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Effects of prenatal bisphenol-A exposure and postnatal overfeeding on cardiovascular function in female sheep

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Abstract

Bisphenol-A (BPA) is a widely used endocrine-disrupting chemical (EDC). Prenatal exposure to BPA is known to affect birth weight, but its impact on the cardiovascular system has not been studied in detail. In this study, we investigated the effects of prenatal BPA treatment and its interaction with postnatal overfeeding on the cardiovascular system. Pregnant sheep were given daily s.c. injections of corn oil (control) or BPA (0.5 mg/kg/day in corn oil) from day 30 to 90 of gestation. A subset of female offspring of these dams were overfed (OF group) to increase bodyweight to ~30% over that of normal fed (NF group) controls. Cardiovascular function was assessed using non-invasive echocardiography and cuff blood pressure monitoring at 21 months of age. Ventricular tissue was analyzed for gene expression of cardiac markers of hypertrophy and collagen at the end of the observation period. Prenatal BPA exposure had no significant effects on blood pressure (BP) or morphometric measures. However, it increased ANP gene expression in the ventricles, and reduced collagen expression in the right ventricle. Overfeeding produced a marked increase in body weight and BP. There were compensatory increases in left ventricular area and internal diameter. Prenatal BPA treatment produced a significant increase in interventricular septal thickness when animals were overfed. However, it appeared to block the increase in BP and left ventricular area caused by overfeeding. Taken together these results suggest that prenatal BPA produces intrinsic changes in the heart that are capable of modulating morphological and functional parameters when animals become obese in later life.

Keywords

Endocrine disruptors; cardio-metabolic programming; blood pressure; echocardiography; cardiac gene expression

INTRODUCTION

Bisphenol-A (BPA) is a weak estrogen that is present as a ubiquitous contaminant in our environment (1). It is used in the manufacture of a variety of plastic products and can leach from plastics when they are subjected to heat, or when plastics are degraded (2) (3). It has also been found in air, dust and water sources (4). Pregnant women are likely to be exposed to BPA through dermal contact (5), consumption of canned beverages and foods, and inhalation (6). As a result, BPA is present in maternal circulation (7), and is known to cross the placenta to reach the fetus and impact fetal growth (8). It is also excreted through breast milk (9). Therefore, exposure to BPA can occur *in utero* and during early postnatal life (10), when organ systems are differentiating. Prenatal BPA exposure is known to affect a number of organs however, its effects on the heart have not been studied in detail.

Most studies examining the effects of BPA on the heart or cardiac myocytes have been in rodents. These studies have found that BPA exposure induces arrhythmogenicity in ventricular myocytes most probably due to altered calcium mobility across the sarcoplasmic reticulum (11). In vitro studies have shown that acute BPA treatment can affect cardiac impulse propagation and increase the risk for complete heart block (12). Moreover, BPA treatment sustains ventricular arrhythmias in female rats that are subjected to ischemia and reperfusion and there appears to be a synergistic effect between BPA and estradiol-17 β in inducing arrhythmias (13). This effect is probably mediated through estrogen receptors (13, 14). In another study involving male mice, BPA exposure following ischemia induced inflammatory changes and reduced the ability of the heart to undergo remodeling (15). Although these studies indicate that BPA can affect the heart, we are not clear if prenatal exposure to BPA can affect the heart as well.

In the present study, we used a sheep model that has a similar developmental trajectory as humans (16). Also, cardiac development in the ovine fetus parallels that of the human fetus (17). Sheep are also good models for studying cardiovascular changes after intrauterine compromise (18). Ovine heart subjected to intrauterine hypoxia has higher levels of inflammatory markers, collagen deposition and matrix metalloproteinases (18) very similar to what is seen with BPA exposure after ischemia (15). Therefore, we hypothesized that prenatal BPA exposure in sheep might compromise cardiac function in the offspring resulting in altered remodeling parameters. More recent evidence suggests that outcomes resulting from early developmental insults can be exacerbated by the postnatal environment (19). We have previously demonstrated that prenatal BPA exposure followed by overfeeding of the offspring in adulthood can affect insulin sensitivity and increase adipose tissue deposition in sheep (20). We hypothesized that overfeeding in adulthood can further impair cardiac function and that this effect would be accompanied by molecular changes that reflect hypertrophy and reduced compliance.

