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Effects of maternal bisphenol A on behavior, sex steroid and thyroid hormones levels in the adult rat offspring

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ABSTRACT

Aims: Bisphenol A (BPA), an endocrine disruptor used in industrial applications, has been detected in both placenta and milk. We studied the effects of BPA exposure during pregnancy and lactation on body composition, palatable food intake, biochemical, hormonal and behavioral profiles of young and adult Wistar rat offspring.
Main methods: Female rats were divided into: control, BPA10 (10 μg/kg/day) and BPA50 (50 μg/kg/day). BPA was administered by gavage to dams from gestation until the end of lactation. Euthanasia occurred at weaning [postnatal day (PN) 21] or adulthood (PN180).
Key findings: At weaning, BPA10 female pups had higher plasma cholesterol and triacylglycerol. BPA10 male pups showed lower plasma T3. BPA10 pups of both sexes had higher plasma progesterone, testosterone and estradiol. At adulthood, females of both BPA groups had lower food intake and higher insulinemia, whereas males had lower visceral fat, lower progesterone and testosterone concentrations. BPA10 females and males had lower T4 levels, while only males showed lower estradiol. BPA50 females showed lower fat mass, higher lean mass and lower corticosteronemia, while males had lower food intake. In the feeding study, BPA10 males ate more fat at 30 min, while BPA10 females and males ingested less fat after 12 h. BPA10 females showed hyperactivity while both groups showed less exploration.
Significance: Maternal exposure to BPA during gestation and lactation, even at low doses, induces life-long changes in the regulation of metabolic homeostasis of the progeny, affects sex steroids and thyroid hormones levels, compromises behavior, but does not lead to obesity or dyslipidemia.

1. Introduction

Adverse events during the intrauterine and postnatal periods can modify gene expression and lead to permanent changes in metabolism and hormonal regulation [1,2]. Maternal exposure, during pregnancy and breastfeeding, to insults such as inadequate nutrition, social stress and environmental pollutants can induce obesity and its comorbidities later in life [3–5]. Studies in humans have identified that environmental pollutants are potential risk factors in the development of several diseases [6,7]. Substances defined as endocrine disrupting chemicals (EDCs) or "exogenous agents" have been associated with numerous health problems, such as reproductive dysfunction, obesity, diabetes, among others disturbances [8].

Bisphenol A (BPA) is one of the most used EDCs worldwide since it is a starting material for the synthesis of plastics (such as toys, tools, flame retardants, antioxidants and pesticides). It has bioaccumulation characteristics [8–10] and an annual production that exceeds 3.5 million tons [11]. The U.S. Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA) established a BPA reference dose of 50 μg/kg BW/day [12,13], while the European Food Safety Authority (EFSA) estimated as tolerable a daily intake (TDI) of 4 μg BPA/kg BW/day [14].

BPA is considered a xenoestrogen, capable of binding to endogenous estrogen receptors [15,16]. Besides, it can bind to receptors of testosterone [17,18], thyroid hormones [19,20] and glucocorticoids [21]. BPA has been detected in amniotic fluid, neonatal blood, placenta,

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umbilical cord blood and breast milk, showing that this compound can pass from mother to child in pre- and postnatal life [22]. Exposure to BPA during pregnancy induces increased body mass in the offspring during infancy and at adulthood [23]. In the rat offspring exposed to 1 mg/l BPA, alterations in the expression of adipogenic genes were observed at PN21 [24]. BPA exposure during gestation and lactation induces a greater expression of UCP1 (uncoupling protein 1) and lipid accumulation in the brown adipose tissue (BAT) in the offspring [25]. In the liver, Rönn et al. [26] identified increased hepatic lipid content after maternal exposure to BPA associated with a fructose diet. Also in rats, the exposure to low doses of BPA during the critical period of development impairs growth, fertility, hormone levels, behavioral and neural functions [27,28].

Despite all the data that are already available concerning the effects of BPA, no previous study has been designed to evaluate the short- and long-term effects of exposure of BPA specifically during the period encompassing both gestation and breastfeeding; most studies use a continuous BPA exposure protocol that lasts until the animals are euthanized. Here, we investigated the short and long-term effects of perinatal exposure to low doses of BPA on several parameters: body mass, body composition, palatable food preference, hormonal, biochemical and behavioral. We also investigated, for the first time, the mechanism by which food intake may be altered in terms of palatable food preference. Our results elucidate the effects of BPA on tissues and systems not previously investigated, and highlight the importance of eliminating this substance from the manufacture of plastic products.

2. Materials and methods

2.1. Animals

Experiments were carried out in accordance with the protocols approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro and in accordance with the Brazilian Law no. 11.794/2008. Wistar rats were housed in a temperature-controlled room (23 ± 1 °C) with artificial dark–light cycles (lights on at 7:00 a.m., lights off at 7:00 p.m.), with free access to water and standard chow. During the entire experimental period, the rats, as well as all material used during the experiments and analyses, were kept on polysulfone cages (BPA-free).

2.2. Experimental model of BPA administration during perinatal life

Thirty adult nulliparous female rats were mated with male ones (2:1). The estrous cycle was monitored in order to allow for the identification of pregnancy. Pregnant dams were placed in individual cages and randomly assigned into one of three groups: 1) CON (control, n = 9): dams in this group received water with ethanol 0.1%; 2) BPA10 (n = 9): dams in this group received 10 μg/kg/day of bisphenol A (Sigma Aldrich, Sao Paulo, Brazil) diluted in ethanol 0.1%; 3) BPA50 (n = 12): dams in this group received 50 μg/kg/day. Treatment fluids were administered by gavage during the gestation and breastfeeding periods.

At birth, considered as postnatal day 1 (PN1), litters were adjusted to eight pups per dam (four females and four males per litter). The dams' body masses and food intake were recorded daily during pregnancy and lactation. The offspring's body masses were monitored every four days from birth to euthanasia. From weaning (PN21) onwards, all offspring received standard diet for rodents (Nuvilab, Sogorb, São Paulo, Brazil). The offspring's chow consumption was monitored every fourth day from PN21 to PN180. The estrous cycle of the adult female offspring was evaluated every morning from PN150 to PN180: All females showed a regular estrous cycle and they were euthanized during diestrous.

2.3. Milk collection and analysis

At PN20, dams were separated from the pups for 2 h and milk samples were collected. To induce milk secretion, synthetic oxytocin (5 UI/ml, UCB, Jaboticabal, São Paulo, Brazil) was administered and 10 min later, dams were lightly anesthetized with xylazine (Xilazin®, 7 mg/kg BW) and ketamine (Cetamin®, 70 mg/kg BW). Milk was collected in a microtube by manually massaging the thoracic and abdominal teats. Milk samples were used for the quantification of carbohydrates, total protein and total fat [29]. Carbohydrate content was measured by a colorimetric method using picric acid (Vetec Química fina LTDA, Rio de Janeiro, Brazil). Lactose content was quantified by a colorimetric method using the standard curve of synthetic lactose (Sigma-Aldrich Co, St. Louis, MO, EUA). Total protein content was estimated by the colorimetric method described by Peterson [30], using bovine serum albumin (Sigma, St. Louis, MO, USA) as a standard. Total lipid content was determined by a colorimetric method using a commercial kit according to manufacturer's instructions (Bioclin, BH, Minas Gerais, Brazil). Milk energy was calculated using the calories from each macronutrient: Carbohydrates and total protein were multiplied by 4 and fat was multiplied by 9 to obtain the caloric value in Kcal and KJ.

2.4. Behavioral tests – EPM and OF

The elevated plus maze (EPM) was performed to assess anxiety-like behavior at PN160, following a previously reported protocol [31]; animals were tested between 2:00 p.m. and 6:00 p.m. The EPM is shaped like a plus sign and consists of two “open” arms (OA, 50 cm long × 10 cm wide, no walls) and two “closed” arms (CA, 50 cm long × 10 cm wide, with 40 cm high walls) arranged perpendicularly and elevated 40 cm above the floor. Each test began with the animal being placed on the center of the equipment facing an open arm. Animals were allowed 5 min to explore. All tests were video-recorded and the recorded images were used to assess the animals' behavior. The total time spent in and the number of entries into the open and closed arms, and the central area (CN) of the maze were noted. The percentage of time spent in the open arms [%Time OA: Time OA / (Time OA + Time CA)] and the percentage of open arms entries [%Entries OA: Entries OA / (Entries OA + Entries CA)] were used as anxiety measures. Ethologically derived measures were also evaluated: 1) The number of CA entries (Entries CA) was used as a measure of locomotor activity; 2) the percentage of time spent in the center of the maze (%Time CN: Time CN / total time) was used as a measure of decision-making; 3) stretching was used as a measure o risk assessment; 4) head-dipping was used as a measure of exploration. The EPM was cleaned with paper towels soaked in 50% ethanol and dried before each trial.

The open field (OF) was performed at PN161, using a previously reported protocol [32]. The OF was used to assess locomotor activity and consisted of a white wooden box (57 cm long × 57 cm wide × 59 cm high). Its floor was divided by lines into 16 same-sized squares (14.25 × 14.25 cm; 12 outer and 4 inner) that allowed the definition of central and peripheral areas. The OF tests were carried out 24 h after the EPM. At the beginning of the session, each rat was placed in the periphery of the arena and its activity was recorded for 5 min. The locomotor activity was quantified on the basis of the number of rectangles crossed by the animals in the center (CN) and in the periphery (P). A valid crossing was considered when the animal crossed the line with all four paws. The OF was cleaned with paper towels soaked in 50% ethanol and dried before each trial.

2.5. Feeding behavior study

At PN178, animals were submitted to the food challenge test with a high sugar and a high fat diet, respectively HSD and HFD. Males and females were fasted from 8:00 a.m. until 8:00 p.m. The palatable food was offered at night, during the rat's active period, and the animals

could freely self-select between the two palatable diets. The HSD had a 38% higher content of sucrose when compared to the standard chow and the HFD had a 20% higher content of saturated fat when compared to the standard chow. In each cage, the food bin was divided in two same-sized sides by a barrier; the HSD was placed on one side, and the HFD was placed on the other side. After a 12 h-fasting period, the palatable chows were offered continuously for 12 h. The consumption evaluations were performed 30 min and 12 h after the palatable chows were initially placed in the bins. The diets consumption was considered to be the difference between the initial and final weight of the chows present in each cage [33].

2.6. Oral glucose tolerance test – OGTT

At PN170, one female and one male offspring per litter per group were fasted overnight (12 h) and a basal blood sample was harvested from the tail tip (T = 0). Then, rats received 2 g/kg glucose 50% (Pró-químicos, Rio de Janeiro, Brazil) dissolved in saline solution (0.9% NaCl) by gavage. Additional blood samples were obtained at 15, 30, 60 and 120 min. Plasma glucose concentrations were recorded using a handheld glucometer (ONETOUCH ULTRA®, Johnson & Johnson, São Paulo, Brazil) [34].

