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Paternal bisphenol A exposure in mice impairs glucose tolerance in female offspring

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Abstract

Humans are ubiquitously exposed bisphenol A (BPA), and epidemiological studies show a positive association between BPA exposure and diabetes risk, but the impact of parental exposure on offspring diabetes risk in humans is unknown. Our previous studies in mice show disruption of metabolic health upon maternal BPA exposure. The current study was undertaken to determine whether exposure in fathers causes adverse metabolic consequences in offspring. Male C57BL/6J mice were exposed to BPA in the diet beginning at 5 weeks of age resulting in the following dietary exposure groups: Control (0 µg/kg/day), Lower BPA (10 µg/kg/day) and Upper BPA (10 mg/kg/day). After 12 weeks of dietary exposure, males were mated to control females. Mothers and offspring were maintained on the control diet. Post-pubertal paternal BPA exposure did not affect offspring body weight, body composition or glucose tolerance. However, when fathers were exposed to BPA during gestation and lactation, their female offspring displayed impaired glucose tolerance in the absence of compromised in vivo insulin sensitivity or reduced ex vivo glucose-stimulated insulin secretion. Male offspring exhibited normal glucose tolerance. Taken together,

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Author contributions

C. S. Rashid and R. A. Simmons designed the experiments; R. A. Simmons and M. S. Bartolomei supervised the experiments; C. S. Rashid and A. Bansal performed the experiments; C. Mesaros measured serum BPA; C. S. Rashid analyzed the data and wrote the manuscript; all authors revised the manuscript and approved the final version.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

these studies show there is an early window of susceptibility in which paternal BPA exposure can cause sex-specific impairments in glucose homeostasis.

Introduction

Bisphenol A (BPA; 4,4'-(propane-2,2-diyl)diphenol) was identified as an estrogenic compound in 1938 (Dodds, 1938), and has been since used in the manufacturing of polycarbonate plastics and epoxy resins. Ingestion and skin contact are the two common routes of exposure to BPA; with ingestion being the predominant route (Liao and Kannan, 2013). The 2003–2004 (Calafat et al., 2008) and 2013–2014 (Lehmle et al., 2018) National Health and Nutrition Examination Surveys (NHANES) detected BPA in 93% and 96% of urine samples, respectively, indicating widespread environmental exposure to this estrogenic compound in the US population. Human epidemiological studies from around the world have established a positive association between urinary BPA exposure levels and adverse metabolic health including obesity (Carwile and Michels, 2011; Ko et al., 2014; Shankar et al., 2012; Wang et al., 2012) and type 2 diabetes (Lang et al., 2008; Shankar and Teppala, 2011). Interestingly, BPA exposure has been significantly associated with diabetes in overweight, obese, as well as in normal weight individuals, indicating that BPA exposure is related to dysglycemia independent of adiposity (Shankar and Teppala, 2011).

Multiple studies in the field of Developmental Origins of Health and Disease (DOHaD) show that offspring are vulnerable to nutritional or toxicological challenges during periods of developmental plasticity, and that exposures during this time can increase the risk of obesity and diabetes in adulthood (Fall, 2011; Fernandez-Twinn et al., 2019; Lauritzen et al., 2018). It is well established that offspring are more likely to become obese or diabetic if their mother was obese or diabetic (Meigs et al., 2000). More recently, investigations have explored the possibility that the paternal environment may similarly affect health in offspring (Ornellas et al., 2017; Rando and Simmons, 2015; Soubry, 2018). There is a paucity of epidemiological studies investigating the effects of paternal environmental exposure on offspring metabolic health (Li et al., 2016).

Most evidence of paternal effects comes from animal studies. Male rats consuming a high fat diet become obese and diabetic, and when sired to healthy females, the female offspring exhibit impaired glucose tolerance and attenuated *in vivo* glucose-stimulated insulin secretion (GSIS) without insulin resistance (Ng et al., 2010). Paternal low protein diet causes impaired glucose tolerance in both male and female offspring in mice (Watkins and Sinclair, 2014).

