Gestational bisphenol A exposure impacts hepatic lipid metabolism in male offspring rats by regulating mTOR/CRTC2/SREBP1

Qiaoqiao Yang
Shenyang Medical College
Yaping Mao
Shenyang Medical College
Juan Wang
Shenyang Medical College
Haiyang Yu
Shenyang Medical College
Xuan Zhang
Shenyang Medical College
Xiucong Pei
Shenyang Medical College
Zhiwen Duan
Shenyang Medical College
Chunling Xiao
Shenyang Medical College
Mingyue Ma (mymacmu@163.com)
Shenyang Medical College https://orcid.org/0000-0003-4440-1618

Research Article

Keywords: bisphenol A, gestational exposure, offspring, liver, lipid metabolism, mTOR, CRTC2, SREBP1

Posted Date: June 27th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1756683/v1

Abstract

Lipid metabolism is an important biochemical process in the body. Recent studies have found that environmental endocrine disruptors play an important role in the regulation of lipid metabolism. Bisphenol A (BPA), a common environmental endocrine disruptor, has adverse effects on lipid metabolism, but the mechanism is still unclear. This study aimed to investigate the effects of gestational BPA exposure on hepatic lipid metabolism and its possible mechanism in male offspring. The pregnant Sprague-Dawley rats were exposed to BPA (0, 0.05, 0.5, 5 mg/kg/day) from day 5 to day 19 of gestation to investigate the level of triglyceride (TG) and the expression of liver lipid metabolism-related genes in male offspring rats. The results showed that compared with the control group, the TG level in serum and liver in BPA-exposed groups was increased. And the expressions of liver fatty acid oxidation related genes, such as peroxisome proliferators-activated receptor α (PPARα) and carnitine palmitoyl transferase 1α (CPT1α), were down-regulated. However, the expressions of fatty acid synthesis related genes, such as sterol regulatory element binding proteins 1 (SREBP-1), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD-1), were up-regulated. The increased protein levels of mTOR and p-CRTC2 suggested that CREB-regulated transcription coactivator 2 (CRTC2) might be an important mediator in the mTOR/SREBP-1 pathway. In conclusion, these results demonstrated that mTOR/CRTC2/SREBP-1 could be affected by gestational BPA exposure, which may involve in the lipid metabolic disorders in later life.

1 Introduction

Environmental endocrine disruptors (EEDs) pollution has become an important issue in the field of public health. In particular, the application of phenolic environmental estrogens (PEEs) has attracted extensive attention in recent years. As a typical PEEs, bisphenol A (BPA) is used to manufacture polycarbonate plastics and epoxy resins, such as food containers, sports equipment, medical and dental equipment, children's toys, and thermal paper receipts (Adeyi et al. 2019; Li et al. 2020; Pan et al. 2021). Demand for BPA is growing worldwide, with global consumption of BPA expected to rise to 10.6 million metric tons this year from 7.7 million metric tons in 2015 (Sonavane et al. 2019). As the polymer degrades, BPA can be released from plastic products into the external environment. Currently, it has been detected in food, water, air and soil, and humans can be exposed to BPA through ingestion, inhalation and skin contact (Li et al. 2019). Studies have shown that the urine of more than 90% of the population in western countries contains BPA, and the level of BPA in workers engaged in BPA production or use is much higher than that in the general population, even reaching 492,000 ng/m^3 (Abraham et al. 2020; Castellini et al. 2020; Heinala et al. 2017). Due to the mimic activity of estrogen, anti-androgen and thyroid hormone, BPA may cause adverse effects on multiple internal systems of human, such as reproductive, nervous, immune and metabolic systems (Cimmino et al. 2020; Ma et al. 2020). What’ more, BPA can also cross the placental barrier and affect the growth and development of fetus (Sun et al. 2002). As the expression of BPA metabolizing enzymes in fetal liver needs to be delayed until after birth, fetuses and newborns may be at

a higher risk of exposure to BPA than older children and adults (Nakajima et al. 2012). The adverse effects of BPA exposure early in life are now a major health concern.

BPA has been shown to act as an obesogen and to disrupt lipid metabolism. The existing epidemiological studies have shown that BPA exposure can increase the risk of obesity and may be positively correlated with urine BPA concentration (Moon et al. 2021). Moreover, in animal experiments, BPA can cause liver fat accumulation in both acute and chronic exposure (Sun et al. 2020). It has even been shown that BPA may accelerate fat formation at non-observed adverse effect levels (NOAEL) (Li et al. 2020). Studies also have shown that prenatal BPA exposure can impair liver lipid metabolism in female offspring (Tonini et al. 2021). And after prenatal BPA exposure, alterations in lipid profiles are more prominent in the offspring than those in the dams, suggesting that exposure to BPA in utero is more serious than adult exposure (Nguyen et al. 2022). Lipid metabolic disorders may lead to obesity, type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), atherosclerosis and other diseases (DeBose-Boyd 2018). However, the mechanism of BPA exposure during pregnancy on lipid metabolism of male offspring is rarely studied.

The sterol regulatory element binding proteins (SREBPs), including SREBP-1 and SREBP-2, are a family of transcription factors that regulate lipid biosynthesis. SREBP-1 has been confirmed to mainly regulate fatty acid synthesis, and SREBP-2 is involved in cholesterol synthesis (Shimano 2000). Studies have shown that BPA can interfere with the binding of SREBP-1 to their SREs through DNA methylation in the 5' side region of carnitine palmitoyl transferase 1 (CPT1), thus affecting fatty acid β-oxidation (Guan et al. 2019). It was also reported that BPA could decrease the DNA methylation level of SREBP-2 in mice, and upregulate the expression of genes related to cholesterol synthesis in liver, which led to liver lipid accumulation and liver steatosis (Li et al. 2019; Ke et al. 2016).

mTOR (Mammalian target of Rapamycin) is a serine/threonine phosphoside kinase belonging to the PI3K related kinase family. mTOR signaling pathway has been implicated in regulation of lipid synthesis in liver. Studies have found that estrogen combined with ESRs inhibits fat synthesis and promotes fat hydrolysis by inhibiting the PI3K/Akt/mTOR signaling pathway and promoting the expression of AMPK in lipolytic pathway (Monteiro et al. 2014; Rogers et al. 2009). A recent study showed that BPA mainly inhibited the phosphorylation of AMPK, thereby activating the mTOR/SREBP-1c pathway and the expression of downstream adipogenesis related factors (Sun et al. 2020). In addition, researches have demonstrated that CREB-regulated transcription coactivator 2 (CRTC2), as a critical mediator of mTOR, may play a key role in mTOR-dependent regulation of SREBP-1 (Qian et al. 2021; Han et al. 2015).

These findings prompt us to investigate whether CRTC2 can mediate the mTOR/SREBP1 pathway, which may be an important pathway for the effects of gestational BPA exposure on lipid metabolism in male offspring.

2 Material And Method

2.1 Animals and treatments

The experimental animals were 9-week-old Sprague-Dawley (SD) rats purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Certificate No.SCXK2020-0001, Liaoning of China). The animals were raised in SPF Experimental Animal Center of Shenyang Medical College, and the breeding conditions met the relevant requirements of "Environment and Facilities for Experimental Animals" (GB14925-2010); indoor relative temperature 25 ± 2℃, humidity 55 ± 5%, 12 h/12 h day-night light cycle, free eating and drinking (all drinking bottles were glass bottles). After one week of adaptive feeding, female and male rats were randomly mated in a 2:1 ratio. Pregnancy was confirmed by vaginal smear. The date of microscopic sperm observation was gestation day 0 (GD0) (Ma et al. 2020). Forty pregnant rats were randomly divided into 4 groups with 10 rats in each group. The rats were orally gavaged with corn oil containing 0, 0.05, 0.5, and 5 mg/kg/d BPA respectively, from GD5 to GD19. BPA (> 99%, TCI, Japan) was dissolved in corn oil. The day of birth was denoted as postnatal day (PND) 1, and male pups at PND21 and 56 were selected as research objects in this experiment. The serum and tissues were collected and stored at -80℃ for further study.

