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ORIGINAL ARTICLE

2,2′,4,4′,5,5′-Hexabromophenyl ether (BDE-153) causes abnormal insulin secretion and disorders of glucose and lipid metabolism in mice

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Abstract

Background: Environmental polybrominated diphenyl ether (PBDE) exposure may be associated with diabetes and obesity. 2,2′,4,4′,5,5′-Hexabromodiphenyl ether (BDE-153) is one of the most abundant and widely distributed homologs of PBDEs detected in humans. This study investigated the effects of BDE-153 on the expression of adipokines and glucose and lipid metabolism.

Methods: Adult male C57BL/6 mice were divided into five BDE-153 groups and one control group. After BDE-153 exposure for 4 weeks, the levels of biochemical indexes and the mRNA and protein expression levels of leptin, adiponectin, peroxisome proliferators activated receptors gamma (PPARγ), and AMPKα were measured. The histomorphological changes of liver and pancreas tissues were observed.

Results: After BDE-153 exposure, the weight of mice in the medium–high-dose group at different exposure times was lower than that in the control group (p all <0.05), and the body weight decreased slightly with the increase of the dose of BDE-153. BDE-153 caused the disorder of glucose and lipid metabolism in mice, the weight of liver and pancreas increased, lipid droplets accumulated in liver cells, and the positive rate of insulin staining increased in a dose-dependent manner. BDE-153 also interfered with the expression of PPARγ, AMPKα, and adipokines. The results of restrictive cubic splines (RCS) showed that there were a nonlinear dose–response relationship between the exposure dose of BDE-153 and the expression levels of PPARγ, AMPKα, and adipokines.

Conclusion: Our results suggest that BDE-153 may interfere with the expression of adipokines and the secretion of insulin by affecting the expression of PPARγ and AMPKα, which play a key role in glucose and lipid metabolism, leading to the occurrence of glucose and lipid metabolism disorder.

Keywords: 2,2′,4,4′,5,5′-Hexabromodiphenyl ether; Adipokines; AMPKα; Glucose and lipid metabolism; PPAR-γ; Restrictive cubic splines

1. INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are global organic pollutants that persist in the environment. They are lipophilic, highly resistant to degradation and easily released from products into the indoor and outdoor environments, resulting in bioaccumulation in the human food chain. It was reported that they are present in human fat, blood, and milk.[1-3] Environmental PBDE exposure may be associated with metabolic diseases such as diabetes and obesity.[4-6] Obesity, in turn, is strongly correlated with metabolic diseases such as insulin resistance, type 2 diabetes, and hypertension. Obesity plays a key role in the development and treatment of these diseases. Therefore, PBDEs at a certain pathological number and volume in adipocytes lead to disordered lipogenesis and lipid metabolism. This may, in turn, affect the function of metabolic disease-related factors, which can lead to the development of diseases. Adipocytes secrete many cytokines, such as leptin, resistin, adiponectin, and tumor necrosis factor α. These factors not only affect adipocyte differentiation and proliferation, but also regulate insulin sensitivity and affect glucose and lipid metabolism.[7]

Peroxisome proliferators activated receptors gamma (PPARγ) is a key factor in transcriptional regulation.[8,9] It can promote adipocyte differentiation and regulate the number of adipocytes at the transcriptional level. Bisphenol A, polychlorinated biphenyls, and PBDEs bind as exogenous ligands to PPAR-γ resulting in interference with the adipocyte differentiation process and altered to sensitivity to glucose. This causes disordered glycan and lipid metabolism and eventually obesity.[10,11] Adiponectin is mainly produced in and secreted by adipocytes.[12] It consists of 244 amino acids encoding for the carboxy terminal globular domain, amino terminal collagen domain, and complement 1q structure. Leptin is a peptide hormone mainly secreted by white adipose tissue. It promotes energy expenditure and fat metabolism. In individuals with normal blood glucose levels, adipose hyperplasia results in

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increased secretion of leptin. This accelerates the process of fat metabolism, thereby dynamically balancing the body's fat volume. Diabetes is usually accompanied by leptin resistance and disordered fat metabolism regulation, resulting in fat accumulation and development of hyperlipidemia. AMPK is a key regulator of energy homeostasis in the body.[13] In vivo experiments have shown that endocrine-disrupting chemicals regulate lipid metabolism by activating the AMPK metabolic pathway.[14]