Our previous studies have shown that maternal dietary BPA exposure at doses relevant to human exposure levels causes increased adiposity, impaired glucose tolerance and β -cell dysfunction in male offspring (Bansal et al., 2017; Susiarjo et al., 2015). The current study uses the same relevant doses and route of exposure to elucidate the consequences of paternal BPA exposure on offspring obesity and glucose homeostasis.

Methods

Experimental Animals

All animal work was performed with the approval of the University of Pennsylvania Institute of Animal Care and Use Committee. For post-pubertal exposure, four-week-old male C57BL/6J mice were purchased from Jackson Laboratory and allowed one week of acclimatization. At five-weeks of age, mice were separated into three groups with equal body weights and placed on one of three purified diets. The base diet was low-phytoestrogen 7% corn oil diet (TD95092 -Teklad, USA) supplemented with either 0 µg BPA/kg food (Control), 50 µg BPA/kg food (Lower BPA), or 50 mg BPA/kg food BPA (Upper BPA). Based upon the rate of food consumption, BPA exposure was estimated to 10 µg BPA/kg body weight (Lower BPA), and 10 mg BPA / kg body weight (Upper BPA) per day. These doses of BPA and respective diets are comparable to human exposure levels and have been used in previous studies investigating the effects of maternal BPA exposure on offspring phenotypes (Bansal et al., 2017; Susiarjo et al., 2015). Post-puberty exposed male mice had *ad libitum* access to food and water, and were weighed weekly. After 12 weeks of dietary BPA exposure, male mice were mated with eight-week-old virgin C57BL/6J female breeders for 5 days at which time both dams and the sires consumed Control diet. Dams and their offspring were maintained on the Control diet. For developmental paternal BPA exposure, we employed an established breeding paradigm (Bansal et al., 2017; Susiarjo et al., 2015). Briefly, dams were fed BPA-containing diets beginning 2 weeks prior to mating and continuing until the offspring were weaned. Their male offspring, referred to as developmentally exposed sires throughout this study, were weaned onto Control diet. At 12 weeks of age developmentally exposed sires were mated to virgin females. All offspring were maintained on Control diet and both male and female offspring were phenotyped. Researchers measuring BPA levels in serum, conducting body composition analysis, GTTs, and islet perfusions were experimentally blinded.

Serum BPA measurement

Male mice exposed to BPA post-puberty were fasted 4 hours. Mice were sacrificed via CO₂ inhalation and blood was collected via cardiac venipuncture. Blood was allowed to clot for 20 minutes at room temperature before serum was separated by centrifugation and collected. Serum was stored at -80°C until analysis. BPA was measured by LC-MS as previously described (Singh et al., 2000; Susiarjo et al., 2013). Briefly, serum was measured with a glass syringe and transferred to a 10 mL glass test tube containing 1 mL of Optima water spiked with 2 ng of ¹³[C₁₂]-BPA (Cambridge Isotope Labs, USA), (20 µL×100 pg/µL sol in acetonitrile) for use as an internal standard. The same procedures were performed with 40 µL of water for blank controls. After equilibrating for 10 min at room temperature, the samples were extracted with ethyl ether (3 mL). The organic layer was then evaporated to dryness under nitrogen, and the BPA in the residue was converted to 2,3,4,5,6-pentafluorobenzyl (PFB) derivatives. The alcohol moiety was derivatized as PFB ether under sodium hydroxide. PFB-bromide, sodium hydroxide and solvents were purchased from Sigma-Aldrich. Normal-phase chiral chromatography for LC/MS experiments was performed using a Waters Alliance 2690 HPLC system (Waters Corp., USA). A Chiralpak AD-H column (250×4.6 mm i.d., 5 µm; Daicel Chemical Industries, Ltd., Japan) was used