2.7. Intraperitoneal pyruvate tolerance test – iPTT

At PN173, the same animals that were submitted to the OGTT were used for the iPTT. After 12 h of fasting, basal glycemia was determined. Then, sodium pyruvate 50% (Pró-químicos, Rio de Janeiro, Brazil) dissolved in saline solution (0.9% NaCl) was intraperitoneally administered (2 g/kg BW), and blood was collected 15, 30, 60 and 120 min after the injection [35]. Plasma glucose concentration was recorded.

2.8. In vivo body composition evaluation – nuclear magnetic resonance (NMR)

The total fat mass and the total lean mass (free-fat mass) of PN180 offspring (both sexes) were evaluated by using a NMR equipment (Minispec LF90 TD-NMR, Bruker, Rheinstetten, Germany) for small animals. A quality control check of internal voltages, temperature, magnets, and parameters was performed using a standard provided by the manufacturer. Non-anesthetized rats were placed in a clear, plastic cylinder and kept immobile by insertion of a tight-fitting plunger into the cylinder. Then, the tube was inserted in the chamber of the NMR for approximately 2 min (duration of the scan). The technician was blind as to group assignment. Results are shown as grams (g) of fat mass and g of lean mass.

2.9. Euthanasia

At weaning (PN21), one female and one male offspring from each litter were killed after 2 h of fasting. The respective dam was killed after 8 h of fasting. The remaining offspring (three females and three males per litter) were killed at PN180: The animals were anesthetized with a non-lethal dose of a 2:1 solution of xylazine (Xilazin®, 100 mg/kg BW) and ketamine (Cetamin®, 50 mg/kg BW) and then killed by cardiac puncture. The blood was collected in heparinized tubes and centrifuged (1500 ×g/20 min, 4 °C) to obtain plasma, which was kept frozen (−20 °C) until assaying. The tissues were dissected out and immediately frozen (−80 °C). The central adiposity was quickly determined by weighing the mesenteric, epididymal and retroperitoneal depots (visceral fat mass, VFM). Data were expressed in percent of 100 g of body mass.

2.10. Plasma determinations

At the end of lactation, fasting glycemia was measured in the dams and two pups (one male and one female). Also, at PN180, the fasting glycemia of the remaining offspring was measured. Plasma glucose concentrations were measured from blood obtained from the tail tip in the day of euthanasia by the use of a handheld glucometer (ONETOUCH ULTRA®, Johnson & Johnson, São Paulo, Brazil). The plasma obtained after the euthanasia was used to measure triacylglycerol (TAG) and cholesterol (CHOL) concentrations by using Biosystem® commercial test kits (Bioclin, Belo Horizonte, Brazil), following the manufacturer's instructions. The plasma concentrations of each hormone (dams and offspring) were determined by radioimmunoassay (RIA). Samples were measured in a single assay. Progesterone, testosterone, estradiol, total T4, free T3, insulin and corticosterone were determined by RIA kits according to the manufacturer's instructions (MP Biomedicals, LLC, NY, USA), with the range of detection between 0.15 and 80 ng/ml, 0.1 and 10.0 ng/ml, 10 and 3000 pg/ml, 0.3 and 11 ng/dl, 50 and 800 ng/dl, 5.5 and 310 μIU/ml, 25 and 1000 ng/ml, respectively.

2.11. Lipids analysis

Liver samples (1 mg) were subjected to lipid extraction by the method of Bligh and Dyer [36], with modifications. Briefly, after incubation with intermittent shaking for 1 h in chloroform-methanol-water solution (2:1:0.8, v/v), samples were centrifuged (1500 ×*g* for 20 min at 4 °C). Then, the supernatant was collected, and chloroform and water (1:1 v/v) were added and mixed. After centrifugation (1500 ×*g* for 20 min at 4 °C), the organic phase (containing the lipids) was removed and dried by nitrogen storm. Total neutral lipids were developed by High-Performance Thin Layer Chromatography (HPTLC) using a DC Silica gel 60 plate (Merck Millipore, HE, Germany). Lipids were revealed submerging the plates in a *Charring* solution (3% $CuSO_4$ and 8% H_3PO_4 v/v) for 10 s, and then drying and heating to 110 °C for 10 min [37]. HPTLC plates were analyzed by densitometry using the Image Master software (TotalLab, Auckland, New Zealand).

2.12. Statistical analysis

All data were compiled as means and standard errors of the means (SEM). The comparisons between the groups were carried out by Graph Pad Prism 6.0 for Windows statistical software (GraphPad Software, Inc. San Diego, CA, USA): One-way ANOVAs were followed by Bonferroni's multiple comparison tests. Based on the non-monotonic dose-response effect of BPA, values for each BPA group were individually compared with the control group. Male and female offspring metabolic programming effects were evaluated separately. Differences were considered significant at $p < 0.05$. For the EPM and OF results, the Kolmogorov–Smirnov one sample test (K–S) was used to assess the normality of the distributions of each of the variables. ANOVAs were used to analyze the offspring's behavior in the EPM and OF: Treatment was used as the between-subjects factor. Fisher Protected Least Significant Difference tests were used *post hoc*. Significance was assumed at the level of $p < 0.05$. For the analysis of the feeding behavior, HSD and HFD were evaluated separately by one-way ANOVAs followed by Bonferroni's multiple comparison tests.

3. Results

3.1. Dams during gestation and lactation

During BPA exposure, dams had no change in body mass and food intake either during gestation or lactation. Dams' VFM showed no alteration at the end of lactation (Table 1). At weaning, the dams' glycemia and TAG content were similar among the groups, but the BPA50 group showed higher plasma CHOL (27% *vs.* CON; Table 1). Table 1

Table 1
Dams' biometric, plasma and milk parameters of dams.

	CON	BPA 10	BPA 50
Dams during gestation			
AUC body mass (g)	5194 ± 375	5559 ± 272	5553 ± 231
AUC food intake (g)	440 ± 22	418 ± 16	434 ± 17
Dams at the end of lactation			
AUC body mass (g)	5993 ± 141	5727 ± 200	5940 ± 130
AUC food intake (g)	1012 ± 25	931 ± 33	994 ± 28
Visceral fat mass (g/100 g BW)	1.6 ± 0.3	1.7 ± 0.2	1.6 ± 0.1
Dams' plasma at the end of lactation			
Glycemia (mg/dl)	77.0 ± 3.3	80.7 ± 1.4	78.8 ± 3.3
TAG (mg/dl)	14.4 ± 1.1	12.6 ± 0.8	11.8 ± 1.0
CHOL (mg/dl)	74.0 ± 4.3	88.3 ± 6.2	97.0 ± 6.6[#]
Dams' milk at the end of lactation			
Lipid (g/100 ml)	44.2 ± 5.6	38.1 ± 4.2	31.4 ± 5.2
Carbohydrate (g/100 ml)	3.8 ± 0.2	3.2 ± 0.4	3.9 ± 0.2
Protein (g/100 ml)	13.1 ± 0.4	14.7 ± 1.0	12.9 ± 0.6
Kcal	465.2 ± 50.0	412.1 ± 38.6	350.2 ± 46.6
KJ	1946.0 ± 209.3	1724.0 ± 161.7	1465.0 ± 194.9

AUC: area under the curve, CHOL: cholesterol, TAG: triacylglycerol. Groups: CON – Dams who received water with ethanol (0.1%) by intragastric gavage; BPA 10 – Dams who received BPA diluted in ethanol 0.1% (10 μg/kg BW/day, gavage); BPA 50 – Dams who received BPA diluted in ethanol 0.1% (50 μg/kg BW/day, gavage) during gestation and lactation. Values represent means ± SEM of 9–12 dams/group. One-way ANOVA followed by Bonferroni post-test.
[#] $p < 0.05$ BPA 50 *vs.* CON.

Table 2
Dams' hormone profile at weaning.

	CON	BPA 10	BPA 50
Progesterone (ng/ml)	59.6 ± 12.8	68.1 ± 25.0	72.7 ± 17.0
Testosterone (ng/ml)	0.8 ± 0.12	0.7 ± 0.20	0.7 ± 0.05
Estradiol (pg/ml)	82.1 ± 6.0	71.0 ± 4.1	70.2 ± 3.7
T3 (ng/dl)	62.5 ± 9.6	52.1 ± 4.4	62.2 ± 5.7
T4 (ng/dl)	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Insulin (μIU/ml)	27.2 ± 6.3	31.1 ± 2.2	26.4 ± 2.5
Corticosterone (ng/ml)	1125.0 ± 314.3	1362.0 ± 585.9	570.0 ± 213.9

Groups: Control – Dams who received water with ethanol (0.1%) by intragastric gavage; BPA10 – Dams who received BPA diluted in ethanol 0.1% (10 μg/kg BW/day, gavage); BPA50 – Dams who received BPA diluted in ethanol 0.1% (50 μg/kg BW/day, gavage) during gestation and lactation. Values represent means ± SEM of 9–12 dams/group.

also describes the milk biochemical composition. The groups did neither differ in terms of lipid, carbohydrate, and protein contents, nor in milk energy at the end of lactation period. At the end of the breastfeeding period, dams had no change in plasma progesterone, testosterone, estradiol, thyroid hormones, insulin and corticosterone concentrations (Table 2). In the HPTLC analyses, we observed an increase in liver free (both BPA groups) and esterified CHOL (BPA50 group), and a reduction in TAG (BPA10 group) and free fatty acids (both BPA groups) (Table 3).

3.2. Offspring at weaning

3.2.1. Females

Female offspring had no alterations in the daily body mass of females during lactation (Fig. 1A), and in VFM and glycemia at weaning (respectively Fig. 1B, C). However, plasma CHOL (25% *vs.* CON; Fig. 1D) and TAG were increased in the BPA10 group at PN21 (59% *vs.* CON; Fig. 1E). Both BPA groups showed higher hepatic esterified CHOL and TAG, while the BPA50 group had lower hepatic free fatty acids (Table 3). As depicted in Table 6, at weaning, the BPA10 female offspring had higher plasma progesterone (1.7-fold increase *vs.* CON), testosterone (2.4-fold increase *vs.* CON) and estradiol (1.7-fold increase *vs.* CON) concentrations. However, plasma T3, T4, insulin and corticosterone levels were similar among the groups at PN21.