2.2 General indicator assay

At PND21 and 56, the male offspring rats were weighed, anesthetized and decapitated with ether. The liver was immediately dissected and weighed, and then the organ coefficient was calculated. Calculation formula: Organ coefficient (%) = organ wet weight (g)/ body weight (g)×100%.

2.3 Lipid content assay

The content of TG in serum and liver was detected by double-antibody one-step sandwich enzyme linked immunosorbent assay ELISA kit (mlbio, China). The absorbance (OD) value was measured at 450nm using a microplate reader, and the sample concentration was calculated.

2.4 Real-time PCR

Trizol (Vazyme, China) was used to extract total RNA from male offspring's liver tissue (six in each group), then the cDNA was synthesized using PrimeScript RT kit (Takara, Japan). The SYBR Premix Ex Taq II kit (Takara, Japan) was used to mix cDNA and primers, and real-time fluorescence quantitative PCR was performed with ABI 7500 fast Real-Time PCR System (California, USA). The housekeeping gene *GAPDH* was used as an internal control. The relative mRNA expression of each gene was calculated by $2^{-\Delta\Delta CT}$ method. Gene-specificity primers were shown in Supplementary Table 1.

2.5 Western blotting

RIPA with phosphatase inhibitors and PMSF (Beyotime, China) was used to lyse liver tissue (six in each group), and then protein concentration was detected by BCA assay kit (Beyotime, China). Western Blotting was used to detect the samples after boiling and denaturation. The protein samples (50μg) were separated by 7.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS – PAGE) (Epizyme, China), and then transferred onto the nitrocellulose membranes. After that, blocking of the membranes

with 5% skimmed milk powder or 5% BSA was done; followed by incubation with primary antibodies (CPT1α: 15184-1-AP, mTOR: 66888-1-Ig, CRTC2: 12497-1-AP, β-actin: 20536-1-AP, Proteintech, China; Phospho-mTOR: AP0094, SCD-1: A16429, ABclonal, China; Phospho-CRTC2: AF8328, peroxisome proliferators-activated receptor α (PPARα): AF5301, SREBP-1: AF6283, Affinity, China) for overnight at 4°C. Secondary antibody goat anti-mouse (SA00001-1, Proteintech, China) and goat anti-rabbit (SA00001-2, Proteintech, China) were incubated at room temperature for 1 h. The gel imaging system (Tanon-5200 Muti, Tanon, China) was used to take pictures. Image J was used to quantify the gray value of protein bands. The relative protein expression was normalized to β-actin.

2.6 Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by One-way ANOVA followed by LSD or Dunnett's test using SPSS 20.0 software (Illinois, USA). $p < 0.05$ was regarded as statistically significant.

3 Results

3.1 Gestational BPA exposure affected the body weight and liver coefficient of male offspring

From birth to sexual maturity, all offspring rats were in a good state and no abnormal appearance, posture and behavior, and ate normally. At PND21, there was no significant difference in body weight and liver coefficient of male offspring between the BPA exposed groups and the control group ($p > 0.05$). However, at PND56, the body weight of male rats was decreased in the BPA exposed groups, especially in the 0.5 mg/kg group ($p < 0.05$). Moreover, the liver coefficient of male rats was increased significantly in 0.05 and 0.5 mg/kg groups compared to the control group ($p < 0.05$) (Fig. 1).

3.2 Gestational BPA exposure increased the level of TG in male offspring

To explore the effects of gestational exposure to BPA on lipid metabolism in male offspring, we examined the TG level in serum and liver tissue. As shown in Fig. 2A, serum TG level in BPA exposed groups was significantly higher than that in the control group ($p < 0.05$). In addition, the liver TG level was also significantly higher in BPA exposure groups, which were basically consistent with the changes of TG level in serum ($p < 0.05$) (Fig. 2B).

3.3 Gestational BPA exposure decreased the expression of liver fatty acid oxidation related genes in male offspring

On the basis of alteration of TG levels, we hypothesized that BPA mainly affected TG content through fatty acid oxidation and fatty acid synthesis. Therefore, we detected the expression levels of two key genes in fatty acid oxidation pathway, PPARα and CPT1α. As shown in Fig. 3A and B, at PND21, the mRNA expression of PPARα was significantly decreased in all BPA exposure groups compared with the control group, and the mRNA expression of CPT1α was significantly decreased only in the 5 mg/kg group. Meanwhile, the protein levels of PPARα and CPT1α were significantly decreased in 5 mg/kg group ($p < 0.05$). At PND56, the mRNA and protein expression of PPARα decreased significantly in 0.5 mg/kg

and 5 mg/kg BPA exposure groups, while the protein expression of CPT1α decreased significantly only in 5 mg/kg BPA exposure group ($p < 0.05$) (Fig. 3C and D).

3.4 Gestational BPA exposure increased the expression of liver fatty acid synthesis related genes in male offspring

Furthermore, we detected the expression of liver fatty acid synthesis related factors. As shown in Fig. 4A, at PND21, compared with the control group, the mRNA expressions of SREBP-1, ACC1, FAS and SCD-1 were significantly increased in BPA exposure groups ($p < 0.05$). Consistently, at PND56, the mRNA expression of these factors in BPA exposure groups were also significantly increased ($p < 0.05$) (Fig. 4C). The protein levels of SREBP-1 and SCD-1 were detected, which were also significantly increased in BPA exposed groups at PND21 (Fig. 4B) and PND56 (Fig. 4D).

3.5 Gestational BPA exposure affected the liver fatty acid synthesis by regulating mTOR/CRTC2 pathway in male offspring

Since CRTC2 could be phosphorylated by mTOR, which caused SREBP1 processing and enhancement of de novo lipogenesis, we furtherly investigated the effects of gestational BPA exposure on mTOR/CRTC2 pathway. At PND21, the mRNA expression of mTOR in 0.05 mg/kg group was significantly increased than that in control group ($p < 0.05$), and the mRNA expression of CRTC2 in all BPA exposure groups were significantly increased ($p < 0.05$) (Fig. 5A). Although the phosphorylation of mTOR did not change significantly, the protein levels of mTOR in BPA exposure groups were significantly increased ($p < 0.05$), meanwhile, the phosphorylation of CRTC2 in 0.05 and 0.5 mg/kg groups was significantly increased ($p < 0.05$) (Fig. 5B). At PND56, compared with the control group, the mRNA expression of mTOR was significantly increased in the 5 mg/kg group ($p < 0.05$), and the mRNA expression of CRTC2 in 0.05 and 0.5 mg/kg groups was significantly increased ($p < 0.05$) (Fig. 5C). Notably, the protein levels of mTOR and the phosphorylation of CRTC2 were also significantly increased in 0.5 and 5 mg/kg groups ($p < 0.05$) (Fig. 5D), which indicated that gestational BPA exposure might affect the liver fatty acid synthesis in male offspring through the mTOR/CRTC2/SREBP-1 pathway.