MATERIALS AND METHODS

Animals and treatment

Mature Suffolk ewes 2–3 years of age were purchased from local farmers and maintained at the Sheep Research Facility, University of Michigan, Ann Arbor, MI. They were maintained under natural photoperiod and fed 0.5 kg of shelled corn and 1.5–2 kg of alfalfa hay/animal/day. Animals were bred during the breeding season using protocols approved by the Animal care and use committee, University of Michigan. Experiments were performed in accordance with the Guide for the care and use of agricultural animals in research and teaching. The experimental protocol for this experiment was recently published (20). Briefly, pregnant ewes were given daily subcutaneous injections of corn oil (control) or BPA (Sigma, St. Louis, MO; 0.5 mg/kg/day in corn oil) from day 30 to 90 of gestation. This window was chosen based on a previous study where pregnant sheep were exposed to excess testosterone, an estrogen precursor, and this caused hypertension in the female offspring (21). The circulating concentrations of BPA achieved with this dose in the umbilical artery averages 2.62 ± 0.52 ng/ml at gestation day 90 (21) and is close to that observed in the maternal circulation of US women (7). Only female offspring of these animals were used in this study making sure that only one female offspring from each dam was used in the experiment. When the lambs were 14 weeks old, a subset of female offspring of these dams were fed *ad libitum* (overfed group-OF group). Diet for the overfed group included additional corn and followed a previously published overfeeding regimen from 14 weeks of age to the end of the experiment (22). This resulted in animals increasing their bodyweight (BW) to ~30% over that of controls. The remaining animals were fed a normal diet as described above (Normal fed-NF group). This resulted in four treatment groups, control–NF (n=6), Control-OF (n=7), BPA–NF (n=7) and BPA-OF (n=7).

Assessing cardiovascular function

Cardiovascular function of adult females was assessed using non-invasive echocardiography at 21 months of age. Blood pressure was measured using a cuff placed on the thigh and a digital BP monitor with the animal in standing position. Animals were restrained manually and a small (3 X 3”) area of skin was clipped and cleaned with alcohol on the thoracic wall on either side, behind the elbow region. Ultrasound gel was applied over the clipped area. The forelimbs were pulled forward to visualize the heart on the echocardiogram. A 5 mHz ultrasonic probe was used in conjunction with the echo device (Vivid-I, GE Healthcare, Little Chalfont, UK) and the EchoPac software. The probe was placed on the 4 or 5th intercostal space with the animal in standing position to examine the heart. Besides two-dimensional echocardiography, Doppler studies (color Doppler and spectral Doppler) were also performed to assess valve function, blood flow velocity and direction. An electrocardiogram (ECG) was connected simultaneously with the leads placed in all the four limbs to measure electrical changes within the heart. The parameters measured included systolic, diastolic and mean blood pressure, aortic diameter and circumference, pulmonary arterial diameter, left ventricular posterior wall thickness, internal diameter and area, left atrial diameter, interventricular septal thickness, cardiac output, ejection fraction, stroke volume, fractional shortening, end systolic and end diastolic volume, ejection fraction and heart rate.

Organ weights

Animals were sacrificed at 22 months of age using Fatal plus (Vortech Pharmaceuticals, Dearborn, MI). We collected various organs including the heart, lung, liver, adrenals, kidneys and spleen and obtained their wet weights. Heart was dissected and flash frozen in a dry ice bath and stored at -80°C until further processing.