3.2.2. Males

No changes were observed in daily body mass of males during lactation (Fig. 2A). VFM, glycemia and plasma lipid profile were also similar among groups (respectively Fig. 2B, C, D, E). In the liver (Table 3), free CHOL was reduced in the BPA50 group, while TAG and free fatty acids were increased in the BPA10 group. Both BPA groups showed lower hepatic esterified CHOL. In Table 6, at weaning, the male offspring had higher plasma progesterone (1.6 fold-increase *vs.* CON), testosterone (70% *vs.* CON) and estradiol concentrations (42% *vs.* CON). In addition, male pups of the BPA10 group had lower plasma T3 concentration (−29% *vs.* CON), with no changes in plasma T4, insulin and corticosterone concentrations among the groups at PN21.

3.3. Offspring at adulthood

3.3.1. Females

The ANOVAs failed to indicate differences between the control and BPA (both concentrations) females concerning the main variables derived from the open field (Locomotor Activity; Fig. 3A) and elevated plus maze (%Time OA and %Entries OA; Fig. 3C and E) tests. As for the ethological variables in the EPM (Table 4), locomotor activity ($F_{2,24} = 3.9$, $p = 0.035$) and head-dipping ($F_{2,24} = 5.6$, $p = 0.010$) were affected by bisphenol-exposure in females: BPA10 animals showed more Entries CA than CON ones ($p = 0.038$), and both BPA10 and BPA50 animals showed less head-dipping events than CON ones ($p = 0.026$ and $p = 0.03$, respectively). Adult female offspring had no alterations in fasting glycemia before OGTT or iPTT as well as at 15, 30, 60 and 120 min after the administration of glucose (Fig. 4A) and sodium pyruvate (Fig. 4C), respectively. Concerning the feeding study in adult females, all female groups ingested more HFD than HSD. After 30 min, the BPA10 group consumed more HSD and less HFD that of the CON ones, while the BPA50 group consumed more HSD and HFD diets that of the CON ones (Fig. 5A). After 12 h, HSD consumption was reduced in the BPA50 group and remained lower in BPA10 group (Fig. 5B). At PN180, the female offspring had no change in body mass (Table 5). BPA50 females showed lower fat mass (−19% *vs.* CON; Table 5) and higher lean mass (3% *vs.* CON; Table 5), although the VFM was similar among the groups (Table 5). Female offspring of both BPA groups had lower chow consumption (BPA10: −6% *vs.* CON; BPA50: −5% *vs.* CON; Table 5), but no changes in plasma CHOL and TAG were detected (Table 5). The plasma concentrations of progesterone, testosterone and estradiol showed no change among the experimental groups at PN180 (Table 6). Plasma T3 levels showed no change in the adult female offspring (Table 6), but T4 levels of the BPA10 group was lower (−51% *vs.* CON; Table 6). Both BPA groups had higher concentration of plasma insulin (BPA10: 102% *vs.* CON; BPA50: 1.3-fold increase *vs.* CON; Table 6). Corticosterone concentration was lower in the BPA50 group (−65% *vs.* CON; Table 6).

3.3.2. Males

The ANOVAs failed to indicate significant differences between the control males and both BPA groups regarding the open field and elevated plus maze (Fig. 3B Table 4). At adulthood, the glycemia of male offspring showed no changes before and after glucose (Fig. 4B) and sodium pyruvate (Fig. 4D) administration at OGTT and iPTT, respectively. At PN178, during the 30-min period of the palatable diets study, all male groups ingested more HFD than HSD. At 30 min, the BPA10 group consumed more HSD and HFD diets than the CON group (Fig. 5C). At 12 h, the profile was maintained only for the consumption of HSD diet (Fig. 5D). PN180 male offspring had no alterations in body

Table 3
Hepatic lipid profile at weaning.

	CON	BPA 10	BPA 50
Dams			
Free cholesterol	0.01122 ± 0.0003	0.02820 ± 0.0006**	0.02636 ± 0.002**
Esterified cholesterol	0.1439 ± 0.04	0.1717 ± 0.01	0.3018 ± 0.01**
Triacylglycerol	0.04931 ± 0.0007	0.03153 ± 0.003*	0.04323 ± 0.003
Free fatty acids	0.04367 ± 0.005	0.02614 ± 0.002**	0.06066 ± 0.002**
Female offspring			
Free cholesterol	0.01075 ± 0.0005	0.01061 ± 0.0009	0.009297 ± 0.0003
Esterified cholesterol	0.1092 ± 0.006	0.1322 ± 0.008**	0.1781 ± 0.008**
Triacylglycerol	0.005037 ± 0.0005	0.01098 ± 0.0009**	0.01310 ± 0.0008**
Free fatty acids	0.01006 ± 0.0006	0.009835 ± 0.0007	0.007748 ± 0.0004**
Male offspring			
Free cholesterol	0.1028 ± 0.005	0.1032 ± 0.003	0.08033 ± 0.007*
Esterified cholesterol	0.1502 ± 0.01	0.09098 ± 0.003**	0.1148 ± 0.01**
Triacylglycerol	0.02411 ± 0.0006	0.02937 ± 0.0011**	0.02329 ± 0.0016
Free fatty acids	0.06638 ± 0.002	0.07843 ± 0.004**	0.05685 ± 0.003

Hepatic lipids profile (μg/μg protein). Groups: Control – Dams and pups of dams who received water with ethanol (0.1%) by intragastric gavage; BPA 10 – Dams and pups of dams who received BPA diluted in ethanol 0.1% (10 μg/kg BW/day, gavage); BPA 50 – Dams and pups of dams who received BPA diluted in ethanol 0.1% (50 μg/kg BW/day, gavage) during gestation and lactation. Values represent mean ± SEM of 2–6 dams or pups/group. One-way ANOVA followed by Bonferroni post-test.

* $p < 0.05$ BPA10 or BPA50 *vs.* CON.

** $p < 0.001$ BPA10 or BPA50 *vs.* CON.

mass, fat mass and lean mass. The VFMs of both the BPA10 and BPA50 animals were lower (respectively −28% *vs.* CON, −27% *vs.* CON; Table 5), however, only the BPA50 group showed lower food intake (−5% *vs.* CON; Table 5). Plasma CHOL and TAG content of the male offspring were similar among all experimental groups (Table 5). Regarding the plasma hormones of the adult male offspring at PN180 (Table 6), both BPA groups had lower progesterone (−50% *vs.* CON) and testosterone (−36% *vs.* CON) concentrations, but only the BPA10 group showed lower estradiol levels (−25% *vs.* CON). The BPA10 group had lower plasma T4 concentration (−60% *vs.* CON). Circulating levels of T3, insulin and corticosterone had no alterations in the male offspring.

4. Discussion

Several studies suggest that lifelong exposure to BPA favors the emergence of diseases such as obesity and diabetes, as well as induces behavioral and reproductive changes [38]. The harmful effects of BPA exposure during critical periods of development, such as pregnancy and lactation, on the reproductive function are also evident. For this reason, BPA use has been banned from a number of children's products [39]. Here, we investigated the effects of maternal exposure to BPA on male and female rat offspring at different stages of life (PN21 and PN180). We identified changes in lipids (increased TAG and serum CHOL), hormones (progesterone, testosterone, estradiol, thyroid hormones, insulin, and corticosterone) and behavior (food preference for fatty diet, hyperactivity and reduced exploration), which occur in a sex-specific dependent manner, even after BPA withdrawal at PN21.

Maternal BPA exposure during pregnancy and lactation changes the composition of breast milk, reducing or increasing the milk lipid composition at doses of 0.6 and 52 μg BPA/kg/day, respectively [40]. We did not find changes in milk composition (lipids, proteins and carbohydrates) or in their caloric value, corroborating data from Santos-Silva et al. [41], who exposed Wistar rats to either 5 or 50 μg BPA/kg/day exclusively during lactation and did not observe changes in milk composition.

The influence of BPA exposure on body mass, adiposity and food intake is still controversial. Some authors have reported that BPA exposure in any stage of life is correlated to increased body mass [42,43]. However, other researchers have suggested that perinatal BPA exposure does not alter the body mass of the offspring at weaning or at adulthood [44–46]. Santos-Silva et al. [41] observed lower food intake and body mass in the adult male offspring of BPA-exposed rats only during lactation. Here, we did not observe changes in body mass, although both male and female offspring had lower food intake. We also did not find alterations in adipose mass in either the dams or the young offspring. However, the adult female offspring showed lower total adiposity, but with normal VFM, indicating a relative decrease in subcutaneous fat mass. Considering that the increase in the ratio of VFM/SAT is suggestive of metabolic syndrome, these animals could be at risk. Conversely, BPA males had lower VFM, without changes in total fat, suggesting a lower VFM/SAT ratio. Our data corroborate that of Anderson et al. [47], who also observed lower adiposity in the adult female offspring of rats exposed to BPA (50 μg BPA/kg/BW). Santos-Silva et al. [41] observed a reduction in VFM/SAT in the adult male offspring of rats exposed to the same dose of BPA exclusively during lactation.

BPA exposure at doses either below or above the recommended dose of 50 μg/kg during early life has been correlated with high levels of TAG and free fatty acids in male and female rodent offspring [39]. Adult mice exposed to BPA (doses of 50 and 500 μg/kg/day) showed increased expression of lipogenic enzymes (acetyl-CoA carboxylase and fatty acid synthase), and, as a consequence, accumulation of hepatic TAG [48]. Recently, Gao et al. [49] demonstrated that perinatal BPA exposure induces dyslipidemia in both male and female offspring, with regulation of hepatic and adipose genes. In the human cell line HepaRG, by using a protocol that simulates perinatal exposure to BPA, an induction of steatosis and accumulation of TAG was observed [50]. In our model of perinatal exposure, we also observed higher levels of plasma CHOL and TAG in rats exposed to the higher dose of BPA (BPA50) and also in the BPA10 PN21 female offspring. We detected an increase in TAG, free and esterified CHOL in the liver of the dams and female offspring (PN21). In the male offspring, the observed increase in TAG and free fatty acids also reinforces this hypothesis. These changes are compatible with the dyslipidemia of these animals and suggest the development of hepatic steatosis. Thus, we confirmed that perinatal exposure to BPA is capable of inducing dyslipidemia, probably through molecular changes in the adipose and liver tissues. The mechanisms resulting from this anomaly require further investigation, since it may be occurring due to: increased lipid biosynthesis, decreased lipolysis, or a defect in the lipid transport carried out by lipoproteins. Since, at adulthood, neither BPA group showed alterations in serum lipids, we decided not to evaluate hepatic TAG and CHOL.