4 Discussion

It is estimated that by 2035, about 592 million people worldwide will be affected by T2DM (Guariguata et al. 2014). It has been reported that obesity (body mass index > 30 kg/m^2) is a major independent risk factor for T2DM and an important cause of NAFLD (Ganz et al. 2014). In the United States alone, 35.7 percent of adults and about 17 percent of children are obese (Rachdaoui 2020). Lipid metabolism disorders have a significant impact on morbidity and mortality of metabolic diseases, which have become major global public health challenges. EEDs capable of disrupting endocrine regulation have been recognized as Environmental Obesogens (Heindel et al. 2019). The study has shown that gestational urinary BPA concentration was associated with the prevalence of obesity in children and adolescents (Braun et al. 2019). Therefore, the present study focused on the effects of BPA exposure

during pregnancy, a critical period of organ formation, and 0.05 mg/kg (Tolerable daily intake, TDI) and 5 mg/kg (NOAEL) were selected as the lowest and highest exposure doses respectively. 0.05 mg/kg was also recognized by FDA as the lifetime safe oral dose of BPA (Yu et al. 2020; Uchtmann et al. 2020).

In present study, the liver organ coefficient of male offspring at PND56 was higher than that in the control group, and was significant in the 0.05 and 0.5 mg/kg groups. Organ coefficient, as a common indicator in toxicology experiments, can not only reflect the toxicity of poisons but also observe the possibility of histopathological changes from the side, and help to find toxic target organs. In general, their elevation suggests that exposure to BPA may cause damage to offspring's organs. Relevant experiments have shown that BPA exposure can cause mild cellular swelling and steatosis in rat liver tissues (Huang et al. 2021), and similar results were obtained in two other studies on vertebrates (Tian et al. 2021; Mi et al. 2021).

TG is an important form of energy storage and oxidative energy supply in the body, and is often used as a key indicator to determine fatty acid biosynthesis in lipid metabolism. In this study, we measured the content of TG in serum and liver. The results showed that gestational BPA exposure increased the level of TG in male offspring at PND21 and 56, and the increase trend of TG in serum and liver was basically the same. Our results are consistent with those of several other epidemiological or animal studies (Liao et al. 2021; Meng et al. 2019; Wang et al. 2020), which demonstrated that intrauterine BPA exposure can lead to TG accumulation in male progeny, indicating the role of promoting adipogenesis. Two important pathways involved in the regulation of liver lipid metabolism are fatty acid synthesis and fatty acid oxidation, among which PPAR plays a very important role in fatty acid oxidation. PPARα is highly expressed in liver and promotes fatty acid oxidation by stimulating the transcription of rate-limiting enzyme CPT1 (Qin et al. 2021). In this study, we found that mRNA and protein expressions of PPARα and CPT1α were decreased in the liver at both stages of 5 mg/kg BPA exposure, suggesting that BPA inhibits liver fatty acid oxidation to increase TG accumulation, which is consistent with previous study (Grasselli et al. 2013).

In addition, to further investigate whether BPA-induced effects were related to the expression of key genes and proteins in lipid synthesis, SREBP-1 and its regulated genes SCD-1, ACC1, FAS were detected. Results showed that the mRNA expressions of these factors were significantly increased at both stages. SCD-1 is a key control point for lipid synthesis in the liver and can catalyze the formation of saturated fatty acids into monounsaturated fatty acids. During fat synthesis, monounsaturated fatty acids are more likely to become substrates of ACAT (ACYL-CoA cholesterol acyltransferase) and DGAT (Diacylglycerol acyltransferase) than saturated fatty acids, which produce cholesterol esters and TG (Ravaut et al. 2020). Therefore, we detected the protein levels of SCD-1 and its key regulatory factor SREBP-1, and found that they were consistent with the results of mRNA. These results suggest that BPA can induce SREBP-1 activation in male offspring, thereby increasing the expression of downstream key genes and leading to TG accumulation in vivo. Similar effects have been reported in the previous studies (Guan et al. 2019; Somm et al. 2009). Zhang et al also found that inhibition of SREBP-1 expression could effectively reduce TG accumulation (Zhang et al. 2019). At present, existing evidence fully shows that the expression of

SREBP-1 is positively correlated with the content of TG, which is also an important target of BPA exposure affecting lipid metabolism.

Mammalian mTOR is an important regulator of cell proliferation, differentiation, apoptosis and protein synthesis, and also plays an important role in lipid metabolism. Our results suggest that 0.05 mg/kg BPA exposure increases the p-mTOR/mTOR ratio at PND56, which is consistent with the well-known pattern of mTOR regulating fatty acid synthesis (Lin et al. 2019). In the 0.5 and 5 mg/kg groups, the total protein level of mTOR increased, while the phosphorylation level did not change significantly. We also found that the phosphorylation level of CRTC2 at PND21 and 56 was significantly increased in 0.5 and 5 mg/kg groups, suggesting that mTOR may phosphorylates CRTC2 and then regulates the expression of SREBP-1. Previous studies on LO2 cells in vitro have also shown that phosphorylation of CRTC2 activates nuclear SREBP-1 activity and subsequent adipogenesis (Hu et al. 2019), but the difference is that phosphorylation of mTOR is not well defined. Zhang et al. (Zhang et al. 2018) found that in adipose tissue, mTORC1 plays a corresponding regulatory role through phosphorylation of CRTC2. Other studies also reported that CRTC2 knockdown or overexpression did not affect the phosphorylation of mTOR (Li et al. 2019), which strongly supported our research results. However, the effect of BPA on mTOR/CRTC2 pathway has not been reported. Our data demonstrate that gestational BPA exposure may increase the liver fatty acid synthesis by regulating mTOR/CRTC2/SREBP-1 pathway in male offspring. In addition, in this study, the expression results of mTOR and CRTC2 at mRNA and protein levels are inconsistent, which may be due to the influence of post-transcriptional regulation.

In summary, the present study proved that gestational BPA exposure could affect liver lipid levels by disturbing lipid metabolism, including inhibition of fatty acid oxidation and promotion of fatty acid synthesis. We first proposed that the mTOR/CRTC2/SREBP-1 pathway may play an important role in the effects of prenatal BPA exposure on liver fatty acid synthesis in male offspring. In this process, as a downstream mediator of mTOR, CRTC2 may play a potential role in the regulation of SREBP-1 and thus promoting lipid synthesis, which provides a novel insight into the established correlations between early-life BPA exposure and lipid metabolism disorders. Furthermore, these data might partially indicate that prenatal BPA exposure caused lasting effects to basal gene expression of lipid metabolism in adult rat liver. This will strengthen the evidence for prevention of exposure to environmental chemicals during pregnancy.

Declarations

Authors'contribution Qiaoqiao Yang(QY), Juan Wang(JW), Yaping Mao(YM), Haiyang Yu(HY), Xuan Zhang(XZ), Xiucong Pei(XP), Zhiwen Duan(ZD), Chunling Xiao(CX), Mingyue Ma(MM*)

QY, YM, HY and MM* were involved in the conception and design of the study. QY, JW, YM, and MM*were involved in the data execution and interpretation. QY, JW, XZ and MM*were involved in the investigation and methodology. QY and MM*were involved in the roles/writing - original draft, writing. XP, ZD, CX, XZ and MM*were involved in the funding acquisition, supervision, validation, review and editing.

Funding This research was funded by Liaoning Natural Science Foundation (Grant No.2019-ZD-0317), and Science and technology innovation fund of Shenyang medical college (Grant No. Y20210508).

Data availability All data are mentioned in the body of manuscript, tables, and figure.

Compliance with ethical standards

The experimental protocol was approved by the Animal Ethics Committee of Shenyang Medical College (Permit number: SYYXY2020092002), and all animal studies were carried out in accordance with the Guidelines for Animal Experimentation.

Consent to participate All participates agreed to participate in this study and signed the informed consents.

Consent for publish Not applicable.