2,2′,4,4′,5,5′-Hexabromodiphenyl ether (BDE-153) is one of the most abundant and widely distributed homologs of PBDEs detected in humans.[15] The presence of BDE-153 in the Chinese population (including children) has increased over time, with BDE-153 currently as the most predominant PBDE in the general population.[16] Toxicokinetic studies of 2,2′,4,4′-tetrabromodiphenyl ether, 2,2′,4,4′,5-pentabromodiphenyl ether, 2,2′,4,4′,6-pentabromodiphenyl ether, and BDE-153 revealed significantly higher concentrations of BDE-153 in milk and adipose tissue compared with the other three PBDEs. Moreover, BDE-153 was metabolized and excreted by the body at a slower rate than the other PBDEs.[17] Therefore, in the current study, BDE-153 was selected to explore its effects on adipokines and glucose and lipid metabolism and the possible mechanisms associated with its effects.

2. METHODS

2.1. Animals and groups

Thirty-six specific pathogen free grade, 6–8-week old, healthy, male mice (body weight between 18 and 22 g) were purchased from the animal experimental center at Xinjiang Medical University (Xinjiang, China). Animal experiments were approved by the ethics committee of the First Affiliated Hospital of Xinjiang Medical University (No. IACUC-20170214-107). Animals were kept at an ambient temperature of 20°C to 22°C, a humidity of 40% to 70%, and 12 h/12 h light/dark cycle. After 1 week of acclimation, taking into account body weight, mice were randomly assigned to six different groups (six mice per group): low, medium-low, medium, medium–high, high and control, which corresponded to BDE-153 doses of 5, 25, 50, 100, and 200 mg/kg, respectively, with administration of corn oil in the control group. Mice in each group were continuously gavaged at a speed of 0.1 mL/10 g body weight for 4 weeks. The body weight of mice was recorded every week, and the growth of mice was calculated. After the gavage period, the mice were fasted overnight. The following day, the mice were sacrificed by cervical dislocation after blood collection by eye picking. Liver, pancreas, and adipose tissue samples were then isolated. The samples were stored in the freezer at –80°C until further use.

2.2. Intraperitoneal glucose tolerance test

Mice in each group were fasted for 8 hours at the end of the second week of the staining period. Glucose solution was prepared and administered according to the weight of mice (2 g/kg). The blood glucose values were measured in samples of tail vein blood at 0, 30, 60, and 120 minutes after intraperitoneal injection of the glucose solution. The area under the blood glucose curve (GAUC) was calculated as follows:

$$\begin{aligned}\text{GAUC} = {} & 0.5 \times \text{blood glucose at 0 min} \\ & + \text{blood glucose at 30 min} + 1.5 \\ & \times \text{blood glucose at 60 min} \\ & + \text{blood glucose at 120 min}\end{aligned}$$

2.3. Determination of organ coefficients, liver pathology, and immunohistochemical staining of pancreas

The color, texture, and volume of each organ were noted and organs were weighed. The weight of each organ was calculated as follows: organ coefficient = organ weight (g)/body weight (g) × 100%. Paraffin sections of 2 μm (liver) and 3 μm (pancreas) were subsequently prepared. After the sections were stained, the pathological changes in the liver and pancreas tissues were observed. Ten images of high-power microscopic fields were randomly made in each pancreatic tissue section, and the rate of positive insulin staining was analyzed using Image J software.

2.4. Determination of biochemical indexes in serum

Serum triglyceride (TG), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) levels were measured according to the instructions of each kit by the manufacturer (all the kits were purchased from Nanjing Jiancheng Biological Company, Nanjing, China). Serum levels of leptin, adiponectin, and insulin were measured by dual-antibody sandwich ELISA assay.