Quantitative RT-PCR for Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), collagen-1 (COL1) and collagen-3a1 (COL3A1)

RNA from ventricular tissue was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified with deoxyribonuclease treatment. To assess the quality of extracted RNA, a Nanodrop Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used. Samples with low quality RNA (assessed by OD 260/280 ratio) were excluded from further analysis. First strand cDNA was synthesized from RNA using Superscript III first strand synthesis system (Invitrogen Cat. No: 18080-051). cDNA was prepared from 1 μg total RNA. Concentrations of cDNA over 1000 fold range was amplified using each target gene and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) specific primers to determine the final concentration at which the each primer sequence for both housekeeping gene and gene of interest were equally and $>90\%$ efficient. The primer sequences were obtained from Aitken et al., 1999 (23) for ANP and BNP and primers for COL1 and COL3A1 were designed in our lab using BLAST (Table 1).

The Sybr Green real time PCR assay was performed using the cDNA generated. A negative reverse transcriptase control was run for each sample to rule out genomic DNA contamination of isolated RNA. Quantitative RT PCR reactions were run on a 7200 Real Time PCR instrument (Applied Biosystems, Foster City, CA), and were performed in triplicates. Power Sybr Green (Thermo Fisher Scientific, Waltham, MA) was used to detect the sheep specific primer sequences of genes. The efficiency of the primers used was determined by generating a standard curve for each. Melting curves were performed to validate the outcomes of the PCR product. Results of the assay were quantified using the cycle threshold (CT) values of each sample. The CT values of each gene of interest were compared against GAPDH. Only values less than 35 were considered positive. The fold change was calculated by the $2^{-\text{CT}}$ method (24).

Statistical analysis

All parameters were analyzed by two-way ANOVA followed by Tukey-Kramer posthoc test. All data were expressed as mean $\pm$ SEM. $P < 0.05$ was considered to be significant.

RESULTS

Body Weight

Birth weights (kg; Mean $\pm$ S.E.) of female lambs from control-NF (5.47 ± 0.23), BPA-NF (4.65 ± 0.48), control-OF (5.55 ± 0.29) and BPA-OF groups (5.06 ± 0.29) were not different from each other. Body weight (kg; Mean $\pm$ S.E.) of control-NF and BPA-NF groups at the end of the study were 78.5 ± 1.5 and 81.2 ± 3.5 , respectively and did not differ significantly (Fig. 1A). There was a significant diet effect with overfeeding producing a marked increase in

body weight both in control (108.5 ± 2.6) and prenatal BPA-treated sheep (103.5 ± 1.9 ; $p < 0.0001$).

Organ weight

Although there were significant, albeit modest, effects on heart weight with overfeeding (Fig 1B), there were no changes when heart weight was normalized to body weight (Fig 1C). There was a significant reduction in kidney weight to body weight ratio with prenatal BPA exposure. Although postnatal overfeeding decreased this ratio, it could not completely reverse the effect of prenatal BPA exposure. Prenatal BPA exposure also reduced lung weight, but these changes were masked by the effects of overfeeding (Table 4).

Functional parameters: Heart rate and blood pressure

Neither prenatal BPA treatment nor postnatal overfeeding had an effect on the heart rate (Fig 2A). However, a significant diet ($F=7.79$) and diet $\times$ BPA treatment interaction ($F=8.056$) was evident relative to systolic blood pressure (SBP) (Fig 2B). Posthoc analyses found SBP (mmHg, Mean $\pm$ S.E.) increased significantly in the control-OF group (144.8 ± 11.1) relative to control-NF (93.8 ± 9.6 ; $p < 0.05$) but not in the BPA-OF group.

A modest overall BPA effect ($F=4.533$), pronounced overfeeding effect ($F=10.78$), and a significant diet $\times$ BPA interaction ($F=14.55$) was evident relative to diastolic blood pressure (DBP) (Fig 2C). Posthoc analyses found DBP (mmHg; Mean $\pm$ S.E.) was significantly elevated in the control-OF group (97.8 ± 7.9) compared to the control-NF (53.6 ± 4.2), and BPA-NF groups (63.8 ± 2.9). Interestingly, DBP in the BPA-OF (60.8 ± 7.9) group did not differ from control or BPA-NF groups. Relative to mean blood pressure (MBP), ANOVA showed a significant overfeeding ($F=10.034$; $p=0.004$) and overfeeding $\times$ BPA interaction ($F=8.584$; $p=0.0078$) (Fig 2D). Post hoc analyses found overfeeding increased MBP (mmHg) in the control-OF (120.8 ± 5.7) compared to control-NF (72.5 ± 7.8) and BPA-NF (87.2 ± 8.7) groups. The obesity-related increase in MBP seen in control-OF group was not evident in the BPA-OF group (88.8 ± 10.3).