Competing interests The authors declare no competing interests.

References

1. Abraham A, Chakraborty P (2020) A review on sources and health impacts of bisphenol A. Rev Environ Health 35: 201-210. https://doi.org/10.1515/reveh-2019-0034
2. Adeyi AA, Babalola BA (2019) Bisphenol-A (BPA) in foods commonly consumed in southwest Nigeria and its human health risk. Sci Rep 9: 17458. https://doi.org/10.1038/s41598 -019-53790-2
3. Braun JM, Li N, Arbuckle TE, Dodds L, Massarelli I, Fraser WD, Lanphear BP, Muckle G (2019) Association between gestational urinary bisphenol A concentrations and adiposity in young children: the MIREC study. Environ Res 172: 454-461. https://doi.org/10.1016/j.envres.2019.02.038
4. Carman GM (2021) Lipid metabolism has been good to me. J Biol Chem 297: 100786. https://doi.org/10.1016/j.jbc.2021.100786
5. Castellini C, Totaro M, Parisi A, D'Andrea S, Lucente L, Cordeschi G, Francavilla S, Francavilla F, Barbonetti A (2020) Bisphenol A and male fertility: myths and realities. Front Endocrinol (Lausanne) 11: 353. https://doi.org/10.3389/fendo.2020.00353
6. Chen G (2021) The interactions of insulin and Vitamin A signaling systems for the regulation of hepatic glucose and lipid metabolism. Cells 10: 2160. https://doi.org/10.3390/cells10082160
7. Cimmino I, Fiory F, Perruolo G, Miele C, Beguinot F, Formisano P, Oriente F (2020) Potential mechanisms of Bisphenol A (BPA) contributing to human disease. Int J Mol Sci 21: 5761. https://doi.org/10.3390/ijms21165761
8. DeBose-Boyd RA (2018) Significance and regulation of lipid metabolism. Semin Cell Dev Biol 81: 97. https://doi.org/10.1016/j.semcdb.2017.12.003
9. Ganz ML, Wintfeld N, Li Q, Alas V, Langer J & Hammer M (2014) The association of body mass index with the risk of type 2 diabetes: a case-control study nested in an electronic health records system in

the United States. Diabetol Metab Syndr 6: 50. https://doi.org/10.1186/1758-5996-6-50

10. Grasselli E, Cortese K, Voci A, Vergani L, Fabbri R, Barmo C, Gallo G, Canesi L (2013) Direct effects of bisphenol A on lipid homeostasis in rat hepatoma cells. Chemosphere 91: 1123-1129. https://doi.org/10.1016/j.chemosphere.2013.01.016
11. Guan Y, Zhang T, He J, Jia J, Zhu L, Wang Z (2019) Bisphenol A disturbed the lipid metabolism mediated by sterol regulatory element binding protein 1 in rare minnow Gobiocypris rarus. Aquat Toxicol 207: 179-186. https://doi.org/10.1016/j.aquatox.2018.12.011
12. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014) Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 103: 137-149. https://doi.org/10.1016/j.diabres.2013.11.002
13. Han J, Li E, Chen L, Zhang Y, Wei F, Liu J, Deng H, Wang Y (2015) The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. Nature 524: 243-246. https://doi.org/10.1038/nature14557
14. Heinala M, Ylinen K, Tuomi T, Santonen T, Porras SP (2017) Assessment of occupational exposure to bisphenol A in five different production companies in Finland. Ann Work Expo Health 61: 44-55. https://doi.org/10.1093/annweh/wxw006
15. Heindel JJ, Blumberg B (2019) Environmental obesogens: mechanisms and controversies. Annu Rev Pharmacol Toxicol 59: 89-106. https://doi.org/10.1146/annurev-pharmtox- 010818-021304
16. Hu J, Hong W, Yao KN, Zhu XH, Chen ZY, Ye L (2019) Ursodeoxycholic acid ameliorates hepatic lipid metabolism in LO2 cells by regulating the AKT/mTOR/SREBP-1 signaling pathway. World J Gastroenterol 25: 1492-1501. https://doi.org/10.3748/wjg.v25.i12.1492
17. Han J, Li E, Chen L, Zhang Y, Wei F, Liu J, Deng H, Wang Y (2015) The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. Nature 524: 243-246. https://doi.org/10.1038/nature14557
18. Ke ZH, Pan JX, Jin LY, Xu HY, Yu TT, Ullah K, Rahman TU, Ren J, Cheng Y, Dong XY, Sheng JZ, Huang HF (2016) Bisphenol A exposure may induce hepatic lipid accumulation via reprogramming the DNA methylation patterns of genes involved in lipid metabolism. Sci Rep 6: 31331. https://doi.org/10.1038/srep31331
19. Kopp R, Martinez IO, Legradi J, Legler J (2017) Exposure to endocrine disrupting chemicals perturbs lipid metabolism and circadian rhythms. J Environ Sci (China) 62: 133-137. https://doi.org/10.1016/j.jes.2017.10.013
20. Li J, Wu C, Zhao H, Zhou Y, Cao G, Yang Z, Hong Y, Xu S, Xia W, Cai Z (2019) Exposure assessment of bisphenols in Chinese women during pregnancy: a longitudinal study. Environ Sci Technol 53: 7812-7820. https://doi.org/10.1021/acs.est.9b01281
21. Li P, Zhou C, Li X, Yu M, Li M, Gao X (2019) CRTC2 is a key mediator of amino acid-induced milk fat synthesis in mammary epithelial cells. J Agric Food Chem 67: 10513-10520. https://doi.org/10.1021/acs.jafc.9b04648

22. Li Q, Zhang H, Zou J, Mai H, Su D, Feng X, Feng D (2019) Bisphenol A exposure induces cholesterol synthesis and hepatic steatosis in C57BL/6 mice by down-regulating the DNA methylation levels of SREBP-2. Food Chem Toxicol 133: 110786. https://doi.org/10.1016/j.fct.2019.110786
23. Li Y, Yin S, Yang Y, Chen J, Wu L, Sun C (2020) Sample treatment methods for the determination of phenolic environmental estrogens in foods and drinking water. J AOAC Int 103: 348-364. https://doi.org/10.5740/jaoacint.19-0249
24. Li M, Gao X, Tan L, Miao Y, Fan W, Gao Z, Liu S, Ding C, Shi X, Song S (2020) Effects of bisphenol A at the safe reference dose on abdominal fat deposition in aged hens. Ecotoxicol Environ Saf 206: 111398. https://doi.org/10.1016/j.ecoenv.2020.111398
25. Liao JX, Chen YW, Shih MK, Tain YL, Yeh YT, Chiu MH, Chang SKC, Hou CY (2021) Resveratrol butyrate esters inhibit BPA-induced liver damage in male offspring rats by modulating antioxidant capacity and gut microbiota. Int J Mol Sci 22: 5273. https://doi.org/10.3390/ijms22105273
26. Lin R, Wu D, Wu FJ, Meng Y, Zhang JH, Wang XG, Jia LH (2019) Non-alcoholic fatty liver disease induced by perinatal exposure to bisphenol A is associated with activated mTOR and TLR4/NF-kappaB signaling pathways in offspring rats. Front Endocrinol (Lausanne) 10: 620. https://doi.org/10.3389/fendo.2019.00620
27. Ma L, Yu H, Wang X, Li D, Zhang Y, Pei X, Duan Z, Ma M (2020) The effects of maternal exposure to BPA during pregnancy on the male reproductive system and the testicular microRNA expression profile. Environ Sci Pollut Res Int 27: 17290-17302. https://doi.org/10.1007/s11356-020-08156-x
28. Meng Z, Tian S, Yan J, Jia M, Yan S, Li R, Zhang R, Zhu W, Zhou Z (2019) Effects of perinatal exposure to BPA, BPF and BPAF on liver function in male mouse offspring involving in oxidative damage and metabolic disorder. Environ Pollut 247: 935-943. https://doi.org/10.1016/j.envpol.2019.01.116
29. Merino Salvador M, Gomez de Cedron M, Moreno Rubio J, Falagan Martinez S, Sanchez Martinez R, Casado E, Ramirez de Molina A, Sereno M (2017) Lipid metabolism and lung cancer. Crit Rev Oncol Hematol 112: 31-40. https://doi.org/10.1016/j.critrevonc.2017.02.001
30. Mi K, Chen X, Lu K, Zhu Y, Zhang M, Yang H, Wei W, Zhang Y (2021) Bisphenol A induces hepatic triglyceride level in adult male rare minnow Gobiocypris rarus. Ecotoxicol Environ Saf 213: 112050. https://doi.org/10.1016/j.ecoenv.2021.112050
31. Monteiro R, Teixeira D, Calhau C (2014) Estrogen signaling in metabolic inflammation. Mediators Inflamm 2014: 615917. https://doi.org/10.1155/2014/615917
32. Moon S, Seo MY, Choi K, Chang YS, Kim SH, Park MJ (2021) Urinary bisphenol A concentrations and the risk of obesity in Korean adults. Sci Rep 11: 1603. https://doi.org/10.1038/s41598-021-80980-8
33. Nakajima Y, Goldblum RM, Midoro-Horiuti T (2012) Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: an animal model study. Environ Health 11: 8. https://doi.org/10.1186/1476-069X-11-8
34. Nguyen HT, Li L, Eguchi A, Agusa T, Yamamoto K, Kannan K, Kim EY & Iwata H (2022) Effects of gestational exposure to bisphenol A on the hepatic transcriptome and lipidome of rat dams:

Intergenerational comparison of effects in the offspring. Sci Total Environ 826: 153990. https://doi.org/10.1016/j.scitotenv.2022.153990

35. Pan MH, Wu YK, Liao BY, Zhang H, Li C, Wang JL, Hu LL, Ma B (2021) Bisphenol A exposure disrupts organelle distribution and functions during mouse oocyte maturation. Front Cell Dev Biol 9: 661155. https://doi.org/10.3389/fcell.2021.661155
36. Rachdaoui N (2020) Insulin: The friend and the foe in the development of type 2 diabetes mellitus. Int J Mol Sci 21: 1770. https://doi.org/10.3390/ijms21051770
37. Ravaut G, Legiot A, Bergeron KF, Mounier C (2020) Monounsaturated fatty acids in obesity-related inflammation. Int J Mol Sci 22: 330. https://doi.org/10.3390/ijms22010330
38. Rogers NH, Witczak CA, Hirshman MF, Goodyear LJ, Greenberg AS (2009) Estradiol stimulates Akt, AMP-activated protein kinase (AMPK) and TBC1D1/4, but not glucose uptake in rat soleus. Biochem Biophys Res Commun 382: 646-650. https://doi.org/10.1016/j.bbrc.2009.02.154
39. Qiao A, Zhou J, Xu S, Ma W, Boriboun C, Kim T, Yan B, Deng J, Yang L, Zhang E, Song Y, Ma YC, Richard S, Zhang C, Qiu H, Habegger KM, Zhang J, Qin G (2021) Sam68 promotes hepatic gluconeogenesis via CRTC2. Nat Commun 12: 3340. https://doi.org/10.1038/s41467-021-23624-9
40. Qin H, Song Z, Shaukat H, Zheng W (2021) Genistein regulates lipid metabolism via estrogen receptor beta and its downstream signal Akt/mTOR in hepG2 cells. Nutrients 13: 4015. https://doi.org/10.3390/nu13114015
41. Shimano H (2000) Sterol regulatory element-binding protein-1 as a dominant transcription factor for gene regulation of lipogenic enzymes in the liver. Trends Cardiovasc Med 10: 275-278. https://doi.org/10.1016/s1050-1738(00)00079-7
42. Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, Huppi PS (2009) Perinatal exposure to bisphenol A alters early adipogenesis in the rat. Environ Health Perspect 117: 1549-1555. https://doi.org/10.1289/ehp.11342
43. Sonavane M, Gassman NR (2019) Bisphenol A co-exposure effects: a key factor in understanding BPA's complex mechanism and health outcomes. Crit Rev Toxicol 49: 371-386. https://doi.org/10.1080/10408444.2019.1621263
44. Sun L, Ling Y, Jiang J, Wang D, Wang J, Li J, Wang X, Wang H (2020) Differential mechanisms regarding triclosan vs. bisphenol A and fluorene-9-bisphenol induced zebrafish lipid-metabolism disorders by RNA-Seq. Chemosphere 251: 126318. https://doi.org/10.1016/j.chemosphere.2020.126318
45. Sun SX, Wu JL, Lv HB, Zhang HY, Zhang J, Limbu SM, Qiao F, Chen LQ, Yang Y, Zhang ML, Du ZY (2020) Environmental estrogen exposure converts lipid metabolism in male fish to a female pattern mediated by AMPK and mTOR signaling pathways. J Hazard Mater 394: 122537. https://doi.org/10.1016/j.jhazmat.2020.122537
46. Sun Y, Nakashima MN, Takahashi M, Kuroda N, Nakashima K (2002) Determination of bisphenol A in rat brain by microdialysis and column switching high-performance liquid chromatography with fluorescence detection. Biomed Chromatogr 16: 319-326. https://doi.org/10.1002/bmc.161

47. Tian S, Yan S, Meng Z, Huang S, Sun W, Jia M, Teng M, Zhou Z, Zhu W (2021) New insights into bisphenols induced obesity in zebrafish (Danio rerio): activation of cannabinoid receptor CB1. J Hazard Mater 418: 126100. https://doi.org/10.1016/j.jhazmat.2021.126100
48. Tonini C, Segatto M, Bertoli S, Leone A, Mazzoli A, Cigliano L, Barberio L, Mandala M, Pallottini V (2021) Prenatal exposure to BPA: the effects on hepatic lipid metabolism in male and female rat fetuses. Nutrients 13:1970. https://doi.org/10.3390/nu13061970
49. Uchtmann KS, Taylor JA, Timms BG, Stahlhut RW, Ricke EA, Ellersieck MR, Vom Saal FS, Ricke WA (2020) Fetal bisphenol A and ethinylestradiol exposure alters male rat urogenital tract morphology at birth: confirmation of prior low-dose findings in CLARITY-BPA. Reprod Toxicol 91: 131-141. https://doi.org/10.1016/j.reprotox.2019.11.007
50. Wang B, Wang S, Zhao Z, Chen Y, Xu Y, Li M, Xu M, Wang W, Ning G, Bi Y, Wang T (2020) Bisphenol A exposure in relation to altered lipid profile and dyslipidemia among Chinese adults: a repeated measures study. Environ Res 184: 109382. https://doi.org/10.1016/j.envres.2020.109382
51. Yu H, Ma L, Liu D, Wang Y, Pei X, Duan Z, Ma M, Zhang Y (2020) Involvement of NMDAR/PSD-95/nNOS-NO-cGMP pathway in embryonic exposure to BPA induced learning and memory dysfunction of rats. Environ Pollut 266: 115055. https://doi.org/10.1016/j.envpol.2020.115055
52. Zhang X, Luo Y, Wang C, Ding X, Yang X, Wu D, Silva F, Yang Z, Zhou Q, Wang L, Wang X, Zhou J, Boyd N, Spafford M, Burge M, Yang XO, Liu M (2018) Adipose mTORC1 suppresses prostaglandin signaling and beige adipogenesis via the CRTC2-COX-2 pathway. Cell Rep 24: 3180-3193. https://doi.org/10.1016/j.celrep.2018.08.055
53. Zhang X, Wang S, Hu L, Wang J, Liu Y, Shi P (2019) Gemfibrozil reduces lipid accumulation in SMMC-7721 cells via the involvement of PPARalpha and SREBP1. Exp Ther Med 17: 1282-1289. https://doi.org/10.3892/etm.2018.7046