2.5. Real-time reverse transcription polymerase chain reaction

Frozen liver tissue (~100 mg) was grounded into powder. Total RNA, extracted by adding 1 mL of Trizol reagent, was reverse transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, USA). A QuantiNova SYBR Green PCR Kit (QIAGEN, Germany) was used for the real-time polymerase chain reaction (PCR), and amplification conditions were: 95°C for 2 minutes, 95°C for 5 seconds, and 60°C for 10 seconds. In total, there were 35 to 40 cycles. Then, mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as an internal reference.

2.6. The expression of leptin, adiponectin, PPARγ, and AMPKα proteins in the liver

Protease inhibitors, phenylmethylsulfonyl fluoride, and phosphatase inhibitors were dissolved in RIPA lysate at a ratio of 100:1:1. Total protein was extracted by adding 150 to 250 μL of RIPA lysates to 20 mg of liver tissue. The protein samples were quantified using and American Thermo Fisher Technology BCA Protein Concentration Assay Kit. Protein electrophoresis was performed by the sodium dodecyl sulfate polyacrylamide gel electrophoresis method. The membranes were incubated with antibodies against leptin, adiponectin, PPARγ, AMPKα, and β-actin. The membrane was then treated with chemiluminescence reagents and exposed to film to reveal immunoreactive bands. The gray scale values of the bands were analyzed using Image J software, using the expression of β-actin as the reference.

2.7. Statistical methods

SPSS 20.0 was used for statistical analysis. Quantitative data with normal distribution are expressed as mean ± standard deviation ($\bar{x} \pm s$). When there was homogeneity of variance, one-way analysis of variance (ANOVA) was used and SNK or LSD-t tests or were used for multiple comparisons. The Kruskal-Wallis H tests were used when there was no homogeneity of variance. $p < 0.05$ indicates that the difference was statistically significant. Restrictive cubic spline (RCS) was plotted using R4.0.5 software. A regression fit model was also constructed, and the segmented regression model used the following equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - A) X_2$$

(1) When $X_1 \leq A$, $X_2 = 0$

$$Y = \beta_0 + \beta_1 X_1$$

(2) When $X_1 > A$, $X_2 = 1$,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - A) X_2$$

A is the dose node.

3. RESULTS

3.1. Effect of BDE-153 on the body weight of C57BL/6 mice

Time and group as separate factors had effects on body weight. Pairwise comparisons between the dose groups at different time points showed that at week 1 and 2, mice of the medium–high-dose group lost weight compared with the control group. Mice of the medium–high and the medium groups lost weight compared with the control group at week 3. Body weight was decreased in the high, medium–high, medium, medium-low-dose groups compared with the control group at week 4 (p all < 0.05, Fig. 1).

3.2. Effect of BDE-153 on blood lipids in C57BL/6 mice

Compared with the control group, the serum TG concentration in mice was reduced in the medium-low-dose group, and increased in the high-dose group. Compared with the low, medium-low-dose group, the TG concentration was increased in the high and medium–high-dose groups. Compared with the control, low, and medium-low-dose groups, the serum T-CHO concentration in mice was increased in the high, medium–high, and medium-dose groups. Compared with the middle dose group, the T-CHO concentration was increased in the high and medium–high-dose groups, and was reduced in the low and medium-low-dose groups. The serum HDL-C concentration of mice was decreased in BDE-153 dose groups compared with the control group. The serum LDL-C concentration of mice increased after BDE-153 exposure. (p all < 0.05, Table 1).

3.3. Effect of BDE-153 on serum leptin, adiponectin, and insulin levels in C57BL/6 mice

Serum leptin levels were increased in all dose groups compared with the control group. Compared with the low-dose group, the leptin levels were increased in the high- and medium-dose groups. The leptin levels of the high-dose group were higher than that of the medium-low-dose group. Serum adiponectin levels were reduced in BDE-153 dose groups compared with the control group. Compared with the low-dose group, the adiponectin levels were reduced in the medium–high-dose group. Adiponectin levels were reduced in the medium–high-dose group compared