Structural changes associated with hypertension

A significant effect of overfeeding and not prenatal BPA was evident in left atrial diameter (LAD), aortic diameter (AoD), aortic area and aortic circumference (Table 2). A significant diet $\times$ BPA treatment interaction was also evident in left ventricular internal diameter during systole (LVID systole).

Other functional parameters

Effects of prenatal BPA and overfeeding were not evident in functional parameters such as end diastolic volume, end systolic volume, cardiac output, fractional shortening, ejection fraction, and stroke volume (Table 3).

Morphometric measurements: Left ventricular changes—ANOVA revealed a marked diet $\times$ prenatal treatment interaction ($F=10.734$) in left ventricular area (LVA) (cm^2 ; Mean $\pm$ S.E.) during diastole (Fig 3A). Post hoc analyses found LVA in diastole to be significantly higher in the control-OF group compared with the control- NF group.

Interestingly, prenatal exposure to BPA blocked this overfeeding-induced increase. During systole, a significant overfeeding $\times$ BPA interaction ($F=11.853$; $p=0.0022$) (Fig 3B) was evident in LVA. Post hoc analyses found that the BPA-NF group had significantly increased LVA compared to the BPA-OF group, which was similar to the LVA in the control group.

A significant diet effect was apparent in interventricular septal thickness (IVS) in both diastole and systole (Fig 3 C and D). However post-hoc analyses revealed significant group differences only during systole with the BPA-OF group being thicker than the BPA-NF group (Fig 3D). Except for modest interaction effects in LVID during systole, there were no effects in LVID during diastole. There were no changes in left ventricular posterior wall thickness (LVPW) with BPA treatment or overfeeding (Table 2).

Molecular changes—Impact of prenatal BPA on ventricular ANP and BNP expression (fold change relative to control; Mean $\pm$ S.E.) are shown in Fig 4. ANP expression was significantly elevated in the BPA-NF group compared to control-NF in the left ventricle (Fig 4A). ANP expression was also elevated in the right ventricle of BPA-NF group compared to the control-NF and control-OF groups (Fig 4B). There were no significant changes in BNP expression in both right and left ventricular tissue (Fig 4 C and D). In the left ventricle, there was modest increase in COL1A1 expression ($p=0.05$), and COL3A1 expression with BPA treatment ($p=0.0737$; Fig 5 A and C). However, a marked reduction in COL1A1 and COL3A1 expression were apparent in the right ventricle with BPA treatment ($p<0.01$; Fig 5 B and D).

DISCUSSION

Results from this study involving a large animal model with developmental trajectory similar to human demonstrate that overfeeding leads to marked increases in systolic, diastolic and mean blood pressure. Paradoxically, while prenatal BPA had little effect on the cardiovascular function in the maintenance-fed animals, overfeeding as adults did not increase their diastolic and mean blood pressure to the level seen in the control-OF group. This makes it appear as if prenatal BPA exposure may in fact be beneficial in preventing overfeeding-induced increases in blood pressure. On the other hand, it could merely indicate that there were no additive effects on blood pressure due to overfeeding in BPA-exposed sheep. It could also indicate that prenatal exposure to BPA induces changes in cardiac structure and function that makes the heart less resilient to the effects of overfeeding.