with a flow rate of 1 mL/min. With solvent A being hexanes and solvent B being 2-propanol/methanol (1/1, v/v), the following linear gradient was performed at 30°C: 2% B at 0 min, 2% B at 2 min, 50% B at 14 min, 50% B at 16 min, 2% B at 17 min, and 2% B at 25 min. HPLC-grade hexanes, isopropanol and ethanol were obtained from Fisher Scientific Co, USA. ACS-grade ethanol was obtained from Pharmco, USA. Gases were supplied by BOC Gases (UK). The following SRM transitions were monitored: BPA-PFB, m/z 227 → 113 (collision energy 28 eV), m/z 227 → 212 (collision energy 25 eV), [13C12]-BPA-PFB, m/z 239 → 139 (collision energy 28 eV) and 13[C12]-BPA-PFB, m/z 239 → 224 (collision energy 25 eV). The detection limit for the BPA-PFB was 3 pg on column. The final concentration of BPA in serum was determined by measuring the area ratio of BPA over ¹³C₁₂-BPA peaks, calculated based on the amount of ¹³C₁₂-BPA added and adjusted based on the volume. All data analysis was performed using Xcalibur software, version 2.0 SR2 (Thermo Electron Corporation, USA) from raw mass spectral data.

Body Composition Analysis

One of two methods was used to determine body composition. Lunar PIXImus2 x-ray densitometer (General Electric, USA) was used for dual-energy X-ray absorptiometry (DEXA) body composition analysis in offspring from sires exposed to BPA post-puberty. DEXA was also used on one cohort of animals from offspring from the developmentally exposed sires. In a second cohort of animals, body composition was determined by nuclear magnetic resonance (NMR) utilizing the EchoMRI 3-in-1 Composition Analyzer (Echo Medical Systems, USA). Because two methods were used to determine body composition analysis in offspring from developmentally-exposed sires, body composition data are presented as relative to controls from their respective method of analysis.

Glucose Tolerance Test

Mice were fasted 4–6 hours beginning when the lights came on in the animal housing room (0630) to control for the circadian rhythm. Mice were then injected intraperitoneally with 2g/kg D-glucose (Sigma, USA) dissolved in physiological saline solution. Blood was sampled from the tail vein at 0, 15, 30, 60 and 120 minutes after glucose injection and blood glucose was measured using OneTouch Ultra 2 glucometer (LifeScan, USA).

Insulin Tolerance Test

Mice were fasted 3 hours beginning when the lights came on in the animals housing room. Male mice and female mice were intraperitoneally injected with 1.25 IU/kg or 0.75 IU/kg, respectively, regular human insulin (Novo Nordisk, USA). Blood glucose was sampled via tail venipuncture at 0, 15, 30, and 60 minutes after glucose injection with OneTouch glucometer. Mice were observed for excessive lethargy, seizures, and blood glucose levels below 20 mg/dL. If any of these hypoglycemia indicators were observed, mice were rescued with an injection of ~1g/kg D-glucose in sterile saline and removed from the remainder of the insulin tolerance test.

Islet Isolation

Islets were isolated from female offspring displaying impaired glucose tolerance. Control females were randomly selected. Mice were anesthetized with IP injection of 80 mg/kg ketamine (PAR Pharmaceutical, USA) and 15 mg/kg xylazine (Akorn Animal Health, USA). Pancreases were perfused with chilled Hanks balanced salt solution (Life Technologies, USA) supplemented with 2.5% (wt/vol) bovine serum albumin, 0.35 g/L NaCO₃ and 2 g/L Collagenase P (Roche, USA). Pancreases were excised and incubated at 37°C for 10 minutes with intermittent shaking to disrupt the tissue. Islets were isolated by discontinuous histopaque (Sigma, USA) gradient centrifugation and washed with perfusate solution not supplemented with collagenase as described previously (Bansal et al., 2017; Susiarjo et al., 2015).