Figures

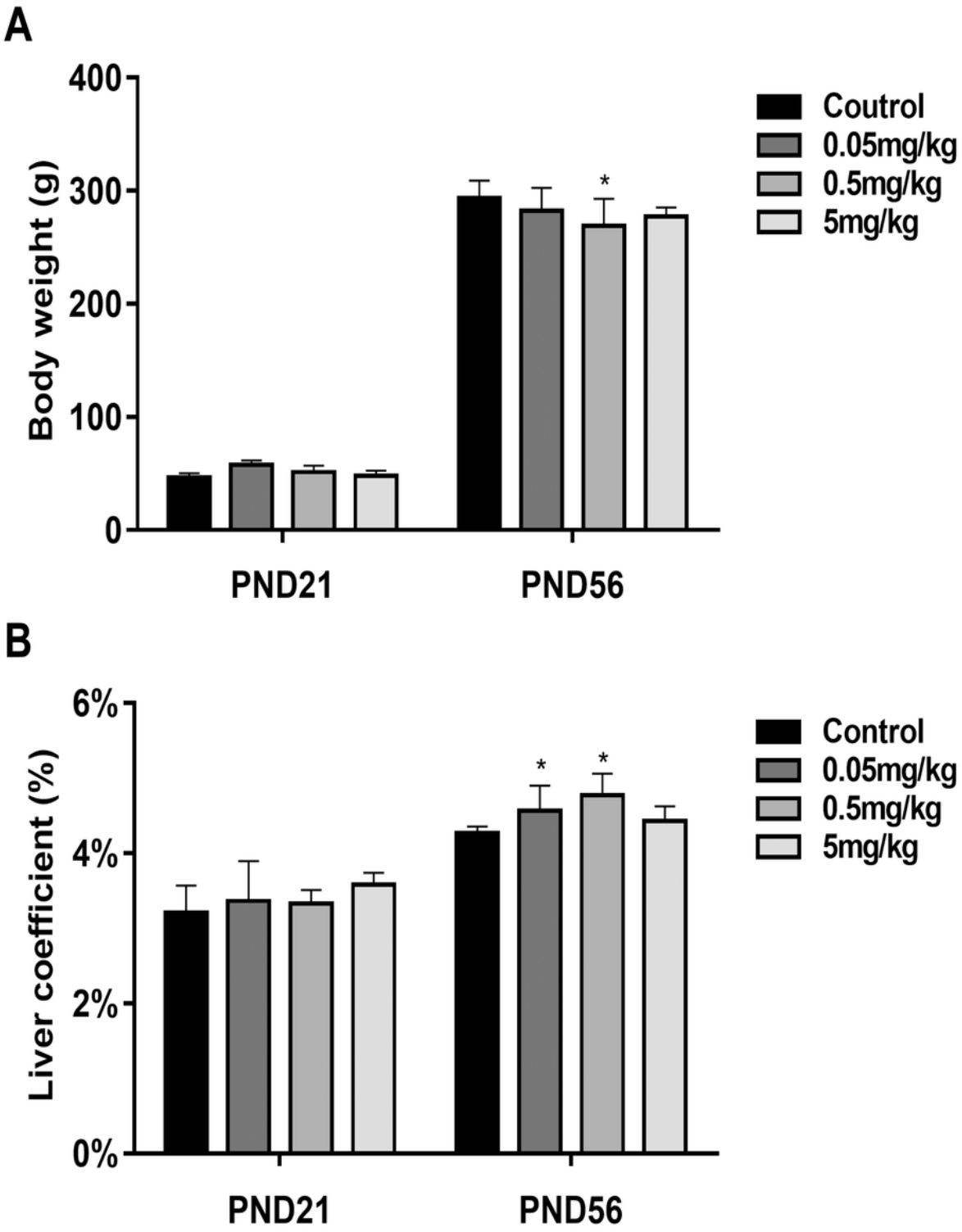

Figure 1

Effects of gestational BPA exposure on general indexes in male offspring. (A) Body weight of male offspring at PND21 and PND56. (B) Liver coefficient of male offspring at PND21 and PND56. Data are shown as means ± SD and analyzed with One-way ANOVA (n = 8). * $p < 0.05$ vs. Control.

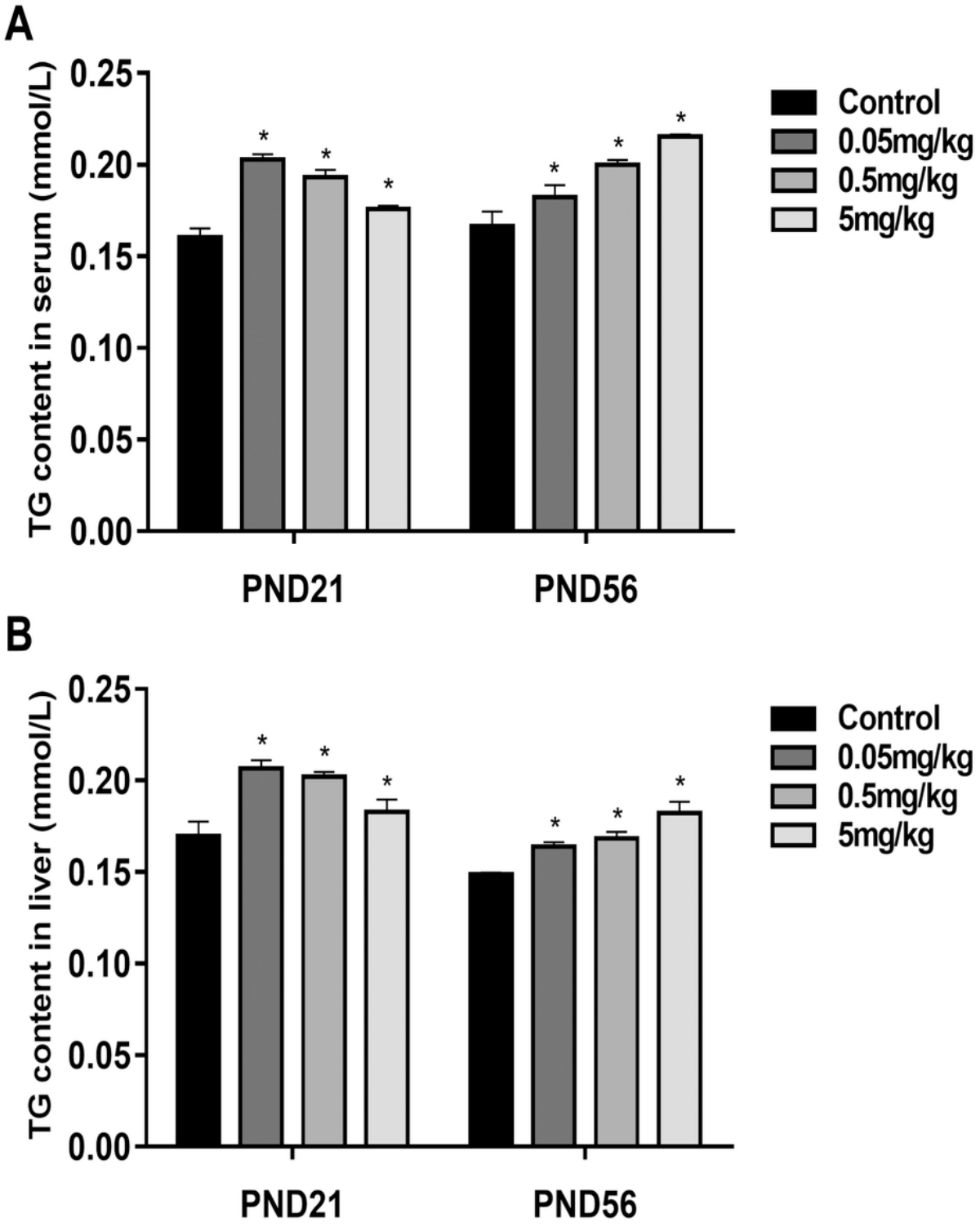

Figure 2

Effects of gestational BPA exposure on TG in male offspring.