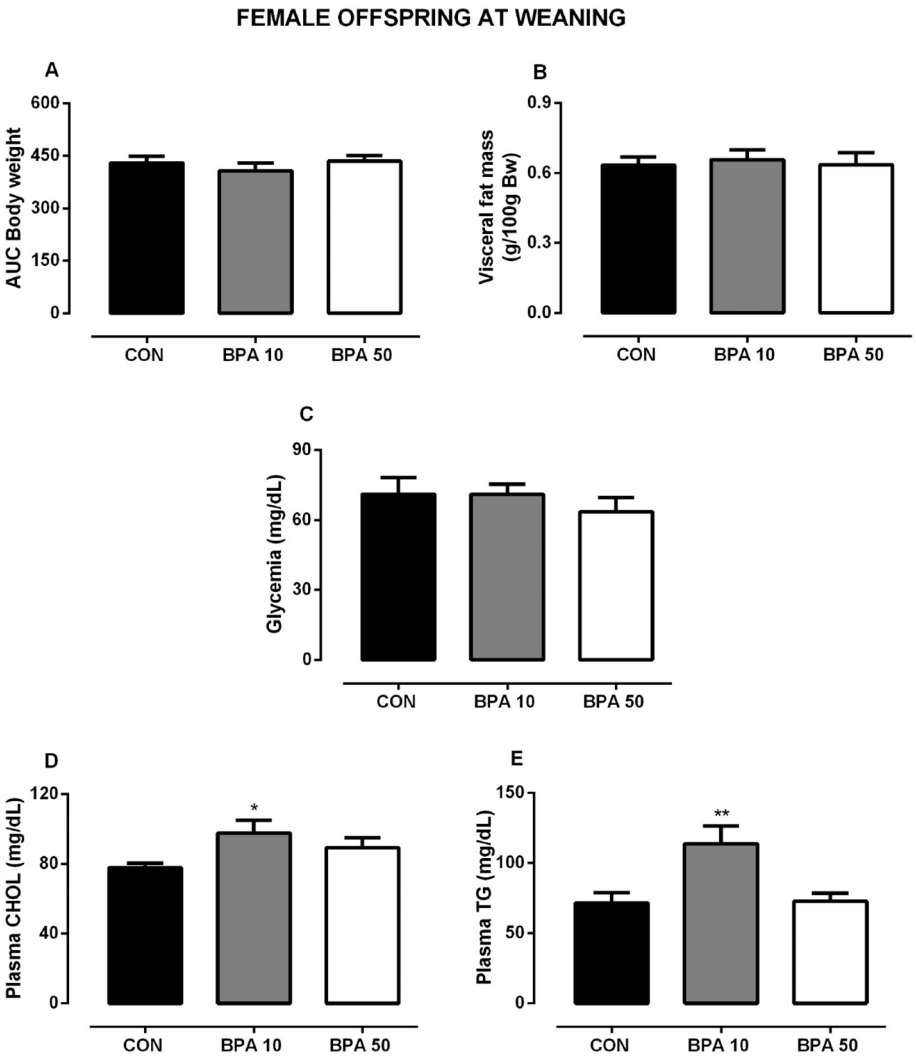

Fig. 1. Effects of oral BPA administration during gestation and breastfeeding on biometric and plasma parameters of the female rat offspring at weaning. Area under the curve (AUC) of daily body mass (A) pups during breastfeeding (PN1–PN21). Visceral fat mass (B), glycemia (C), cholesterolemia (D) and triglyceridemia (E) of female offspring at PN21. Groups: CON (black bar), BPA10 (gray bar) and BPA50 (white bar). Values are given as means ± SEM; n = 9–12 offspring/group. One-way ANOVA followed by Bonferroni post-test. *$p < 0.05$ - BPA10 *vs.* CON; **$p < 0.01$ - BPA10 *vs.* CON.

Wei et al. [51] did not find glycemic alterations after oGTT and iPTT in adult male and female offspring of rats exposed to 250 or 1250 μg/kg/BW of BPA during gestation and lactation. In our study, with lower doses of BPA, we did not observe changes in the fasting glycemia, and in the glycemia after the oGTT and iPTT. Only the adult female offspring had increased serum insulin, which suggests that the BPA action on the secretion of this hormone is sex-dependent. Besides, the increase in insulin levels in the female offspring (PN180) in both BPA groups was followed by hypophagia, corroborating the anorexigenic effect of insulin, which has already been described in the literature [52]. Adult BPA50 males also had hypophagia, however, with no change in circulating insulin, which suggests a mechanism of food intake regulation that is different from that observed in BPA females.

Different studies described that BPA exposure in rodents and humans during gestation and/or childhood interacts with the development of the central nervous system of the offspring of both sexes at different ages, causing behavioral changes, such as attention deficit, hyperactivity, depression, anxiety and aggressive behavior [53–59]. Male and female rodents whose dams were exposed to BPA exhibit depressive-like behavior as well as reduced exploratory behavior [60–63] and impairment in learning and memory [64]. Mice exposed exclusively *in utero* to BPA (2, 20 and 200 μg/kg/day) exhibited changes in DNA methylation in genes expressed in the brain in a dose-dependent manner, resulting in anxiety and dysfunction in reproductive behaviors [65]. Adriani et al. [66] observed that after perinatal exposure to BPA (40 μg/kg), adolescent male and female offspring show hyperactivity and low compulsive behavior, and that only the female offspring shows lower exploration. In our model, with adult rats, there were no changes in the male offspring, while the female offspring showed higher locomotion (hyperactivity) and lower exploration.

Regarding feeding behavior, we did not observe changes in the total dietary intake of lactating dams and their BPA-exposed offspring. The feeding behavior is influenced by the biological rhythm (throughout the 24 h of the day) and by different environmental factors [82]. In addition, insults during the perinatal period are able to modulate the offspring's eating behavior [83–85], which can induce metabolic disorders. In our model, the food preference demonstrated that the lower dose of BPA is able to modulate the food preference of the offspring in a sex-dependent way. In the female offspring, BPA induced lower intake of HFD throughout the test but, in the male offspring, HFD intake was higher during the initial 30 min of the test, without changes when the entire testing period is considered (12 h). These findings suggest that homeostatic mechanisms (hormonal regulators such as leptin, ghrelin and insulin) or hedonic (reward circuit) [86,87] can be regulated by the

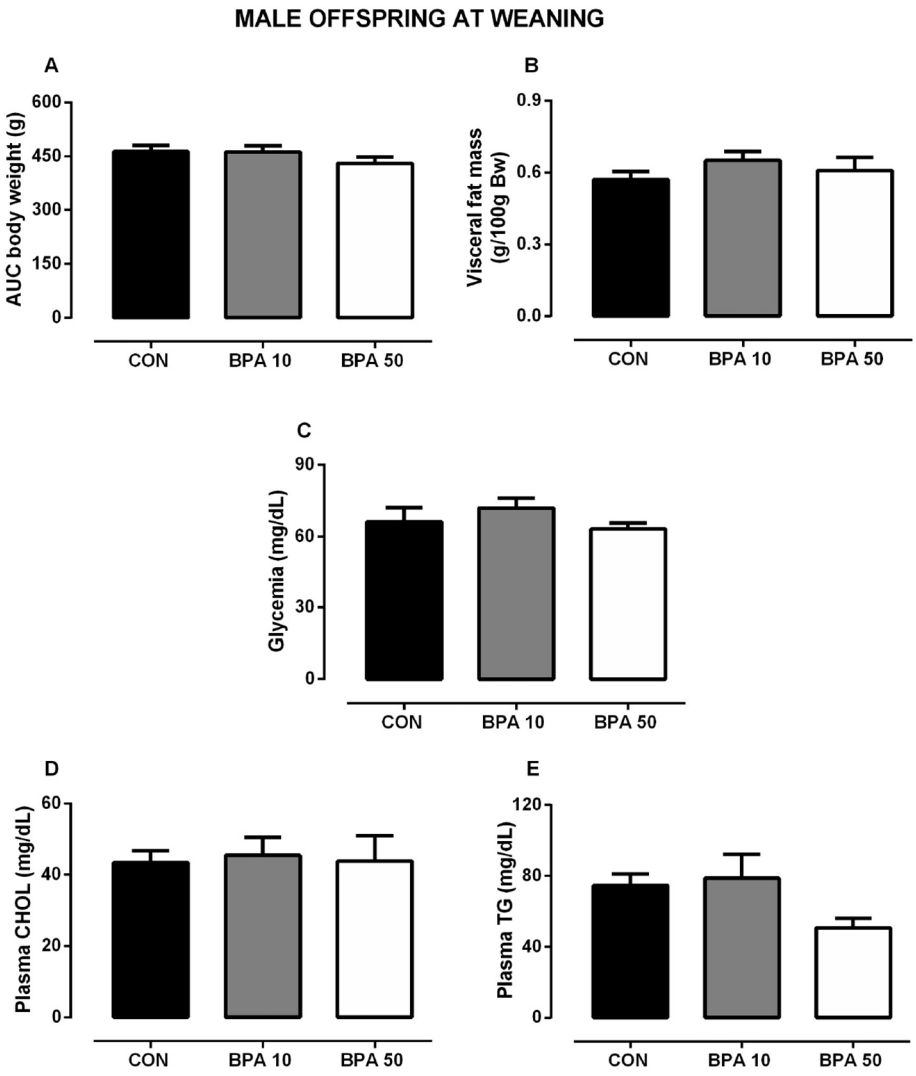

Fig. 2. Effects of oral BPA administration during gestation and breastfeeding on biometric and plasma parameters of the male rat offspring at weaning. Area under the curve (AUC) of daily body mass (A) pups during breastfeeding (PN1–PN21). Visceral fat mass (B), glycemia (C), cholesterolemia (D) and triglyceridemia (E) of male offspring at PN21. Groups: CON (black bar), BPA10 (gray bar) and BPA50 (white bar). Values are given as means ± SEM; n = 9–12 offspring/group.

BPA in a sex-dependent manner. In addition, the higher HSD and HFD consumption at 30 min in males indicates a compulsive behavior for palatable foods. A modulation of the dopaminergic system in our model is possible, since it is directly related to food reward [67]. Narita et al. [68] observed that perinatal BPA exposure induces reward effects through dopamine in mice.

Studies have associated BPA exposure with changes in sex hormones and reproductive disorders [69,70]. In humans [46] and rodents [71,72], exposure to different BPA doses during gestation and/or lactation reduces serum testosterone. The reduction in testosterone levels observed here in the male offspring (PN180) corroborates already reported data, although, at weaning, both male and female offspring had higher levels of testosterone. Such findings demonstrate that the effects of BPA on testosterone occur both at short- and long-term, even long after the end of the exposure to the compound. Thus, for the first time, we show higher concentrations of the three sex steroids in weaned rats.

Several studies have also identified the estrogenic action of BPA [69]. In our model, we identified higher serum levels of progesterone and estradiol in both the male and female offspring (BPA10) at PN21, and reduced serum progesterone levels in the male offspring (BPA10 and BPA50 groups) at PN180, while the adult female offspring had no changes in sex steroid levels. Ma et al. [72] have also identified higher levels of estradiol in adolescent (both sexes) BPA-exposed rats (50, 500 and 2500 mg/kg/day), and Santamaría et al. [73] have found higher levels of progesterone in the adult female offspring of rats exposed to BPA (0.5 or 50 μg/kg/day) during the same critical period.