Impact of prenatal BPA

Prenatal BPA exposure by itself produced significant increases in ANP gene expression in both ventricles compared to the control group. It did not affect other cardiac structural and functional parameters significantly. ANP and BNP are known to increase in response to elevated atrial and ventricular transmural pressure (25) and can be upregulated in heart failure and myocardial infarction (26), (27), (28). ANP and BNP are also markers of ventricular hypertrophy (29). The significant increase in ANP in left ventricles of prenatal BPA treated, offspring compared to control animals could be suggestive of adverse left ventricular programming with BPA but further studies are needed to characterize this. Available evidence suggests that ANP is produced by cardiac myocytes in response to

increased blood pressure (30) and could play a role in preventing cardiac hypertrophy (31). ANP expression in the ventricles of control animals were comparable to a previously published study by Cameron et al (28). The finding that increased ANP expression was also seen in the right ventricle of BPA treated animals suggests the possibility that prenatal BPA treatment adversely programs pulmonary structure and function as reported in rodent and human studies (32, 33), an aspect not investigated in this study.

Besides affecting ANP expression in the ventricles, prenatal BPA exposure also affected collagen expression in these tissues. Collagen I and III are fibrillar collagens that are present in the heart. Fibrillar collagen is an important contributing factor to ventricular compliance during diastole and affects systolic function by modulating myocyte shortening (34). Intrauterine hypoxia is known to increase COL1 and COL3 expression in the ventricles of ovine fetuses and this is believed to compromise systolic and diastolic function (18). In the present study, although COL1A1 and COL3A1 expression were increased modestly in the left ventricle, their expression was reduced in the right ventricle with prenatal BPA exposure. These changes could suggest compensatory mechanisms, nevertheless, could be indicators of altered ventricular compliance and need further investigation.

Impact of postnatal overfeeding

It is well known that excess food intake and high fat diet are detrimental to cardiovascular function due to the associated hemodynamic overload (35). In this model, there is insulin resistance, and increased adipose tissue deposition as a result of overfeeding. There are also marked changes in organ weights especially the kidneys and lungs (Table 4) (20). The marked increase in diastolic blood pressure and the parallel increase in MBP in the control-OF group could be a function of the weight gain in these animals (Fig 1). These increases resulted in the expected compensatory changes in left atrial diameter (Table 1) that matches what is observed in obese patients (36). Moreover, there are marked increases in left ventricular area (LVA) during diastole (Fig 3A) but not in left ventricular internal diameter (LVID; Table 1) in the Control-OF group that suggest ventricular remodeling as observed in obese people (37). The hemodynamic load due to obesity is likely to increase both ventricular preload and afterload that could have pronounced effects on blood pressure (38). Moreover, peripheral resistance and stiffness of larger blood vessels that are frequently observed in obese individuals are believed to contribute to the increase in blood pressure as well (39). Further, obese patients generally have increased heart rate, higher stroke volume and cardiac output (40); parameters that did not change significantly in our model with obesity. The combination of obesity and hypertension leads to a variety of compensatory changes in the circulatory system. The modest increases in aortic area and circumference that were observed in the OF groups in this study are likely to be compensatory changes induced by obesity and associated hypertension.

Interaction between prenatal BPA and postnatal overfeeding

Contrary to our expectation that postnatal obesity would exaggerate the detrimental effects of prenatal BPA, prenatal BPA treatment prevented the increase in diastolic, systolic, and mean blood pressure caused by overfeeding. Moreover, it decreased left ventricular area in diastole and reduced COL expression in the right ventricle. It is likely that the molecular

effects seen with prenatal BPA exposure could have induced structural alterations that interfere with ventricular compliance. Further studies are needed to investigate changes in cardiac myocytes at the ultrastructural and molecular level.

Relevance of BPA dose used in the study

The BPA dose used in this study produces circulating levels of 2.62 ± 0.52 ng/ml in the umbilical artery on day 90 of gestation (21). These levels are comparable what is seen in pregnant women both at delivery and during the first trimester (41). In addition, at the time of study, the body weights of control-NF and BPA-NF were similar as were the control-OF and BPA-OF thus eliminating differences in adiposity between groups contributing to the differences.