(A) Serum TG content of male offspring at PND21 and PND56. (B) Liver TG content of male offspring at PND21 and PND56. Data are shown as means ± SD and analyzed with One-way ANOVA (n = 6). * $p < 0.05$ vs. Control.

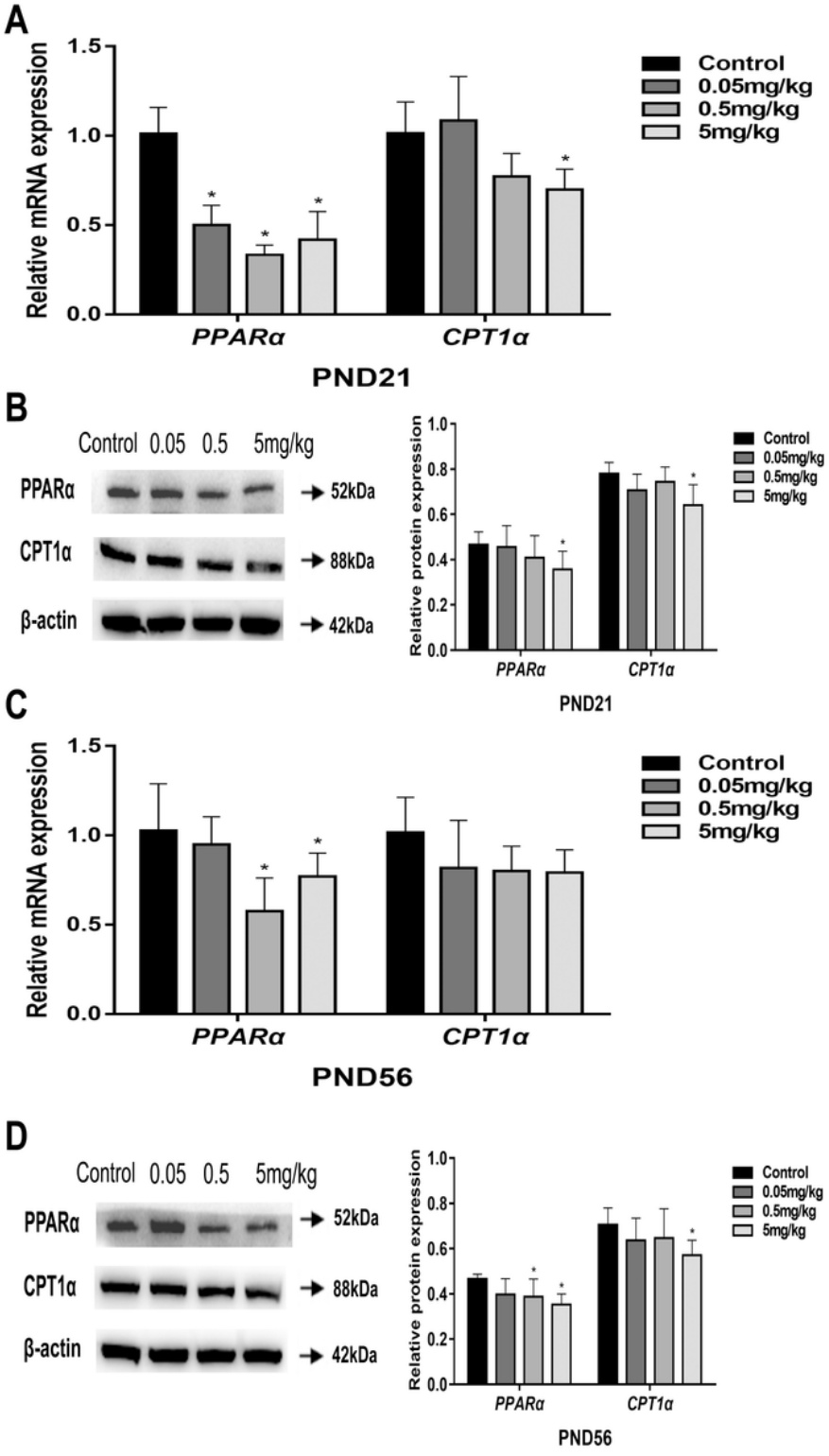

Figure 3

Effects of gestational BPA exposure on expressions of fatty acid oxidation related genes in liver of male offspring.

(A) The mRNA levels of PPARα and CPT1α in male offspring at PND21. (B) Protein levels of PPARα and CPT1α in male offspring at PND21. (C) The mRNA levels of PPARα and CPT1α in male offspring at

PND56. (D) Protein levels of PPARα and CPT1α in male offspring at PND56. Western blot bands represent the detection of the protein from three independent tests. The relative intensities were expressed in the bar chart. All data were normalized to β-actin level within the same lane/blot. Data are shown as means ± SD and analyzed with One-way ANOVA (n = 6). * $p < 0.05$ vs. Control.