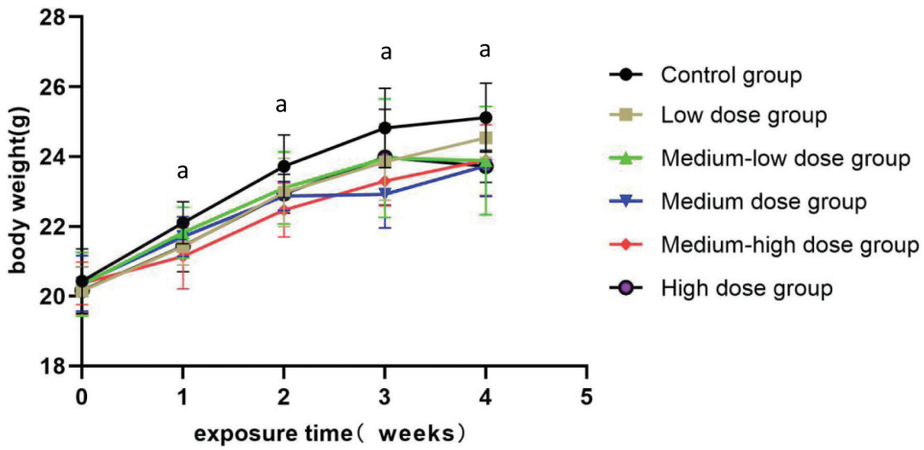

Fig. 1 Effect of BDE-153 on body weight in mice. [a]p < 0.05 compared with the control group. BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether.

Table 1

Comparison of blood lipids levels in mice treated with different doses of BDE-153 ($\bar{x} \pm s$, mmol/L)

Group	TG	T-CHO	HDL-C	LDL-C
Control	0.65 ± 0.05	3.47 ± 0.36	3.08 ± 0.41	1.31 ± 0.39
Low dose	0.57 ± 0.17	3.91 ± 0.60[a]	2.20 ± 0.61[b]	1.60 ± 0.39
Medium-low dose	0.37 ± 0.19[b]	3.43 ± 0.46[a]	2.56 ± 0.76	2.20 ± 0.71[b]
Medium dose	0.72 ± 0.09[c]	5.14 ± 0.53[b,c,d]	1.52 ± 0.50[b,c,d]	2.37 ± 0.98[b]
Medium–high dose	0.89 ± 0.10[c,d]	7.25 ± 0.36[a,b,c,d]	1.01 ± 0.25[b,c,d]	3.15 ± 0.83[a,b,c,d]
High dose	0.91 ± 0.40[b,c,d]	6.96 ± 0.21[a,b,c,d]	1.63 ± 0.27[b,c]	2.41 ± 0.34[b,d]

BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglyceride; T-CHO = total cholesterol.
[a]p < 0.05 compared with the medium-dose group.
[b]p < 0.05 compared with the control group.
[c]p < 0.05 compared with the medium-low-dose group.
[d]p < 0.05 compared with the low-dose group.

with the middle dose group. The serum insulin levels varied after BDE-153 exposure. Compared with the control group, low and medium-low-dose groups, the insulin levels were lower in the middle dose group. The insulin levels of the medium–high-dose group were higher than that of the medium-dose group (p all < 0.05, Table 2).

RCS analysis showed that there was a weak nonlinear inverted U-shaped dose–response relationship between BDE-153 exposure and serum leptin content (Fig. 2A), a weak nonlinear "U" dose–response relationship between BDE-153 exposure and serum adiponectin content (Fig. 2B), and a weak nonlinear "U" dose–response relationship between BDE-153 exposure and serum insulin content (Fig. 2C).

3.4. Effect of BDE-153 on organ coefficient, morphology, and histopathology in C57BL/6 mice

3.4.1. Effects of BDE-153 on weight and organ coefficient of the liver and pancreas in C57BL/6 mice

At the end of BDE-153 treatment, the weight and organ coefficient of the liver increased. Compared with the medium-low-dose group, the weight and organ coefficient of the liver were reduced in the medium–high-dose group. After BDE-153 exposure, the weight of the pancreas in all the groups increased with varying degree, except in the low-dose group. The organ coefficient of the pancreas increased significantly after BDE-153 exposure. Weight and organ coefficient of the pancreas of mice

Table 2

Comparison of serum leptin, adiponectin, and insulin levels in the different groups ($\bar{x} \pm s$)