Conclusions

Taken together, the findings from this study indicate that prenatal BPA exposure alone while having no effects on cardiac structure may be inducing changes at the molecular level that could reduce compliance and prevent compensatory responses to postnatal overfeeding. Alternatively, the findings may suggest that BPA pre-treatment alters the trajectory of cardiac structure/function differentially from overfeeding alone that may be adaptive vs maladaptive. This needs to be further investigated at the molecular level.

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Figure 1.

Panel A: Body weight in sheep from different treatment groups. Control+NF and BPA+NF groups were subjected to prenatal exposure to vehicle (Control) or BPA and placed on a normal diet. Control+OF and BPA+OF groups were subjected to prenatal exposure as above and overfed into adulthood. NF groups demonstrate the effect of prenatal exposures alone, and the OF groups demonstrate the effects of prenatal exposure+postnatal overfeeding. Panel B: Heart weight in sheep from the different treatment groups. Panel C: Heart weight to body weight ratio in the different treatment groups. “a” and “b” indicate significant differences from each other. ($p<0.05$).

Figure 2.

Panel A: Effects of prenatal vehicle or BPA exposure in combination with postnatal normal feeding or overfeeding on heart rate. Panels B, C, and D: Effects on systolic, diastolic and mean blood pressure. Blood pressure was measured using the tail cuff method. Notation differences indicate significant differences between groups ($p<0.05$). Bars with “a,b” indicate that they are not different from bars with “a” and “b”.

Figure 3.

Effect of prenatal vehicle or BPA exposure in combination with postnatal normal feeding or overfeeding on left ventricular area during diastole (Panel A) and during systole (Panel B), and in interventricular septal thickness in diastole (Panel C) and systole (Panel D). Notation differences indicate significant differences between groups ($p<0.05$). Bars with “a,b” indicate that they are not different from bars with “a” and “b”.

Figure 4.

Effect of prenatal vehicle or BPA exposure in combination with postnatal normal feeding or overfeeding on ANP gene expression on the left and right ventricles (Panels A and B respectively) and on BNP gene expression on the left and right ventricles (Panels C and D respectively). Notation differences indicate significant differences between groups ($p < 0.05$). Bars with “a,b” indicate that they are not different from bars with “a” and “b”. Graphs that have no notations indicate that there are no significant differences between groups.

Figure 5.

Effect of prenatal vehicle or BPA exposure in combination with postnatal normal feeding or overfeeding on COL1A1 gene expression on the left and right ventricles (Panels A and B respectively) and on COL3A1 gene expression on the left and right ventricles (Panels C and D respectively). Notation differences indicate significant differences between groups ($p < 0.05$).

Table 1

Primer sequences used for qPCR analysis of ANP, BNP, COL1A1 and COL3A1.

S. No.	Primer name	Primer sequence	Accession number
1	Sheep ANP Fwd	5' ACG ACG CCA GCA TGA GCT CCT TC 3'	Ovine ANP AF037465
2	Sheep ANP Rev	5' GCT GTT ATC TTC AGT ACC GGA A 3'	
3	Sheep BNP Fwd	5' TCC AGC CAC ATG GGC CCC CGG A 3'	Ovine BNP AF037466
4	Sheep BNP Rev	5' CCT GAG CAC ATT GCA GCC CAG GC 3'	
5	Sheep COL1A1 Fwd	5' TCC GTG CCT GGT CCC ATG GGT CC 3'	Ovis aries Collagen1A1 GAAI01000511
6	Sheep COL1A1 Rev	5' GGA CCA CGG GGA CCC ATG GGA CC 3'	
7	Sheep COL3A1 Fwd	5' GGA CCT CAA GGC CCC AAG GGA GAT C 3'	Ovis aries Collagen Type III, Alpha 1 XM_004004514
8	Sheep COL3A1 Rev	5' GGC CCA GGA TAG CCT GCG AGT CC 3'	

Effect of prenatal vehicle or BPA exposure in combination with postnatal normal feeding or overfeeding on morphometric measurements of the heart that are associated with hypertension.

