) and visceral (visc)/SC adipose tissue ratio at the time of the CT scan (means ± SE). C: SC, visc, and total adipose tissue volume (means ± SE) from the 4 groups. *Significant differences between groups (P < 0.05); #large-effect size difference between the BPA + OF and C + OF groups.
sheep found insulin sensitivity to fluctuate throughout development with significant reduction in insulin sensitivity evident early in life relative to controls, which disappears as they approach puberty when controls are also becoming insulin resistant (31). Alternatively, since breeder animals were purchased from different farmers, this finding might highlight the importance in the heterogeneity of the genetic background within the same breed of sheep. This is a common finding in rodent literature, where different mouse strains have a greater susceptibility than others to endocrine disruptors’ impact on metabolic responses (44) or genetic manipulations (4).

Where postnatal overfeeding led to insulin resistance in our previous study, contrastingly, it did not significantly impair insulin sensitivity despite achieving a similar percentage weight gain from overfeeding in the present study (31). Overfeeding induced basal hyperinsulinemia, a typical result of insulin resistance (21). Potentially because of its small sample size, the insulin response to the glucose challenge was numerically, not significantly, higher. Genetic background along with maternal body condition at the time of conception or during pregnancy may have contributed to the differing responses between animal cohorts.

Effects of Prenatal BPA and Postnatal Overfeeding on Metabolic Variables

Epidemiological studies have reported positive associations in higher maternal urinary BPA concentrations with increased adiposity (11, 15) and body mass index (47) in children; however, the effects of prenatal BPA treatment on body weight and subcutaneous, visceral, and total adipose tissue mass were not evident in this study. Although some studies in rodents report that BPA exposure leads to increased body weight (25, 40) and adipose tissue mass (25, 41, 43), their findings are inconsistent (25, 41, 43, 46) and may be sex specific (41). Because most studies in rodents focus on the parametrial rather than the subcutaneous fat depot (25, 43) or fail to report fat depot partitioning (41), it is difficult to relate observations from rodent studies to the present findings.

The finding that postnatal overfeeding increases the visceral/subcutaneous fat mass ratio upon prenatal BPA exposure is novel and previously unreported. Given an increase in the visceral, not subcutaneous, adipose depot and its association with negative metabolic outcomes (20, 38), the higher visceral/subcutaneous ratio may have contributed to the poor metabolic outcomes in prenatal BPA-treated sheep. A high-fat diet was found to reduce fat mass without body mass reduction in prenatal (41), not postnatal (9), BPA-treated rats. These differences across studies may relate to dose and window of BPA exposure, species, strain, sex, and/or nature of dietary intervention. The increase in visceral adipocyte size in overfed BPA females relative to overfed controls appears to be modulated by diet and highlights the obesogenic potential of BPA. Independent of fat distribution and degree of obesity, hypertrophic adipocytes are more lipolytic, and it has been hypothesized that metabolic disturbances may arise from the inability of the adipose tissue to expand through hyperplasia (23). Visceral adipocyte hypertrophy in BPA-treated females without an accompanying increase in visceral adiposity was an unexpected finding, as hyper trophy (17), not hyperplasia (3, 10), of adipose cells is considered to be the main determinant of adipose depot increase. A potential reduction in adipocyte number in visceral adipose depot of BPA-treated females might have contributed to this dissociation, a possibility that could not be tested with dissociated adipocytes such as those used in the present study.

Compared with subcutaneous adipose tissue, the finding that visceral adipose tissue secretes lower amounts of beneficial adipokines such as adiponectin is consistent with earlier findings (54). Overfeeding reduced tissue mRNA adiponectin expression, a surrogate measure for circulating adiponectin (19), which appears to be driven by increased fat deposition in both fat depots. Increased CD68 expression in visceral fat of over-

![Figure 5. Study 2. A: representative images of visceral adipocytes from C, C + OF, BPA, and BPA + OF animals identified by computerized image analyses (red lines). B: cumulative size distributions of area (left) and diameter (right) of adipocytes from all 4 groups (see color legend for group identification). P values refer to the overall effect of prenatal exposure to BPA or postnatal overfeeding (OF) on adipocyte area and diameter.](https://example.com/image.png)
fed females may reflect increased macrophage infiltration. In women, increased visceral fat is a predictor of macrophage infiltration (24). The inverse relationship observed between adiponectin and CD68 in the visceral fat depot is also a common finding in high-fat diets and overfeeding regimens (36). CD68 was also increased in subcutaneous fat of BPA-treated females. Although enhanced immunomodulatory effects (33) and innate immune responses (39) have been reported following BPA exposure, to our knowledge this is the first reported increase in CD68 expression, suggesting increased macrophage infiltration in adipose tissue following prenatal BPA exposure. To what extent these changes contribute in the development of insulin resistance observed in these females remains to be determined. The finding that macrophage infiltration was restricted to subcutaneous adipose tissue may reflect a fat depot-specific susceptibility to BPA or, as seen with other organic pollutants, differences in BPA storage capacity between fat depots (35).

In conclusion, our findings using a precocial, large-animal model indicate that gestational exposure to BPA at levels detected in humans can program metabolic disruptions that are independent of postnatal obesity, leading to insulin resistance, a forerunner of type 2 diabetes.

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DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS


REFERENCES