Group	Leptin (ng/mL)	Adiponectin (ng/mL)	Insulin (ng/mL)
Control	5.60 ± 0.83	2838.50 ± 226.93	0.84 ± 0.20
Low dose	6.37 ± 0.63	2576.13 ± 241.20[a]	0.81 ± 0.19
Medium-low dose	6.90 ± 1.01	2417.75 ± 24.33[a]	0.93 ± 0.10
Medium dose	7.99 ± 0.85[a,b]	2645.25 ± 94.19	0.60 ± 0.17[a,b,c]
Medium–high dose	7.46 ± 1.20[a]	2327.65 ± 163.57[a,b,d]	0.89 ± 0.13[d]
High dose	8.46 ± 1.75[a,b,c]	2547.28 ± 184.68[a]	0.79 ± 0.14

[a] $p < 0.05$ compared with the control group.
[b] $p < 0.05$ compared with the low-dose group.
[c] $p < 0.05$ compared with the medium-low-dose group.
[d] $p < 0.05$ compared with the medium dose group.

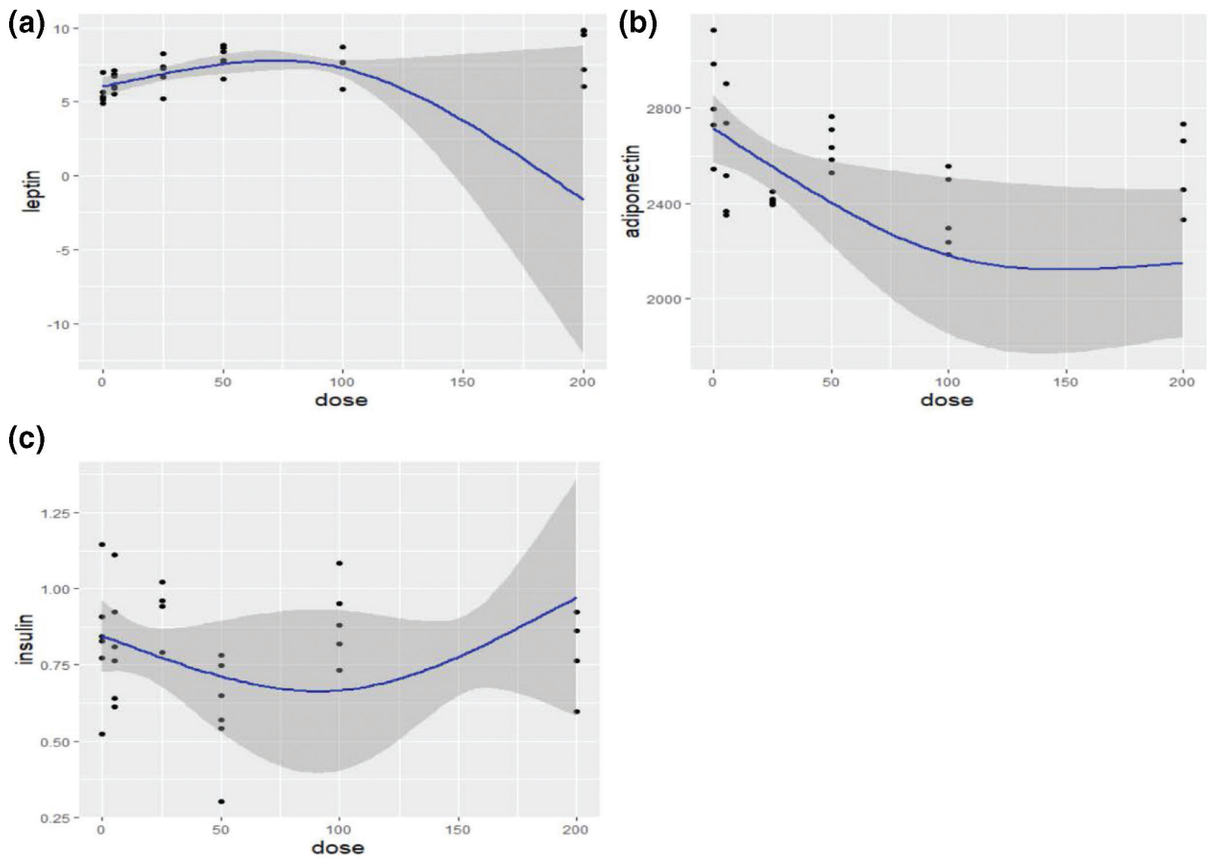

Fig. 2 Dose–response relationship between BDE-153 exposure and the serum leptin (A), adiponectin (B), and insulin (C) levels. BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether.