Table 2

Parameter	Control-NF		BPA-NF		Control-OF		BPA-OF		Prenatal treatment effect	Diet effect	Prenatal Treatment $\times$ Diet interaction
	Original value	Normalized to BSA	Original value	Normalized to BSA	Original value	Normalized to BSA	Original value	Normalized to BSA			
LA diameter	10.05 $\pm$ 0.38 mm	2.716 $\pm$ 0.07	10.18 $\pm$ 0.35 mm	2.746 $\pm$ 0.13	10.52 $\pm$ 0.15 mm	2.46 $\pm$ 0.1 b	10.62 $\pm$ 0.16 mm	2.32 $\pm$ 0.056 a,b	NS	F=11.87 P=0.0026	NS
Ao diameter	3.2 $\pm$ 0.12 mm	0.21 $\pm$ 0.005	3.23 $\pm$ 0.1 mm	0.212 $\pm$ 0.01	3.36 $\pm$ 0.05 mm	0.18 $\pm$ 0.005 a,b	3.4 $\pm$ 0.04 mm	0.185 $\pm$ 0.001 a,b	NS	F=22.689 P=0.0001	NS
LA/Ao ratio	41.13 $\pm$ 1	0.08 $\pm$ 0.003	43.06 $\pm$ 0.8	0.08 $\pm$ 0.003	45.5 $\pm$ 1.7	0.074 $\pm$ 0.003 a,b	42.8 $\pm$ 1.29	0.069 $\pm$ 0.002 a,b	NS	F=22.263 P=0.0001	NS
Aortic area	8.1 $\pm$ 0.6 mm ²	0.54 $\pm$ 0.027	8.3 $\pm$ 0.56	0.545 $\pm$ 0.03	8.82 $\pm$ 0.24	0.47 $\pm$ 0.016 a,b	8.97 $\pm$ 0.24	0.489 $\pm$ 0.008	NS	F=5.78 P=0.026	NS
Aortic circumference		0.67 $\pm$ 0.017		0.67 $\pm$ 0.028		0.56 $\pm$ 0.014 a,b		0.58 $\pm$ 0.005 a,b	NS	F=23.386 P=0.0001	NS
AoD at AoS	3.28 $\pm$ 0.14 cm	0.22 $\pm$ 0.006	3.03 $\pm$ 0.11 cm	0.204 $\pm$ 0.01	3.2 $\pm$ 0.07 cm	0.17 $\pm$ 0.007 a,b	3.3 $\pm$ 0.15 cm	0.181 $\pm$ 0.007 a,b	NS	F=23.595 P=0.0001	NS
AoD at AoV	2.78 $\pm$ 0.15 cm	0.19 $\pm$ 0.007	2.63 $\pm$ 0.05 cm	0.17 $\pm$ 0.006	2.66 $\pm$ 0.05 cm	0.144 $\pm$ 0.003 a,b	2.75 $\pm$ 0.09 cm	0.151 $\pm$ 0.003 a,b	NS	F=31.977 P<0.0001	NS
LVPW dias	13.67 $\pm$ 0.97 cm	0.85 $\pm$ 0.06	11.39 $\pm$ 0.73 cm	0.79 $\pm$ 0.027	13.52 $\pm$ 0.36 cm	0.72 $\pm$ 0.024	15.01 $\pm$ 1.48 cm	0.801 $\pm$ 0.051	NS	NS	NS
LVPW sys	20.45 $\pm$ 1.41 cm	1.26 $\pm$ 0.099	18.98 $\pm$ 0.65 cm	1.19 $\pm$ 0.062	18.33 $\pm$ 1.52 cm	0.98 $\pm$ 0.068	23.41 $\pm$ 2.24 cm	1.21 $\pm$ 0.095	NS	NS	NS
IVS dias	11.48 $\pm$ 0.44 mm	0.73 $\pm$ 0.029	9.98 $\pm$ 0.76 mm	0.695 $\pm$ 0.03	12.65 $\pm$ 0.45 mm	0.655 $\pm$ 0.034	13.04 $\pm$ 0.46 mm	0.714 $\pm$ 0.029	NS	NS	NS
IVS sys	15.17 $\pm$ 1.43 mm	0.97 $\pm$ 0.064	13.38 $\pm$ 1.36 mm	0.908 $\pm$ 0.04	17.96 $\pm$ 1.38 mm	0.938 $\pm$ 0.07	18.81 $\pm$ 1.16 mm	1.029 $\pm$ 0.059	NS	NS	NS