Changes observed in our model indicate again that the exposure period and sex of the offspring are decisive regarding the modulation of sex hormones by BPA. Although changes in sex hormones were observed predominantly in the offspring at PN21, we showed that females have regular estrous cycles. Besides, ovary, uterus and testis masses increased at PN180 only in the BPA10 group (data not shown). These results suggest that hormonal changes during early life, which could influence the development of these tissues, may reflect in morphological changes at adulthood. The mechanism by which BPA affects sex hormones is not yet clear, but studies show that perinatal exposure to BPA has an important influence on hormonal and/or molecular changes occurring in the adult offspring. This interferes directly with fertility, both in males and females [27], highlighting the importance of programming models to better understand these changes.

Most studies report that exposure to BPA induces hypothyroidism in mammals (rodents, sheep and humans) [41,74–77]. Our results corroborate these studies since we have also observed a decrease in T3 and T4 levels in the male offspring (BPA10 group - PN21 and PN180, respectively) and a reduction in T4 in the female offspring (BPA10 group - PN180). Our study suggests that the hypothyroidism could already be

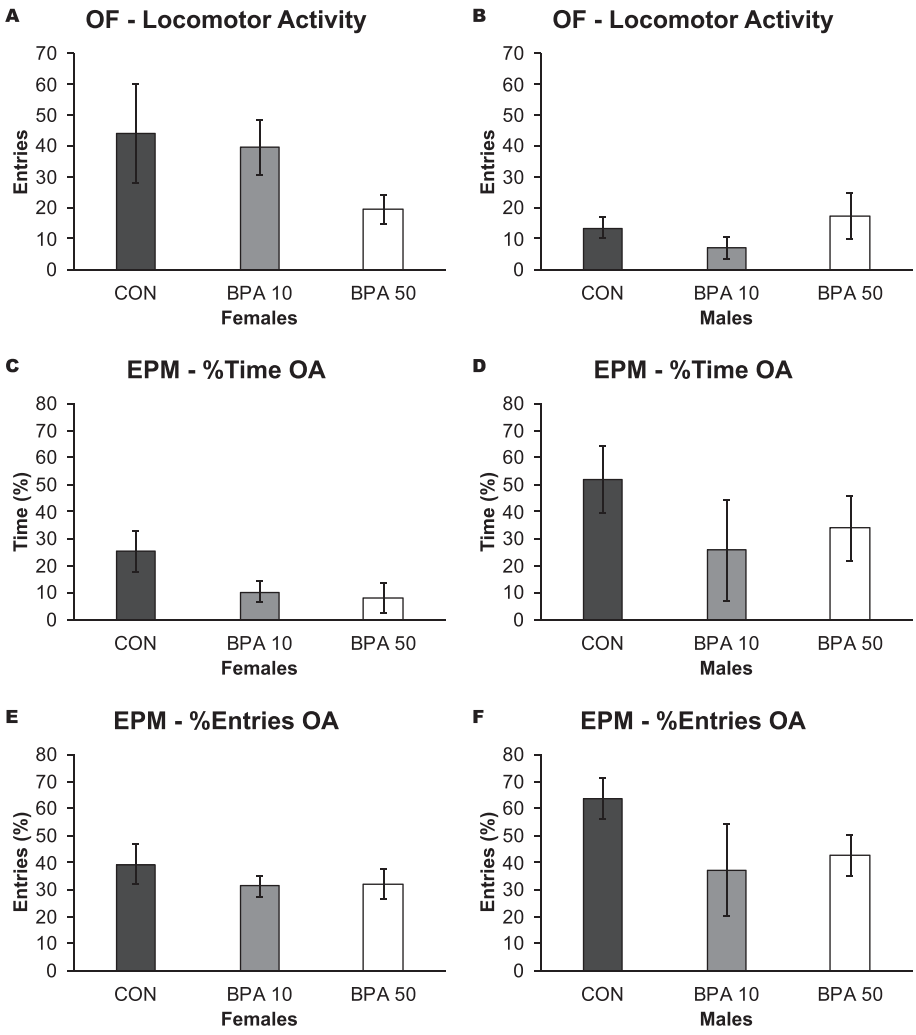

Fig. 3. Behavioral measures in female and male rat offspring at PN180. Locomotor activity assessed in the Open Field (OF: A and B) and anxiety-like behaviors assessed in the Elevated Plus Maze (EPM: C, D, E and F) in females and males. Groups: CON (black bar), BPA10 (gray bar) and BPA50 (white bar). Values are given as means ± SEM; n = 9–12 offspring/group.

Table 4
Additional behavioral measures in female and male rat offspring at PN180 in the Elevated Plus Maze.

	Number of entries CA	% time CN	Number of stretchings	Number of head-dips
Female offspring				
CON	4.1 ± 0.9	9.9 ± 2.7	7.8 ± 1.2	6.4 ± 1.4
BPA 10	8.1 ± 1.6*	12.5 ± 1.4	7.2 ± 1.2	2.9 ± 0.9*
BPA 50	3.6 ± 0.7	5.7 ± 1.3	6.6 ± 1.1	1.3 ± 0.7**
Male offspring				
CON	1.7 ± 0.4	16.3 ± 6.9	5.5 ± 1.3	5.5 ± 1.3
BPA 10	3.0 ± 1.2	9.5 ± 1.5	6.2 ± 1.2	3.6 ± 0.9
BPA 50	3.6 ± 1.1	14.6 ± 2.9	5.9 ± 1.3	3.8 ± 1.0

Groups: Control – Pups of mothers of control group dams; BPA 10 – Pups of mothers of BPA 10 group dams; BPA 50 – Pups of mothers of BPA 50 group dams. Values represent means ± SEM.

* $p < 0.05$ BPA10 or BPA50 *vs.* CON.

** $p < 0.01$ BPA10 or BPA50 *vs.* CON.

present at weaning and last until adulthood, possibly impairing adequate neurological maturation of the offspring, since thyroid hormones are essential for proper brain development [78], a finding that could explain, at least in part, the behavioral changes.

At adulthood (PN180), the female offspring had lower levels of corticosterone. However, Panagiotidou et al. [79] demonstrated that, in rats, perinatal exposure to BPA (40 μg/kg/BW) induces an increase in serum corticosterone only in the adolescent female offspring. These data show that maternal exposure to BPA modulates serum corticosterone only in the female offspring and that this modulation varies according to the age of the offspring.

Previous studies have also shown that 25 (OH) D levels are reduced in pregnant women after environmental exposure to BPA [80]. However, no study has been done on the effects of BPA exposure on programming models. Our study demonstrated, for the first time in rodents, that perinatal exposure to BPA does not induce changes in 25 (OH) D levels in either dams or offspring.

5. Conclusion

Our results lend support to the hypothesis that BPA transfer between mothers and offspring can adversely affect, in a sex-dependent way, the phenotype of the offspring. We have shown that maternal oral BPA exposure induces several short- and long-term biochemical, hormonal and behavioral changes in the offspring. Our study reinforces the concept that exposure to BPA is harmful even if it is restricted to the gestation and lactation periods. Currently, in some countries, BPA is banned from children's products [81]. However, our data suggest that the use of BPA should also be substituted in products used by pregnant

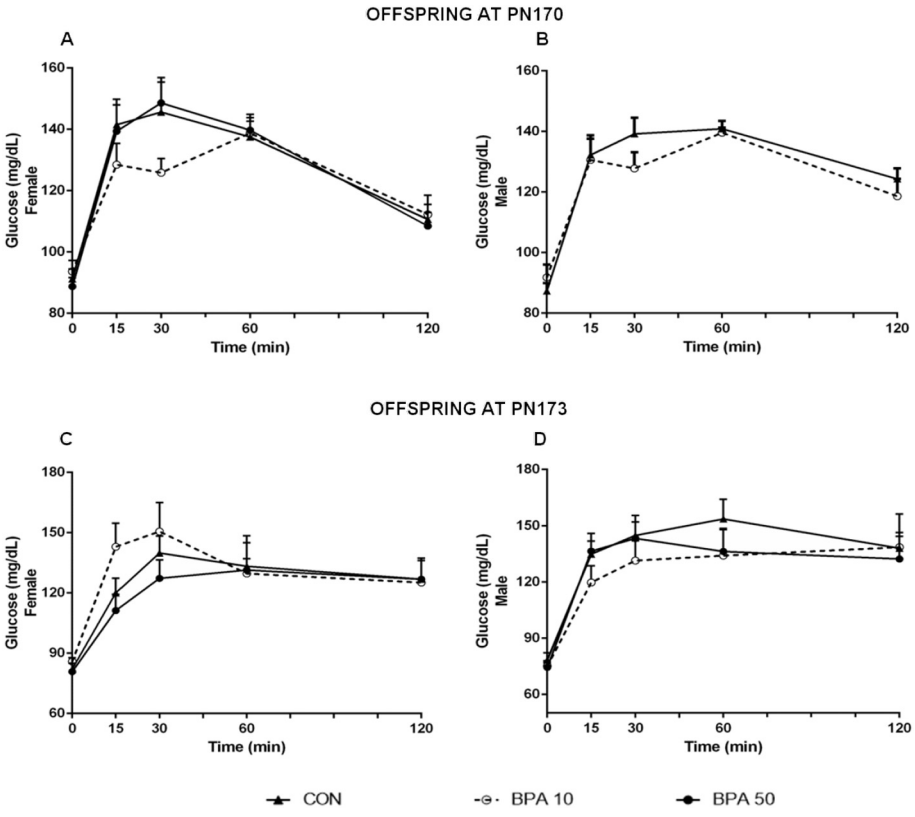

Fig. 4. Effects of oral BPA administration during gestation and breastfeeding on glycemic profile of the adult offspring. Oral glucose tolerance test (OGTT) of female (A) and male (B) rats at PN170. Intraperitoneal pyruvate tolerance test (iPTT) of female (C) and male (D) rats at PN173. Values are given as means ± SEM; n = 9–12 offspring/group.