Islet Perifusion

Isolated islets were cultured for 48 hours in RPMI 1640 (Sigma, USA) supplemented with 10 mM glucose, 10% FBS, and pen/strep. Islet perifusion was performed as previously described (Li et al., 2003). One hundred fifty cultured islets of similar size were handpicked and loaded onto nylon filters and perifused with Krebs-Ringer bicarbonate buffer supplemented with 0.25% bovine serum albumin at a flow rate of 1 mL/min. Perifusate solutions were gassed with 95% O₂/5% CO₂ and maintained at 37 °C. Glucose concentrations in the perifusate was incrementally increased until a concentration of 25 mM was reached at which time glucose-free perifusate solution was used to bring insulin secretion back to baseline. After insulin secretion returned to basal levels, islets were stimulated with KCl to depolarize the cell membranes and stimulate insulin exocytosis via non-metabolic mechanisms. Perfusate samples were collected every minute for insulin quantification. Insulin was measured by homogeneous time resolved fluorescence technology (Cisbio, USA).

Statistical Analysis

For each metabolic experiment in offspring, the sample size (n) represents a litter. GraphPad Prism 6 was used for statistical analysis. One-way ANOVA was performed with post-hoc Dunnett's to correct for multiple testing. Significant differences were defined as p<0.05.

Results

Post-pubertal BPA exposure is associated with an age-dependent impairment of glucose tolerance in male mice

There was no difference in body weight between male mice (sires) exposed to BPA and Controls prior to mating (Figure 1A) or anytime during the study. After offspring were born, a subset of sires was sacrificed to measure fasting (4 hours) unconjugated BPA in serum. Upper BPA sires had significantly elevated serum BPA levels compared to Controls while serum levels in Lower BPA sires were comparable to Controls (Figure 1B). This finding is consistent with serum levels of BPA in maternal exposure to the same BPA diets (Susiarjo et al., 2013). Detection of unconjugated BPA in Control animals signifies the ubiquity of BPA in the environment (Vandenberg et al., 2007). The possibility that indoor air, dust and

drinking water contained BPA and contributed to extra-dietary BPA exposure and subsequent detection cannot be ruled out. BPA pharmacokinetics in mice is similar to humans (Taylor et al., 2011; vom Saal and Welshons, 2014). Indeed, after oral administration of a single dose of BPA, serum levels of unconjugated BPA peak within an hour and drop ~ 7 fold after 4 hours (Taylor et al., 2011; Vandenberg et al., 2007). It is to be expected then that BPA levels in Lower BPA sires would be comparable to Controls. Although BPA-free labware was used in collection and storage of serum, we also cannot eliminate the possibility that BPA contamination occurred during blood processing and measurements. Nonetheless, levels measured are within range of those found in humans (Vandenberg et al., 2007).

Glucose tolerance testing revealed decreased area under the curve in the Lower BPA sires compared to Controls signifying enhanced glucose disposal (Figure 1D). We next tested for insulin resistance using an insulin tolerance test. Although the 15- minute time point in the insulin tolerance test showed decreased glucose levels compared to Controls, there was no difference at 30 or 60 minutes, suggesting overall insulin-dependent glucose disposal was comparable between treatment groups in 6 months old sires (Figure 1E). Glucose tolerance testing at 1 year of age showed dose-specific impairments with the Lower BPA sires exhibiting increased area under the curve compared to Control sires (Figure 1G).

Post-pubertal paternal BPA exposure does not affect offspring body composition or glucose tolerance

Neither male nor female offspring from sires exposed to dietary BPA post-puberty showed differences in body weight from birth to termination of the experiment at 4 months of age (Figure 2A and D). In other developmental programming models, there are often modest lean mass reductions and fat mass increments that mask weight-based determinants of obesity, but nonetheless, have significant implications for metabolic health (Ibanez et al., 2006; Kensara et al., 2005; Sayer et al., 2004; Yates et al., 2012). Therefore, we measured lean mass and fat mass, and presented the findings as a ratio of fat mass to lean mass. At 4 months of age, post-pubertal paternal BPA exposure did not impact body composition in males (Figure 2B) or females (Figure 2E). Post-pubertal paternal dietary BPA exposure did not affect glucose tolerance in either male (Figure 2C) or female (Figure 2F) F1 offspring at 4 months of age.