AoD= aortic diameter, LVPW =Left ventricular posterior wall thickness, IVS= interventricular septal thickness, LVID=Left ventricular internal diameter.

Groups with different alphabetic notations are different from each other. "a,b" indicates no change from "a" and "b". If there are no notations, it indicates that there are no significant changes between groups. NS indicates no significant differences.

Table 3

Effect of prenatal vehicle or BPA exposure in combination with postnatal normal feeding or overfeeding on functional parameters of the heart. NS; non significant

Parameter	Control-NF	BPA-NF	Control-OF	BPA-OF	Prenatal treatment effect	Diet effect	Prenatal Treatment x Diet interaction
End Diastolic volume	100.8±12.7	123.71±11.3	139.14±13.5	115.7±8.4	NS	NS	NS
End systolic volume	41.4±10.7	56±7.3	61.86±6	44±4.1	NS	NS	NS
Stroke Volume	58.8±10	67.57±4.96	77.143±9.89	72.14±7.32	NS	NS	NS
CO (ml/min)	4193.6±599	4051.6±395	4588.7±551	5069±568	NS	NS	NS
Fractional shortening	32.4±4.4	29.1±1.5	29.14±1.9	33.71±2.3	NS	NS	NS
Ejection fraction	59.6±6.9	55.7±2.45	54.85±2.89	61.57±3.2	NS	NS	NS

Table 4

Changes in organ weight: Body weight ratios with prenatal treatment and postnatal overfeeding. Groups with different alphabetic notations are different from each other.

<i>Parameter (Ratios)</i>	<i>Control-NF</i>	<i>BPA-NF</i>	<i>Control-OF</i>	<i>BPA-OF</i>	<i>Treatment effect</i>	<i>Diet effect</i>	<i>Prenatal Treatment x Diet interaction</i>
<i>Heart weight:BW (X10⁻³)</i>	3.59±0.07	3.18±0.25	3.53±0.09	3.33±0.14	NS	NS	NS
<i>Liver weight:BW (X10⁻³)</i>	7.82±0.32	8.11±0.36	8.11±0.19	7.7±0.16	NS	NS	NS
<i>Lung weight:BW (X10⁻³)</i>	8.85±0.46 a	6.26±0.19 b	7.78±0.34 a,b	7.09±0.79 a,b	Yes p=0.003	NS	NS
<i>Spleen weight:BW (X10⁻³)</i>	7.82±0.32	8.11±0.36	8.11±0.19	7.7±0.16	NS	NS	NS
<i>Right adrenal weight:BW (X10⁻²g/kg)</i>	2.61±0.26 a	2.06±0.09 a,b	2.31±0.19 a,b	1.99±0.06 b	Yes p=0.013	NS	NS
<i>Left adrenal weight:BW (X10⁻² g/kg)</i>	2.69±0.27 a	2.18±0.12 a,b	2.28±0.15 a,b	2.04±0.08 b	Yes p=0.029	NS	NS
<i>Right kidney weight:BW (g/kg)</i>	0.89±0.02 a	0.66±0.02 b	0.88±0.03 c	0.74±0.02 c	Yes p<0.001	NS	NS
<i>Left Kidney weight:BW (g/kg)</i>	0.88±0.02 a	0.65±0.02 b	0.85±0.019 c	0.74±0.01 d	Yes p<0.0001	NS	Yes p=0.0126

“a,b” indicates no change from “a” and “b”. If there are no notations, it indicates that there are no significant changes between groups. NS indicates no significant differences.