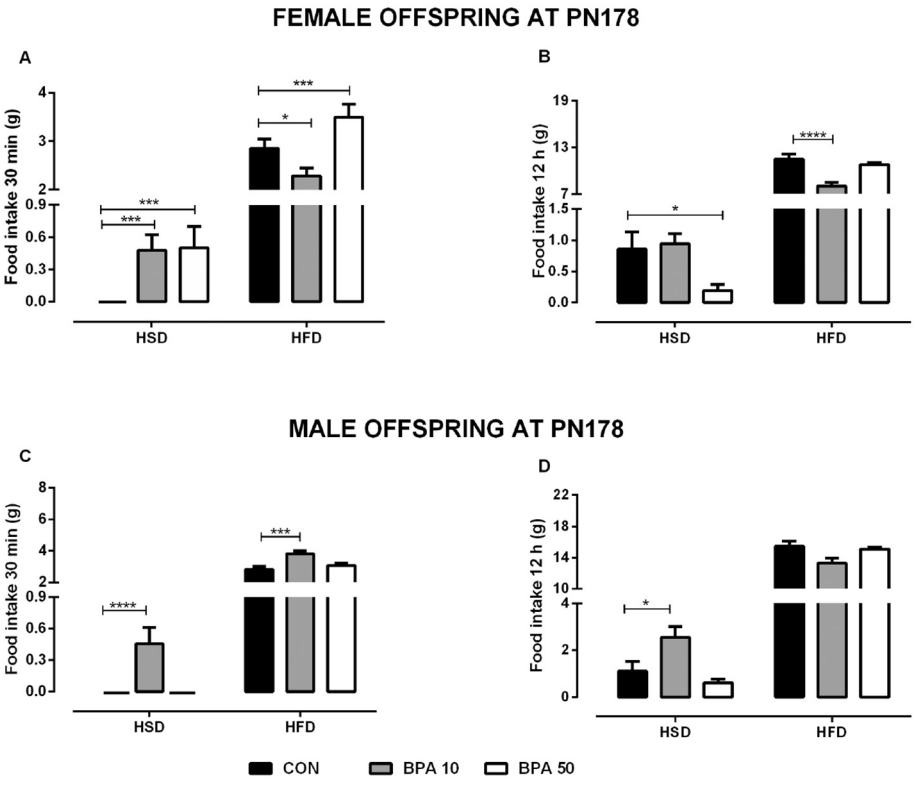

Fig. 5. Effects of oral BPA administration during gestation and breastfeeding on food intake preference of the adult offspring. Food preference as measured by consumption of either HSD or HFD in female and male rats during a 30-min (A, C) or 12-h (B, D) interval, respectively. Values are given as mean ± SEM; n = 9–12 offspring/group. One-way ANOVA followed by Bonferroni post-test. $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

and lactating women, particularly when considering its impact on lipid metabolism, hormone levels and behavior of the offspring. This substitution may be an efficient strategy to prevent the incidence of metabolic and reproductive disorders that may be directly related to BPA exposure.

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Table 5
Body composition, plasma CHOL and TG of female and male rat offspring at PN180.

	Body mass (g)	Fat mass (%)	Lean mass (%)	Visceral fat mass (%)	Food intake (g)	CHOL (mg/dl)	TAG (mg/dl)
Female offspring							
CON	269.3 ± 5.6	13.1 ± 0.9	68.3 ± 0.8	3.7 ± 0.4	780.1 ± 8.4	135.6 ± 7.2	52.8 ± 8.0
BPA 10	260.2 ± 4.9	11.3 ± 0.6	70.0 ± 0.5	3.5 ± 0.2	737.1 ± 3.4****	115.3 ± 6.2	43.5 ± 5.8
BPA 50	267.9 ± 4.7	10.6 ± 0.5*	70.3 ± 0.4*	3.6 ± 0.2	740.7 ± 5.1****	124.1 ± 8.3	47.5 ± 4.3
Male offspring							
CON	447.6 ± 12.4	15.1 ± 1.2	67.1 ± 0.9	6.7 ± 0.7	1055 ± 13.8	44.5 ± 4.3	43.4 ± 6.0
BPA 10	444.2 ± 8.9	14.2 ± 1.2	68.4 ± 0.8	4.8 ± 0.2**	1053 ± 6.0	47.0 ± 2.7	36.0 ± 7.1
BPA 50	440.3 ± 7.9	14.2 ± 0.7	68.3 ± 0.6	4.9 ± 0.2**	1003 ± 6.7***	40.0 ± 1.5	45.0 ± 5.2

CHOL: cholesterol, TAG: triacylglycerol. Groups: Control – Pups of mothers of control group dams; BPA 10 – Pups of mothers of BPA 10 group dams; BPA 50 – Pups of mothers of BPA 50 group dams. Food intake represents the sum of total chow consumption from PN21 to 180. Values represent means ± SEM of 9–12 offspring/group. One-way ANOVA followed by Bonferroni post-test.

* $p < 0.05$ - BPA 10 or BPA50 *vs.* CON.
** $p < 0.01$ - BPA 10 or BPA50 *vs.* CON.
*** $p < 0.001$ - BPA 10 or BPA50 *vs.* CON.
**** $p < 0.0001$ - BPA 10 or BPA50 *vs.* CON.

Table 6
Hormones of female and male rat offspring at weaning and adulthood.

	Progesterone (ng/ml)	Testosterone (ng/ml)	Estradiol (pg/ml)	T3 (ng/dl)	T4 (ng/dl)	Insulin (μIU/ml)	Corticosterone (ng/ml)
Female – weaning							
CON	4.0 ± 0.7	0.29 ± 0.02	21.5 ± 5.9	69.3 ± 10.6	0.50 ± 0.06	43.4 ± 8.8	519.5 ± 96.7
BPA 10	10.8 ± 1.4***	0.99 ± 0.14****	57.9 ± 5.1****	81.5 ± 12.0	0.46 ± 0.06	37.6 ± 4.3	749.4 ± 68.8
BPA 50	2.8 ± 0.7	0.30 ± 0.02	29.4 ± 3.2	86.4 ± 10.8	0.52 ± 0.06	41.1 ± 4.6	352.9 ± 95.6
Female – PN180							
CON	45.8 ± 3.7	0.37 ± 0.01	89.2 ± 6.6	65.3 ± 5.8	0.95 ± 0.12	11.4 ± 4.3	3401 ± 736.1
BPA 10	36.3 ± 4.7	0.46 ± 0.03	89.5 ± 9.6	65.8 ± 6.1	0.47 ± 0.08*	29.3 ± 2.7*	2076 ± 210.6
BPA 50	39.5 ± 3.4	0.44 ± 0.04	101.6 ± 8.6	71.0 ± 4.8	0.68 ± 0.12	33.4 ± 3.0**	1197 ± 259.7*
Male – weaning							
CON	5.9 ± 0.8	0.45 ± 0.05	51.0 ± 6.2	71.0 ± 5.4	0.36 ± 0.04	51.0 ± 11.1	383.4 ± 49.0
BPA 10	15.3 ± 2.7*	0.77 ± 0.10*	72.2 ± 3.6*	50.3 ± 4.4*	0.37 ± 0.06	28.0 ± 5.4	519.8 ± 122.2
BPA 50	11.0 ± 2.2	0.62 ± 0.05	58.7 ± 6.3	72.2 ± 8.8	0.33 ± 0.04	33.3 ± 5.0	495.3 ± 84.5
Male – PN180							
CON	13.7 ± 3.2	5.8 ± 0.8	80.6 ± 6.8	84.3 ± 9.2	1.4 ± 0.1	16.0 ± 3.2	938.0 ± 147.7
BPA 10	6.8 ± 0.8*	3.6 ± 0.2*	60.1 ± 5.5*	87.5 ± 8.1	0.6 ± 0.1	12.0 ± 4.1	882.5 ± 93.3
BPA 50	6.7 ± 1.1*	3.7 ± 0.4*	65.1 ± 3.3	70.8 ± 8.1	1.4 ± 0.2*	17.2 ± 1.7	998.2 ± 124.0

Groups: Control – Pups of Control group dams; BPA 10 – Pups of BPA 10 group dams; BPA 50 – Pups of BPA 50 group dams. Values represent means ± SEM of 9–12 offspring/group. One-way ANOVA followed by Bonferroni post-test.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
**** $p < 0.0001$ BPA 10 or BPA 50 *vs.* CON.

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References

[1] D.J. Barker, J. Gelow, K. Thornburg, C. Osmond, E. Kajantie, J.G. Eriksson, The early origins of chronic heart failure: impaired placental growth and initiation of insulin resistance in childhood, Eur. J. Heart Fail. 12 (2010) 819–825, https://doi.org/10.1093/eurjhf/hfq069.
[2] N.R. Winder, G.V. Krishnaveni, J.C. Hill, C.L. Karat, C.H. Fall, S.R. Veena, et al., Placental programming of blood pressure in Indian children, Acta Paediatr. 100 (2011) 653–660, https://doi.org/10.1111/j.1651-2227.2010.02102.x.
[3] L.C. Messer, J. Boone-Heinonen, L. Mponwane, L. Wallack, K.L. Thornburg, Developmental programming: priming disease susceptibility for subsequent generations, Curr. Epidemiol. Rep. 2 (2015) 37–51.
[4] J.H. Dasinger, B.T. Alexander, Gender differences in developmental programming of cardiovascular diseases, Clin. Sci. 130 (2016) 337–348, https://doi.org/10.1042/CS20150611.
[5] Y.L. Tain, C.N. Hsu, Interplay between oxidative stress and nutrient sensing signaling in the developmental origins of cardiovascular disease, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18040841.
[6] S.K. Park, Y. Tao, J.D. Meeker, S.D. Harlow, B. Mukherjee, Environmental risk score as a new tool to examine multi-pollutants in epidemiologic research: an example from the NHANES study using serum lipid levels, PLoS One 9 (2014), https://doi.org/10.1371/journal.pone.0098632.
[7] M.S. Burroughs Peña, A. Rollins, Environmental exposures and cardiovascular disease: a challenge for health and development in low- and middle-income countries, Cardiol. Clin. 35 (2017) 71–86, https://doi.org/10.1016/j.ccl.2016.09.001.
[8] X. Li, L. Wang, F. Shen, Q. Zhou, X. Huang, Impacts of exogenous pollutant bisphenol A on characteristics of soybeans, Ecotoxicol. Environ. Saf. 157 (2018) 463–471, https://doi.org/10.1016/j.ecoenv.2018.04.013.
[9] S.A. Johnson, M.S. Painter, A.B. Javurek, M.R. Ellersieck, C.E. Wiedmeyer, J.P. Thyfault, et al., Sex-dependent effects of developmental exposure to bisphenol A and ethinyl estradiol on metabolic parameters and voluntary physical activity, J. Dev. Orig. Health Dis. 6 (2015) 539–552, https://doi.org/10.1017/S2040174415001488.
[10] B.B. Andriana, T.W. Tay, R. Hiramatsu, M.A. Awal, Y. Kanai, M. Kurohmaru, et al., Bisphenol A-induced morphological alterations in Sertoli and spermatogenic cells of immature Shiba goats in vitro: an ultrastructural study, Reprod. Med. Biol. 3 (2004) 205–210, https://doi.org/10.1111/j.1447-0578.2004.00072.x.
[11] X. Huo, D. Chen, Y. He, W. Zhu, W. Zhou, J. Zhang, Bisphenol-A and female infertility: a possible role of gene-environment interactions, Int. J. Environ. Res. Public Health 12 (2015) 11101–11116, https://doi.org/10.3390/ijerph120911101.
[12] L.N. Vandenberg, I. Chahoud, J.J. Heindel, V. Padmanabhan, F.J. Paumgartten, G. Schoenfelder, Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A, Cien. Saude Colet. 17 (2012) 407–434.
[13] A.C. Gore, V.A. Chappell, S.E. Fenton, J.A. Flaws, A. Nadal, G.S. Prins, et al., EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals, Endocr. Rev. 36 (2015) E1–E150, https://doi.org/10.1210/er.2015-1010.
[14] EFSA CEF Panel (EFSA Panel on Food Contact Materials E, Flavourings and Processing Aids), EFSA (European Food Safety Authority). Scientific opinion on the

risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: executive summary, EFSA J. 13 (2015) 1–1040, https://doi.org/10.2903/j.efsa.2015.3978.
[15] J.B. Matthews, K. Twomey, T.R. Zacharewski, In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta, Chem. Res. Toxicol. 14 (2001) 149–157.
[16] T. Kurosawa, H. Hiroi, O. Tsutsumi, T. Ishikawa, Y. Osuga, T. Fujiwara, et al., The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type, Endocr. J. 49 (2002) 465–471.
[17] L.C. Xu, H. Sun, J.F. Chen, Q. Bian, J. Qian, L. Song, et al., Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol in vitro, Toxicology 216 (2005) 197–203, https://doi.org/10.1016/j.tox.2005.08.006.
[18] M. Tanaka, S. Nakaya, M. Katayama, H. Leffers, S. Nozawa, R. Nakazawa, et al., Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth, Hum. Exp. Toxicol. 25 (2006) 369–373, https://doi.org/10.1191/0960327106ht638oa.
[19] K. Moriyama, T. Tagami, T. Akamizu, T. Usui, M. Saijo, N. Kanamoto, et al., Thyroid hormone action is disrupted by bisphenol A as an antagonist, J. Clin. Endocrinol. Metab. 87 (2002) 5185–5190, https://doi.org/10.1210/jc.2002-020209.
[20] J. Chevrier, R.B. Gunier, A. Bradman, N.T. Holland, A.M. Calafat, B. Eskenazi, et al., Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study, Environ. Health Perspect. 121 (2013) 138–144, https://doi.org/10.1289/ehp.1205092.
[21] A. Poimenova, E. Markaki, C. Rahiotis, E. Kitraki, Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A, Neuroscience 167 (2010) 741–790, https://doi.org/10.1016/j.neuroscience.2010.02.051.
[22] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W.V. Welshons, Human exposure to bisphenol A (BPA), Reprod. Toxicol. 24 (2007) 139–177.
[23] B.S. Rubin, M.K. Murray, D.A. Damassa, J.C. King, A.M. Soto, Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels, Environ. Health Perspect. 109 (2001) 675–680.
[24] E. Somm, V.M. Schwitzgebel, A. Toulotte, C.R. Cederroth, C. Combescure, S. Nef, et al., Perinatal exposure to bisphenol a alters early adipogenesis in the rat, Environ. Health Perspect. 117 (2009) 1549–1555, https://doi.org/10.1289/ehp.11342.
[25] J.C. van Esterik, M.E. Dollé, M.H. Lamoree, S.P. van Leeuwen, T. Hamers, J. Legler, et al., Programming of metabolic effects in C57BL/6JxFVB mice by exposure to bisphenol A during gestation and lactation, Toxicology 321 (2014) 40–52.
[26] M. Rönn, J. Kullberg, H. Karlsson, J. Berglund, F. Malmberg, J. Orberg, et al., Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats, Toxicology 303 (2013) 125–132, https://doi.org/10.1016/j.tox.2012.09.013.
[27] F.S. vom Saal, C. Hughes, An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment, Environ. Health Perspect. 113 (2005) 926–933, https://doi.org/10.1289/ehp.7713.
[28] EFSA (European Food Safety Authority), Scientific Opinion on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A, EFSA J. 8 (2010) 1–116.
[29] A.P. Santos-Silva, E. Oliveira, C.R. Pinheiro, A.L. Nunes-Freitas, Y. Abreu-Villaça, A.C. Santana, et al., Effects of tobacco smoke exposure during lactation on nutritional and hormonal profiles in mothers and offspring, J. Endocrinol. 209 (2011) 75–84, https://doi.org/10.1530/JOE-10-0410.
[30] G.L. Peterson, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem. 83 (1977) 346–356.
[31] M.C. Fraga-Marques, E.G. Moura, S. Claudio-Neto, I.H. Trevenzoli, F.P. Toste, M.C. Passos, et al., Neonatal hyperleptinaemia programmes anxiety-like and novelty seeking behaviours but not memory/learning in adult rats, Horm. Behav. 55 (2009) 272–279, https://doi.org/10.1016/j.yhbeh.2008.11.010.
[32] C.C. Filgueiras, A. Ribeiro-Carvalho, F. Nunes, Y. Abreu-Villaça, A.C. Manhães, Early ethanol exposure in mice increases laterality of rotational side preference in the free-swimming test, Pharmacol. Biochem. Behav. 93 (2009) 148–154, https://doi.org/10.1016/j.pbb.2009.04.023.
[33] C.R. Pinheiro, E.G. Moura, A.C. Manhães, M.C. Fraga, S. Claudio-Neto, Y. Abreu-Villaça, et al., Concurrent maternal and pup postnatal tobacco smoke exposure in Wistar rats changes food preference and dopaminergic reward system parameters in the adult male offspring, Neuroscience 301 (2015) 178–192, https://doi.org/10.1016/j.neuroscience.2015.06.001.
[34] N. Peixoto-Silva, E.P. Conceição, J.C. Carvalho, N.S. Lima, J.F. Nogueira-Neto, E. de Oliveira, et al., Does bromocriptine play a role in decreasing oxidative stress for early weaned programmed obesity? Life Sci. 95 (2014) 14–21, https://doi.org/10.1016/j.lfs.2013.12.013.
[35] S.P. Frankenfeld, L.P. de Oliveira, D.L. Ignacio, R.G. Coelho, M.N. Mattos, A.C. Ferreira, et al., Nandrolone decanoate inhibits gluconeogenesis and decreases fasting glucose in Wistar male rats, J. Endocrinol. 220 (2014) 143–153, https://doi.org/10.1530/JOE-13-0259.
[36] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.
[37] J.I. Ruiz, B. Ochoa, Quantification in the subnanomolar range of phospholipids and neutral lipids by monodimensional thin-layer chromatography and image analysis, J. Lipid Res. 38 (1997) 1482–1490.
[38] S. Eladak, T. Grisin, D. Moison, M.J. Guerquin, T. N'Tumba-Byn, S. Pozzi-Gaudin, et al., A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound, Fertil. Steril. 103 (2015) 11–21, https://doi.org/10.1016/j.fertnstert.2014.11.005.
[39] P.N.H. Wassenaar, L. Trasande, J. Legler, Systematic review and meta-analysis of early-life exposure to bisphenol A and obesity-related outcomes in rodents, Environ. Health Perspect. 125 (2017) 106001, https://doi.org/10.1289/EHP1233.
[40] G.A. Altamirano, M. Munoz-de-Toro, E.H. Luque, A.L. Gomez, M.B. Delconte, L. Kass, Milk lipid composition is modified by perinatal exposure to bisphenol A, Mol. Cell. Endocrinol. 411 (2015) 258–267, https://doi.org/10.1016/j.mce.2015.05.007.
[41] A.P. Santos-Silva, E.G. de Moura, C.R. Pinheiro, E. O, P.C. Lisboa, Short-term and long-term effects of bisphenol A (BPA) exposure during breastfeeding on the biochemical and endocrine profiles in rats, Horm. Metab. Res. 50 (2018) 491–503, https://doi.org/10.1055/a-0628-6708.
[42] H. Mackay, Z.R. Patterson, R. Khazall, S. Patel, D. Tsirlin, A. Abizaid, Organizational effects of perinatal exposure to bisphenol-A and diethylstilbestrol on arcuate nucleus circuitry controlling food intake and energy expenditure in male and female CD-1 mice, Endocrinology 154 (2013) 1465–1475, https://doi.org/10.1210/en.2012-2044.
[43] S. Legeay, S. Faure, Is bisphenol A an environmental obesogen? Fundam. Clin. Pharmacol. 31 (2017) 594–609, https://doi.org/10.1111/fcp.12300.
[44] B.C. Ryan, A.K. Hotchkiss, K.M. Crofton, L.E.J. Gray, In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats, Toxicol. Sci. 114 (2010) 133–148, https://doi.org/10.1093/toxsci/kfp266.
[45] S.A. Williams, E. Jasarevic, G.M. Vandas, D.A. Warzak, D.C. Geary, M.R. Ellersieck, et al., Effects of developmental bisphenol A exposure on reproductive-related behaviors in California mice (*Peromyscus californicus*): a monogamous animal model, PLoS One 8 (2013) 55698, https://doi.org/10.1371/journal.pone.0055698.
[46] K.K. Ferguson, K.E. Peterson, J.M. Lee, A. Mercado-García, C. Blank-Goldenberg, M.M. Téllez-Rojo, et al., Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys, Reprod. Toxicol. 47 (2014) 70–76, https://doi.org/10.1016/j.reprotox.2014.06.002.
[47] O.S. Anderson, K.E. Peterson, B.N. Sanchez, Z. Zhang, P. Mancuso, D.C. Dolinoy, Perinatal bisphenol A exposure promotes hyperactivity, lean body composition, and hormonal responses across the murine life course, FASEB J. 27 (2013) 1784–1792, https://doi.org/10.1096/fj.12-223545.
[48] A. Marmugi, S. Ducheix, F. Lasserre, A. Polizzi, A. Paris, N. Priymenko, et al., Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver, Hepatology 55 (2012) 395–407, https://doi.org/10.1002/hep.24685.
[49] L. Gao, H.N. Wang, L. Zhang, F.Y. Peng, Y. Jia, W. Wei, et al., Effect of perinatal bisphenol A exposure on serum lipids and lipid enzymes in offspring rats of different sex, Biomed. Environ. Sci. 29 (2016) 686–689, https://doi.org/10.3967/bes2016.092.
[50] J.G. Boucher, S. Ahmed, E. Atlas, Bisphenol S induces adipogenesis in primary human preadipocytes from female donors, Endocrinology 157 (2016) 1397–1407, https://doi.org/10.1210/en.2015-1872.
[51] J. Wei, Y. Lin, Y. Li, C. Ying, J. Chen, L. Song, et al., Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet, Endocrinology 152 (2011) 3049–3061, https://doi.org/10.1210/en.2011-0045.
[52] S. Murray, A. Tulloch, M.S. Gold, N.M. A, Hormonal and neural mechanisms of food reward, eating behaviour and obesity, Nat. Rev. Endocrinol. 10 (2014) 540–552, https://doi.org/10.1038/nrendo.2014.91.
[53] H. Inadera, Neurological effects of bisphenol A and its analogues, Int. J. Med. Sci. 12 (2015) 926–936, https://doi.org/10.7150/ijms.13267.
[54] Y.C. Cheng, H.W. Chen, W.L. Chen, C.Y. Chen, G.S. Wang, Occurrence of nonylphenol and bisphenol A in household water pipes made of different materials, Environ. Monit. Assess. 188 (2015) 562, https://doi.org/10.1007/s10661-016-5556-0.
[55] R. Zhou, F. Chen, X. Feng, L. Zhou, Y. Li, L. Chen, Perinatal exposure to low-dose of bisphenol A causes anxiety-like alteration in adrenal axis regulation and behaviors of rat offspring: a potential role for metabotropic glutamate 2/3 receptors, J. Psychiatr. Res. 64 (2015) 121–129, https://doi.org/10.1016/j.jpsychires.2015.02.018.
[56] G. Mileva, S.L. Baker, A.T. Konkle, C. Bielajew, Bisphenol-A: epigenetic reprogramming and effects on reproduction and behavior, Int. J. Environ. Res. Public Health 11 (2014) 7537–7561, https://doi.org/10.3390/ijerph110707537.
[57] V. Mustieles, R. Perez-Lobato, N. Olea, M.F. Fernandez, Bisphenol A: human exposure and neurobehavior, Neurotoxicology 49 (2015) 174–184, https://doi.org/10.1016/j.neuro.2015.06.002.
[58] D. Nesan, L.C. Sewell, D.M. Kurrasch, Opening the black box of endocrine disruption of brain development: lessons from the characterization of Bisphenol A, Horm. Behav. 101 (2018) 50–58, https://doi.org/10.1016/j.yhbeh.2017.12.001.
[59] J.T. Wolstenholme, J.A. Taylor, S.R. Shetty, M. Edwards, J.J. Connelly, E.F. Rissman, Gestational exposure to low dose bisphenol A alters social behavior in juvenile mice, PLoS One 6 (2011) e25448, https://doi.org/10.1371/journal.pone.0025448.
[60] T. Fujimoto, K. Kubo, S. Aou, Prenatal exposure to bisphenol A impairs sexual differentiation of exploratory behavior and increases depression-like behavior in rats, Brain Res. 1068 (2006) 49–55, https://doi.org/10.1016/j.brainres.2005.11.028.
[61] E. Jasarevic, P.T. Sieli, E.E. Twellman, T.H. Welsh Jr., T.R. Schachtman, R.M. Roberts, et al., Disruption of adult expression of sexually selected traits by developmental exposure to bisphenol A, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 11715–11720, https://doi.org/10.1073/pnas.1107958108.
[62] X. Xu, X. Hong, L. Xie, T. Li, Y. Yang, Q. Zhang, et al., Gestational and lactational exposure to bisphenol-A affects anxiety- and depression-like behaviors in mice, Horm. Behav. 62 (2012) 480–490.
[63] F. Xin, E. Fischer, C. Krapp, E.N. Krizman, Y. Lan, C. Mesaros, et al., Mice exposed to