Table 3

Comparison of weight and organ coefficient of the liver and pancreas in the different groups ($\bar{x} \pm s$)

Group	Body weight (g)	Liver weight (g)	Pancreatic weight (g)	Liver organ coefficient (%)	Pancreatic organ coefficient (%)
Control	24.57 ± 0.94	1.06 ± 0.18	0.14 ± 0.03	4.32 ± 0.65	0.56 ± 0.13
Low dose	23.72 ± 1.25	3.43 ± 0.56[a]	0.13 ± 0.05	14.45 ± 2.41[a]	0.58 ± 0.24
Medium-low dose	23.72 ± 1.66	3.79 ± 0.85[a]	0.17 ± 0.03	15.86 ± 2.72[a]	0.74 ± 0.16
Medium dose	22.73 ± 0.78	3.23 ± 0.25[a]	0.17 ± 0.03	14.23 ± 1.11[a]	0.74 ± 0.13
Medium–high dose	23.58 ± 0.71	3.00 ± 0.28[a,b]	0.23 ± 0.02[a,b,c,d]	12.72 ± 0.95[a,b]	0.95 ± 0.10[a,b,c,d]
High dose	23.58 ± 0.88	3.44 ± 0.45[a]	0.24 ± 0.03[a,b,c,d]	14.56 ± 1.43[a]	1.00 ± 0.14[a,b,c,d]

[a]$p < 0.05$ compared with the control group.
[b]$p < 0.05$ compared with the medium-low-dose group.
[c]$p < 0.05$ compared with the low-dose group.
[d]$p < 0.05$ compared with the medium-dose group.

in the high and medium–high-dose groups were significantly higher than those in other groups (p all < 0.05, Table 3).

3.4.2. Effect of BDE-153 on liver morphology and liver histomorphology in C57BL/6 mice

The liver in the control group appeared normal: the size was uniform, the surface of the liver was smooth, the texture was soft, and the color was red. Livers of mice of the low and medium-low dose BDE-153 groups had an increased volume, darker color, and less surface smoothness compared with the control group. Compared with the control group, livers of the middle, medium–high, and high-dose BDE-153 groups were significantly larger in volume, had a dark red color, unsmooth surface, and blunt edges. In addition, granular diffuse fine nodules were seen on the liver surface of the mice in the high-dose group. In the control group, hepatocytes were regularly arranged, with close contact and no intracellular lipid droplet aggregation (Supplementary Figure S1, http://links.lww.com/JCMA/A180). In the BDE-153 groups, hepatocytes were enlarged to different degrees. Binuclear or multinucleation was observed in hepatocytes of the low and medium-low-dose groups, and a small number of hepatocytes was degenerated. In the medium, medium–high, and high-dose groups, mice had disrupted liver leaflet structures, increased collagen fibers in the confluence area, unclear cell boundaries, and lipid droplets aggregation. Fiber interval was evident in the high-dose group, and hepatocyte necrosis and local necrosis were observed in some regions (Supplementary Figure S2, http://links.lww.com/JCMA/A180).

3.4.3. Effects of BDE-153 on islet morphology and insulin expression in C57BL/6 mice

The brown stained regions in tissue sections of the pancreas indicate positive staining for insulin. This is the region where the islets are located. The number of islets in tissue sections was stable and islets were relatively normal in size in mice of the control group. With increasing doses of BDE-153, the number of islets gradually increased, the islet area slightly increased, and the morphology was more irregular. The number of islets was slightly increased in tissues of the pancreas in the low-dose group compared with the control group. The number of islets was significantly higher in tissues of the pancreas in the medium-low, medium, medium–high, and high-dose groups than in the control and low-dose groups. Islet volume was significantly increased in tissues of the pancreas of mice in the medium-low, medium, and medium–high-dose groups. A dose-dependent increase was found for positive insulin staining: positive rates of insulin staining in the high, medium–high, medium, medium-low-dose groups were higher than in the control and low-dose groups ($p < 0.05$) (Supplementary Figure S3, http://links.lww.com/JCMA/A180, Table 4).