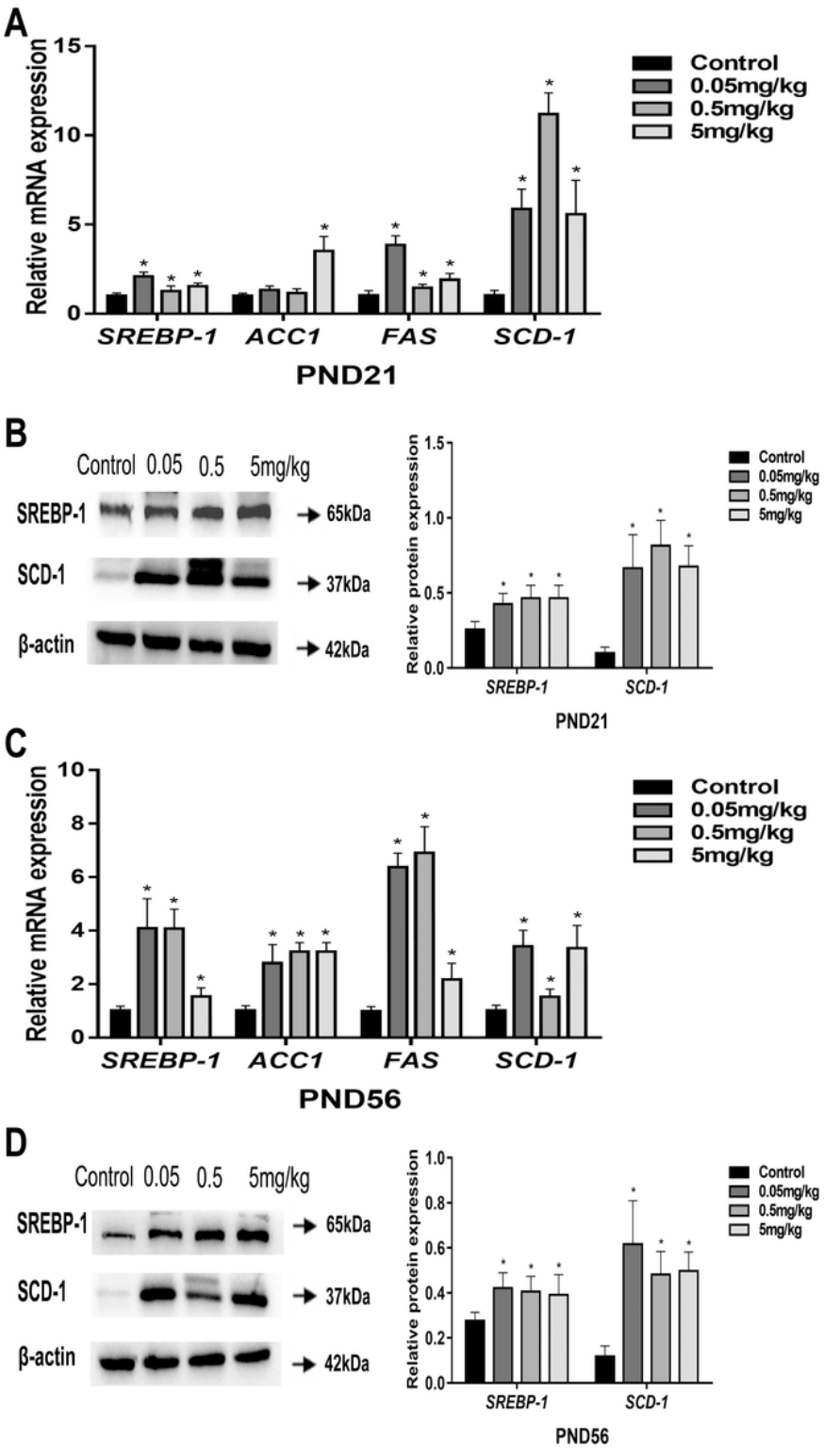

Figure 4

Effects of gestational BPA exposure on expressions of fatty acid synthesis related genes in liver of male offspring.

(A) The mRNA levels of SREBP-1, ACC1, FAS and SCD-1 in male offspring at PND21. (B) Protein levels of SREBP-1 and SCD-1 in male offspring at PND21. (C) The mRNA levels of SREBP-1, ACC1, FAS and SCD-1 in male offspring at PND56. (D) Protein levels of SREBP-1 and SCD-1 in male offspring at PND56. Western blot bands represent the detection of the protein from three independent tests. The relative intensities are expressed in the bar chart. All data were normalized to β-actin level within the same lane/blot. Data are shown as means ± SD and analyzed with One-way ANOVA (n = 6). *$p < 0.05$ vs. Control.

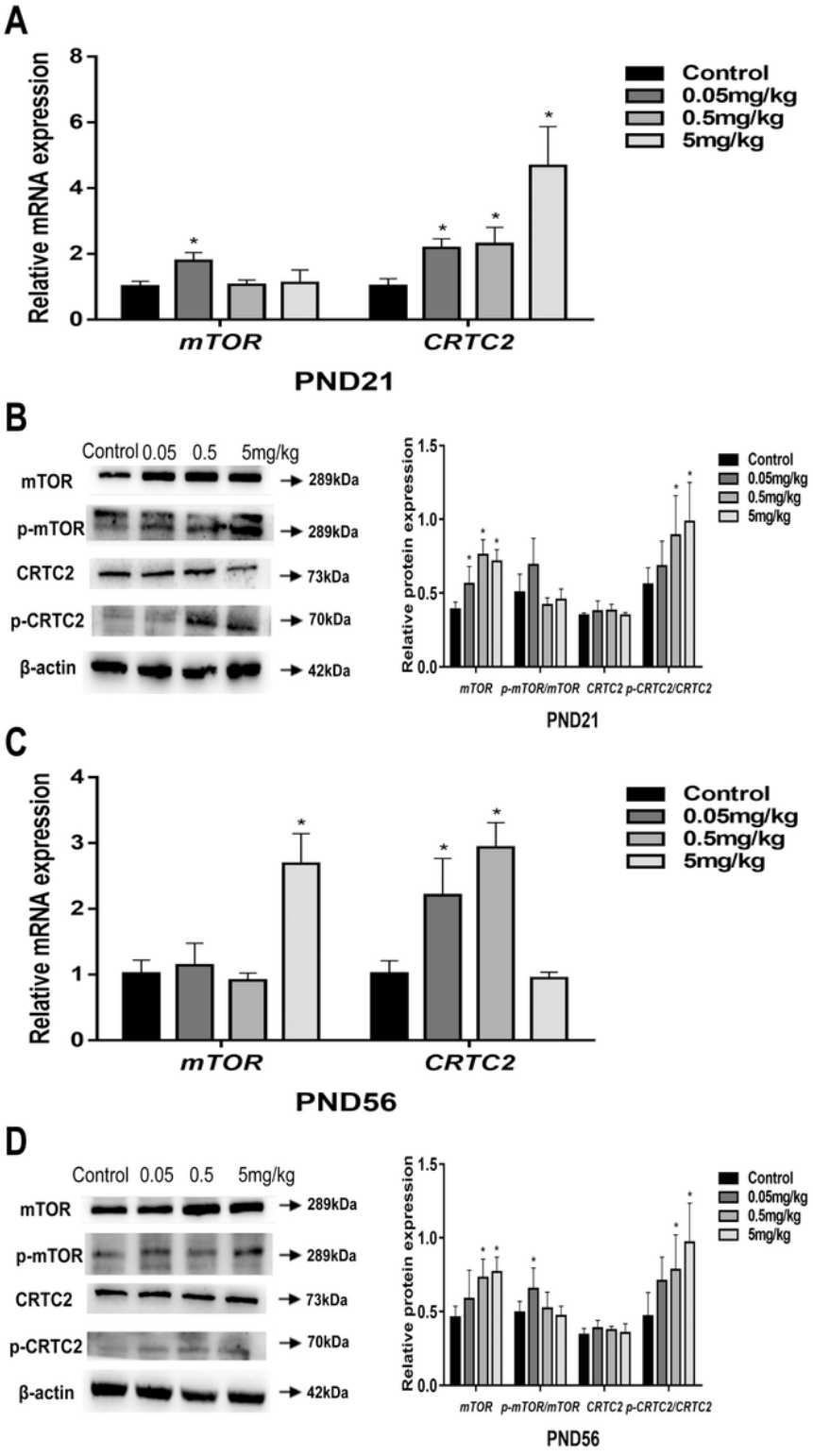

Figure 5

Effects of gestational BPA exposure on mTOR/CRTC2 pathway in male offspring.

(A) The mRNA levels of mTOR and CRTC2 in male offspring at PND21. (B) Protein levels of mTOR, p-mTOR, CRTC2 and p-CRTC2 in male offspring at PND21. (C) The mRNA levels of mTOR and CRTC2 in male offspring at PND56. (D) Protein levels of mTOR, p-mTOR, CRTC2 and p-CRTC2 in male offspring at

PND56. Western blot bands represent the detection of the protein from four independent tests. The relative intensities are expressed in the bar chart. All data were normalized to β-actin level within the same lane/blot, except for the p-CRTC2: mature CRTC2 ratio and the p-mTOR: mature mTOR ratio. Data are shown as means ± SD and analyzed with One-way ANOVA (n = 6). * $p < 0.05$ vs. Control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementTable1.docx