Sires developmentally exposed to BPA produce offspring with sex-specific defects in glucose homeostasis

We have previously shown that developmental exposure to BPA via maternal consumption of these diets from pre-conception through lactation causes obesity, glucose intolerance, and dose-specific glucose-stimulated insulin secretion impairments in male offspring (Bansal et al., 2017; Susiarjo et al., 2015). Using the same exposure paradigm as in those studies, we produced sires developmentally exposed to BPA and mated them to unexposed females. At 4 months of age, male offspring of developmental BPA exposed sires had similar body weights (Figure 3A), body composition (Figure 3B) and glucose tolerance (Figure 3C) between treatment groups. In female F1 offspring, body weights (Figure 4A) and body composition (Figure 4B) were also similar between experimental groups. However, F1

female offspring born to sires developmentally exposed to Lower BPA and Upper BPA showed impaired glucose tolerance (Figure 4C and D).

Female offspring of developmentally exposed to BPA sires have altered insulin secretion

Impaired glucose tolerance is characterized by reduced insulin sensitivity and reduced insulin secretion. Therefore, we performed insulin tolerance tests and islet perfusion studies to determine if the impairment in glucose disposal in female offspring was a consequence primarily of insulin resistance or attenuated glucose-stimulated insulin secretion, respectively. During the insulin tolerance test, 4 of 7 Lower BPA and 2 of 8 Upper BPA female offspring exhibited either blood glucose <20 mg/dL, extreme lethargy, or seizures. These mice were rescued with IP-injection of glucose. Because over half the Lower BPA group were rescued, the ITT was discontinued after the 30 minute time point (Figure 4G). Of note, hypoglycemic episodes were not observed in Control mice. It was concluded based upon the results of the ITT that BPA-exposed female offspring were not significantly insulin resistant.

Because female offspring of the BPA-exposed sires were glucose intolerant without overt insulin resistance, we performed islet perfusion to test whether the impairment in glucose tolerance was the result of attenuated glucose-stimulated insulin secretion. Surprisingly, glucose-stimulated insulin secretion was modestly increased in female offspring born to sires developmentally exposed to BPA, although with the small sample size (n=3), this was not statistically significant (Figure 4I).

Discussion

BPA is a ubiquitous environmental contaminant whose exposure in humans is positively associated with obesity and diabetes. Maternal exposure to BPA in humans is associated with childhood obesity and animal models further show that offspring display diabetic phenotypes such as impaired glucose tolerance, insulin resistance and β -cell insulin secretory defects (Bansal et al., 2017; Susiarjo et al., 2015). Although maternal BPA exposure and its contribution to impairing later life offspring metabolic health is widely studied, the effects of paternal BPA exposure on offspring metabolic health remain unknown. Therefore, the current study was undertaken to determine whether paternal BPA exposure in mice leads to obesity and aberrant glucose homeostasis in offspring. We observed that paternal post-pubertal BPA exposure did not alter offspring metabolic health, but developmental BPA exposure was associated with sex-specific differences in offspring. Female offspring of developmentally exposed fathers exhibited impaired glucose tolerance. Paternal obesity and diabetes increase the risk of these phenotypes in offspring, so we first sought to determine the impact of BPA exposure in fathers under the premise that metabolic disruption in offspring could be secondary to metabolic-disrupting action of BPA in fathers.