bisphenol A exhibit depressive-like behavior with neurotransmitter and neuroactive steroid dysfunction, Horm. Behav. 102 (2018) 93–104, https://doi.org/10.1016/j.yhbeh.2018.05.010.
[64] X.H. Xu, Y.M. Wang, J. Zhang, Q.Q. Luo, Y.P. Ye, Q. Ruan, Perinatal exposure to bisphenol-A changes N-methyl-D-aspartate receptor expression in the hippocampus of male rat offspring, Environ. Toxicol. Chem. 29 (2010) 176–181, https://doi.org/10.1002/etc.18.
[65] M. Kundakovic, K. Gudsnuk, B. Franks, J. Madrid, R.L. Miller, F.P. Perera, et al., Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 9956–9961, https://doi.org/10.1073/pnas.1214056110.
[66] W. Adriani, D.D. Seta, F. Dessì-Fulgheri, F. Farabollini, G. Laviola, Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A, Environ. Health Perspect. 111 (2003) 395–401.
[67] G. Ribeiro, O. Santos, Recompensa alimentar: mecanismos envolvidos e implicações para a obesidade, Revista Portuguesa de Endocrinologia, Diabetes e Metabolismo 8 (2013) 82–88.
[68] M. Narita, K. Miyagawa, K. M, T. Yoshida, T. Suzuki, Changes in central dopaminergic systems and morphine reward by prenatal and neonatal exposure to bisphenol-A in mice: evidence for the importance of exposure period, Addict. Biol. 12 (2007) 167–172, https://doi.org/10.1111/j.1369-1600.2007.00048.x.
[69] R. Rezg, S. El-Fazaa, N. Gharbi, B. Mornagui, Bisphenol A and human chronic diseases: current evidences, possible mechanisms, and future perspectives, Environ. Int. 64 (2014) 83–90, https://doi.org/10.1016/j.envint.2013.12.007.
[70] P. Tarapore, M. Hennessy, D. Song, J. Ying, B. Ouyang, V. Govindarajah, et al., High butter-fat diet and bisphenol A additively impair male rat spermatogenesis, Reprod. Toxicol. 68 (2017) 191–199, https://doi.org/10.1016/j.reprotox.2016.09.008.
[71] B.T. Akingbemi, C.M. Sottas, A. Koulova, G.R. Klinefelter, M.P. Hardy, Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells, Endocrinology 145 (2004) 592–603, https://doi.org/10.1210/en.2003-1174.
[72] S. Ma, W. Shi, X. Wang, P. Song, X. Zhong, Bisphenol A exposure during pregnancy alters the mortality and levels of reproductive hormones and genes in offspring mice, Biomed. Res. Int. (2017), https://doi.org/10.1155/2017/3585809.
[73] C. Santamaría, M. Durando, M. Muñoz de Toro, E.H. Luque, H.A. Rodriguez, Ovarian dysfunctions in adult female rat offspring born to mothers perinatally exposed to low doses of bisphenol A, J. Steroid Biochem. Mol. Biol. 158 (2016) 220–230, https://doi.org/10.1016/j.jsbmb.2015.11.016.
[74] C. Viguié, S.H. Collet, V. Gayrard, N. Picard-Hagen, S. Puel, B.B. Roques, et al., Maternal and fetal exposure to bisphenol a is associated with alterations of thyroid function in pregnant ewes and their newborn lambs, Endocrinology 154 (2013) 521–528, https://doi.org/10.1210/en.2012-1401.
[75] D. Guignard, V. Gayrard, M.Z. Lacroix, S. Puel, N. Picard-Hagen, C. Viguié, Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level representative of human exposure, Chemosphere 182 (2017) 458–467, https://doi.org/10.1016/j.chemosphere.2017.05.028.
[76] R.G. Ahmed, G.H. Walaa, F.S. Asmaa, Suppressive effects of neonatal bisphenol A on the neuroendocrine system, Toxicol. Ind. Health 34 (2018) 397–407, https://doi.org/10.1177/0748233718757082.
[77] M.O. Fernandez, N.S. Bourguignon, P. Arocena, M. Rosa, C. Libertun, V. Lux-Lantos, Neonatal exposure to bisphenol A alters the hypothalamic-pituitary-thyroid axis in female rats, Toxicol. Lett. 285 (2018) 81–86, https://doi.org/10.1016/j.toxlet.2017.12.029.
[78] P. Salazar, P. Cisternas, M. Martinez, N.C. Inestrosa, Hypothyroidism and cognitive disorders during development and adulthood: implications in the central nervous system, Mol. Neurobiol. (2018) 1–12, https://doi.org/10.1007/s12035-018-1270-y.
[79] E. Panagiotidou, S. Zerva, D.J. Mitsiou, M.N. Alexis, E. Kitraki, Perinatal exposure to low-dose bisphenol A affects the neuroendocrine stress response in rats, J. Endocrinol. 220 (2014) 207–218, https://doi.org/10.1530/JOE-13-0416.
[80] L.E. Johns, K.K. Ferguson, D.E. Cantonwine, T.F. M, B. Mukherjee, J.D. Meeker, Urinary BPA and phthalate metabolite concentrations and plasma vitamin D levels in pregnant women: a repeated measures analysis, Environ. Health Perspect. 125 (2017) 1–9.
[81] A. Usman, M. Ahmad, From BPA to its analogues: is it a safe journey? Chemosphere 158 (2016) 131–142, https://doi.org/10.1016/j.chemosphere.2016.05.070.
[82] D.M. Arble, J. Bass, A.D. Laposky, M.H. Vitaterna, F.W. Turek, Circadian timing of food intake contributes to weight gain, Obesity (Silver Spring) 11 (2009) 2100–2102, https://doi.org/10.1038/oby.2009.264.
[83] R. Orozco-Sólis, S. Lopes de Souza, R.J. Barbosa Matos, I. Grit, J. Le Bloch, P. Nguyen, et al., Perinatal undernutrition-induced obesity is independent of the developmental programming of feeding, Physiol. Behav. 96 (2009) 481–492, https://doi.org/10.1016/j.physbeh.2008.11.016.
[84] L.S. Oliveira, L.P. Silva, A.L. Silva, C.P. Magalhães, S.L. Souza, RM. Castro, Effects of early weaning on the circadian rhythm and behavioral satiety sequence in rats, Behav. Process. 86 (2011) 119–124, https://doi.org/10.1016/j.beproc.2010.10.001.
[85] P.C. Lisboa, P.N. Soares, T.C. Peixoto, J.C. Carvalho, C. Calvino, V.S. Tavares, et al., Effects of cigarette smoke exposure during suckling on food intake, fat mass, hormones, and biochemical profile of young and adult female rats, Endocrine 57 (2017) 60–71, https://doi.org/10.1007/s12020-017-1320-7.
[86] M. Lutter, EJ. Nestler, Homeostatic and Hedonic Signals Interact in the Regulation of Food Intake1–3, The Jornal of Nutrition 139 (2009) 629–632, https://doi.org/10.3945/jn.108.097618.
[87] E. Egecioglu, K.P. Skibicka, C. Hansson, M. Alvarez-Crespo, A.P. Friberg, E. Jerlhag, et al., Hedonic and incentive signals for body weight control, Rev. Endocr. Metab. Disord. 12 (2011) 141–151, https://doi.org/10.1007/s11154-011-9166-4.