Table 4

Comparison of rate of positive insulin staining in mice treated with different doses of BDE-153 ($\bar{x} \pm s$)

Group	Positive rate of insulin staining (%)
Control	4.49 ± 0.26
Low dose	4.51 ± 0.14
Medium-low dose	5.38 ± 0.17[a,b]
Medium dose	6.31 ± 0.20[a,b,c]
Medium–high dose	6.97 ± 0.22[a,b,c,d]
High dose	7.16 ± 0.84[a,b,c,d]

BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether.
[a]$p < 0.05$ compared with the control group.
[b]$p < 0.05$ compared with the low-dose group.
[c]$p < 0.05$ compared with the medium-low-dose group.
[d]$p < 0.05$ compared with the medium-dose group.

3.5. Effects of BDE-153 on glucose tolerance in C57BL/6 mice

The effect of different doses of BDE-153 over time on glucose tolerance in mice was analyzed by repeated measurement ANOVA testing. The interaction of group × time was statistically significant ($p < 0.05$), and the effects of BDE-153 among groups and time points were then separately tested. The fasting blood glucose was normal before peritoneal glucose injection, with consistent baseline levels in all groups. After intraperitoneal injection of the glucose solution, the blood glucose of mice in all groups increased first and then decreased. After 30 minutes, the blood glucose of mice in all groups peaked, then decreased to the baseline level after 120 minutes. After 30 minutes of glucose injection, glucose increased at a slower rate in the BDE-153 groups compared with the control group. The blood glucose values of mice in the high and medium-low-dose groups were lower than those in the control group, and the blood glucose level in the high-dose group was lower than that in the low-dose group ($p < 0.05$). After 120 minutes of glucose injection, glucose levels returned to baseline levels. There were no significant differences in the GAUC between the groups (Fig. 3).

3.6. Effect of BDE-153 on the mRNA expression levels of leptin, adiponectin, PPAR-γ, and AMPKα in the liver of C57BL/6 mice

After BDE-153 exposure, the mRNA expression of leptin, adiponectin, PPAR-γ, and AMPKα in mice changed. Compared with the control group, expression of leptin mRNA was downregulated in the liver in the low-dose group and upregulated in the other groups. The expression of leptin mRNA in the high-dose group was upregulated compared with that of the medium-low, low dose, and control groups. The expression

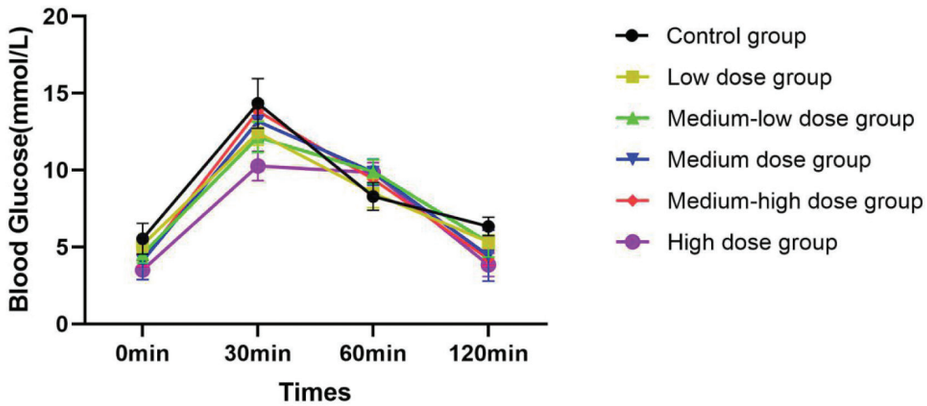

Fig. 3 Curves of blood glucose in different dose groups over time.