In sires exposed to BPA from 5 weeks of age to 1 year of age, increased body weight was not observed at any point during this time. Despite comparable body weights between treatment groups, dose-specific effects were observed regarding glucose tolerance with Lower BPA sires showing enhanced glucose tolerance at 5 months of age and impaired glucose tolerance at 1 year of age. The Upper BPA sires did not show any changes in

glucose tolerance, supporting the non-monotonic effects of endocrine disrupting compounds. These findings are consistent with a previous study showing 12 week BPA exposure in drinking water at levels comparable to the Lower BPA dose in the current study, also does not affect body weight and modestly improves glucose tolerance (Moon et al., 2015). This is in contrast to a study showing increased body weight but comparable glucose tolerance in male mice after 30 days of dietary BPA exposure (Yang et al., 2016). Impaired glucose tolerance was not observed in the present study until a much later age suggesting that BPA's impairment of glucose homeostasis is potentiated by aging-associated mechanisms. The fact that BPA did not elicit diabetic phenotypes prior to mating indicates potential metabolic maladies in offspring would not be a consequence of BPA-induced metabolic phenotypes in fathers. Notwithstanding, rodent studies have demonstrated that experimental treatments that do not elicit metabolic defects in fathers can still initiate diabetic phenotypes in offspring (Anderson et al., 2006; Watkins and Sinclair, 2014).

In the current study, post-pubertal paternal exposure to BPA for 12 weeks prior to mating did not show an appreciable effect on body weight or metabolic health outcomes in male or female offspring. It is noteworthy that we assessed metabolic health of the offspring at 4 months of age. We selected the 4-month time point for offspring because we previously observed metabolic differences at 4 months of age in our maternal BPA exposure model (Susiarjo 2015; Bansal 2017). Therefore, in the current study we wanted to compare whether post-pubertal paternal BPA exposure would alter metabolic health of the offspring at a similar age. Although we did not observe differences at 4 months of age, it is well established that metabolic health deteriorates with age. It is possible that 4 months of age is not sufficient for manifestation of post-pubertal paternal BPA-induced metabolic derangements. This is worthy of future exploration.

In contrast to post-pubertal paternal BPA exposure, male mice developmentally exposed to BPA sired female offspring exhibiting impaired glucose tolerance. The ability of developmentally exposed sires to produce offspring with dysregulated glucose homeostasis may stem from the fact that developmental BPA exposure in males elicits obesity, impaired glucose tolerance and diminished GSIS (Susiarjo et al., 2015). Thus, the presence of diabetes in BPA-exposed fathers may be a prerequisite for phenotypic transmission. This, too, may be sex-specific as previous studies from our laboratories have shown dams developmentally exposed to BPA have normal glucose homeostasis and body composition, yet are still able to transmit BPA-induced obesity and islet deficits to male offspring, but not female offspring (Bansal et al., 2017; Susiarjo et al., 2015). Moreover, our previous studies demonstrated that generational effects of BPA are sex-specific (Bansal et al., 2017; Susiarjo et al., 2015) and this study is no exception as impaired glucose tolerance was only observed in female offspring. This is in contrast to a previous study that found impaired glucose homeostasis in both male and female offspring born to developmentally exposed sires (Mao et al., 2015). The difference in sex-specific phenotypes between the Mao et al. study and our own may be due to differences in dosage (single 40µg/kg/day dose via oral gavage) and/or species (rat). However, together these data support that diabetic phenotypes induced by environmental contaminants can be transmitted to offspring through the paternal lineage.

The female offspring of developmentally exposed sires displayed impaired glucose tolerance in the absence of overt insulin resistance and with concomitant increased *ex vivo* glucose-stimulated insulin secretion. While this is an unusual result, it is not a solitary one. Chen et al. showed that injecting differentially expressed spermatozoal small non-coding RNAs from fathers with high fat diet-induced obesity and glucose intolerance into the zygote recapitulates this unusual glucose homeostasis phenotype (Chen et al., 2016). Interestingly, natural mating of high-fat diet males to control females produces offspring with impaired glucose tolerance concomitant with either insulin resistance (Fullston et al., 2013) or diminished *in vivo* insulin secretion (Ng et al., 2010). Blood glucose levels are modulated by insulin secretion, insulin-mediated glucose uptake and glucose effectiveness, which is the ability of glucose to mediate its own uptake independent of insulin. This insulin-independent glucose uptake is primarily hepatic-mediated and involves both glycogen synthesis and suppression of endogenous glucose production (Alford et al., 2018). We deductively speculate that this impaired glucose tolerance in female offspring may be mediated by attenuated glucose effectiveness, which studies have shown is an independent predictor of type 2 diabetes conversion in at risk individuals (Lorenzo et al., 2010). Additional studies are needed to determine whether paternal BPA exposure indeed diminishes glucose effectiveness.

Regarding transmission of diabetic phenotypes to offspring, it is likely that this occurs neither via cryptic maternal effects nor seminal fluid as diabetic phenotypes have been transmitted to offspring via *in vitro* fertilization (Chen et al., 2016; Sharma et al., 2016). The spermatozoon genome is hypermethylated and changes in its methylation pattern have not been definitively shown to result in transmission of acquired metabolic phenotypes (Shea et al., 2015) possibly because its DNA methylation is lost and re-established after fertilization. This is specifically supported by findings that developmental BPA exposure in male Sprague-Dawley rats causes biologically insignificant alterations in DNA methylation at *Igf2* differentially methylated regions (Mao et al., 2015). It is, therefore, more likely that small non-coding RNAs are the information carriers. Recent studies demonstrated that tRNA fragments from sperm confer to offspring impaired glucose tolerance in the absence of whole body insulin resistance (Chen et al., 2016). Thus, we hypothesize that developmental BPA exposure is altering spermatozoal small non-coding RNAs, which are then sex-specifically affecting glucose homeostasis.

In conclusion, our studies provide additional experimental evidence that endocrine disrupting chemicals adversely affect health via non-monotonic and sex-specific mechanisms, and provide evidence that paternal exposure to environmental contaminants pose a risk to metabolic health of their progeny. We showed that prolonged post-pubertal exposure does not adversely affect offspring glucose homeostasis while developmental BPA exposure in fathers can cause impaired glucose tolerance in female offspring. This provides further evidence that the timing of exposure is important and that gestation and pre-puberty are critical windows of susceptibility in paternal programming. Further studies are needed to determine the mechanism of impaired glucose tolerance and the mechanism of sex-specific generational transmittance of this phenotype.

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Highlights

- Prolonged BPA exposure impairs glucose tolerance in male mice
- Paternal BPA exposure does not influence offspring body composition
- BPA exposure in fathers impairs glucose tolerance in female offspring
- Timing of paternal BPA exposure is a critical determinant of offspring health

Figure 1.

Metabolic phenotyping of male mice exposed post-puberty to dietary BPA. (A) Body weight of sires for the first 4 months on the BPA-containing diets. (B) Fasting serum BPA levels 4 months on respective diets. Glucose tolerance (C), AUC (D), and insulin tolerance (E) at 6 months of age. Glucose tolerance (F) and AUC (G) at 1 year of age. * $p < 0.05$ compared to controls.

Figure 2.

Metabolic phenotype of offspring from sires exposed to BPA post-puberty. Male offspring body weight (A), body composition (B), and glucose tolerance (C) at 4 months of age.

Female offspring body weight (D), body composition (E), and glucose tolerance (F) at 4 months of age.

Figure 3. Metabolic phenotyping of male offspring from sires developmentally exposed to BPA. Body weight (A), body composition (B), and glucose tolerance (C) at 4 months of age. Data represents two independent cohorts.

Figure 4.

Metabolic phenotyping of female offspring from sires developmentally exposed to BPA. Body weight (A), body composition (B), and glucose tolerance with AUC (C and D) at 4 months. Glucose tolerance with AUC (E and F), insulin tolerance (G), and *ex vivo* islet perfusion with AUC (H and I) at 7 months of age. * $p<0.05$ Lower BPA vs Control; ## $p<0.01$ Upper BPA vs Control; \$ indicates $p<0.05$ vs Control using Student's t-test.