Table 5

Comparison of mRNA expression levels of leptin, adiponectin, PPAR-γ, and AMPKα in the liver tissue of mice ($\bar{x} \pm s$)

Group	Leptin	Adiponectin	PPARγ	AMPKα
Control	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Low dose	0.77 ± 0.21	1.07 ± 0.18	0.94 ± 0.11	0.46 ± 0.16[a]
Medium-low dose	1.10 ± 0.79	0.90 ± 0.08	1.03 ± 0.04	0.42 ± 0.07[a]
Medium dose	1.16 ± 0.05	0.77 ± 0.07[a,b]	1.29 ± 0.25[a,b,c]	0.40 ± 0.04[a]
Medium–high dose	1.38 ± 0.24[b]	0.59 ± 0.12[a,b,c]	1.21 ± 0.14[b]	0.29 ± 0.13[a,b,c]
High dose	1.79 ± 0.45[a,b,c]	0.47 ± 0.14[a,b,c,d]	1.41 ± 0.09[a,b,c]	0.36 ± 0.06[a]

PPAR-γ = proliferators activated receptors gamma.
[a]$p < 0.05$ compared with the control group.
[b]$p < 0.05$ compared with the low-dose group.
[c]$p < 0.05$ compared with the medium-low-dose group.
[d]$p < 0.05$ compared with the medium-dose group.

of leptin mRNA of the medium–high-dose group was upregulated compared with that of the low-dose group ($p < 0.05$). Compared with the control group, adiponectin mRNA was upregulated in the low-dose group, but downregulated in the other groups. The expression of adiponectin mRNA was downregulated in the high, medium–high, and medium-dose groups compared with the low and control groups ($p < 0.05$). The expression of adiponectin mRNA was downregulated in the high and medium–high groups compared with the medium-low-dose group ($p < 0.05$). Adiponectin mRNA expression was downregulated in the high-dose group compared with the medium-dose group ($p < 0.05$, Table 5). After BDE-153 exposure, the mRNA expression of PPARγ in the liver of mice in the low-dose group was downregulated, whereas it was upregulated in all other groups. mRNA expression levels of PPARγ in the high- and medium-dose groups were higher than those in the control group. mRNA expression levels of PPARγ in the high, medium–high, and medium-dose groups were higher than those in the low-dose group ($p < 0.05$). Compared with the medium-low-dose group, mRNA expression levels of PPARγ were upregulated in the high- and medium-dose groups ($p < 0.05$). After BDE-153 exposure, mRNA expression of AMPKα was downregulated in the liver of mice. The expression of AMPKα mRNA was downregulated in all groups compared with the control group ($p < 0.05$). The mRNA expression of AMPKα was downregulated in the medium–high-dose group compared with the low-dose group and downregulated in the medium–high-dose group compared with the medium-low-dose group ($p < 0.05$, Table 5).

BDE-153 exposure dose had a weak nonlinear dose–response relationship with leptin mRNA expression (Fig. 4A), a strong nonlinear "U" shaped dose–response relationship with adiponectin mRNA expression (Fig. 4B), a strong nonlinear inverted "U" shaped dose–response relationship with PPARγ mRNA expression (Fig. 4C), a strong nonlinear "U" shaped dose–response relationship with AMPKα mRNA expression (Fig. 4D).

The regression equation of adiponectin was: $Y = \beta 0 + \beta 1 X_1 + \beta 2\ (X_1 - A)\ X_2$

(1) When $X_1 \leq 77.897$, $X_2 = 0$

$$Y = \beta 0 + \beta 1 X_1 = -0.005727X + 1.058908$$

(2) When $X_1 > 77.897$, $X_2 = 1$,

$$Y = \beta 0 + \beta 1 X_1 + \beta 2\ (X_1 - A)\ X_2 = -0.00113X + 0.7008155$$

Adjusted $R^2 = 0.7477$

The A is the dose node.

The regression equation of PPARγ was: $Y = \beta 0 + \beta 1 X_1 + \beta 2\ (X_1 - A)\ X_2$

(1) When $X_1 \leq 50$, $X_2 = 0$

$$Y = \beta 0 + \beta 1 X_1 = 0.005732X + 0.944379$$

(2) When $X_1 > 50$, $X_2 = 1$

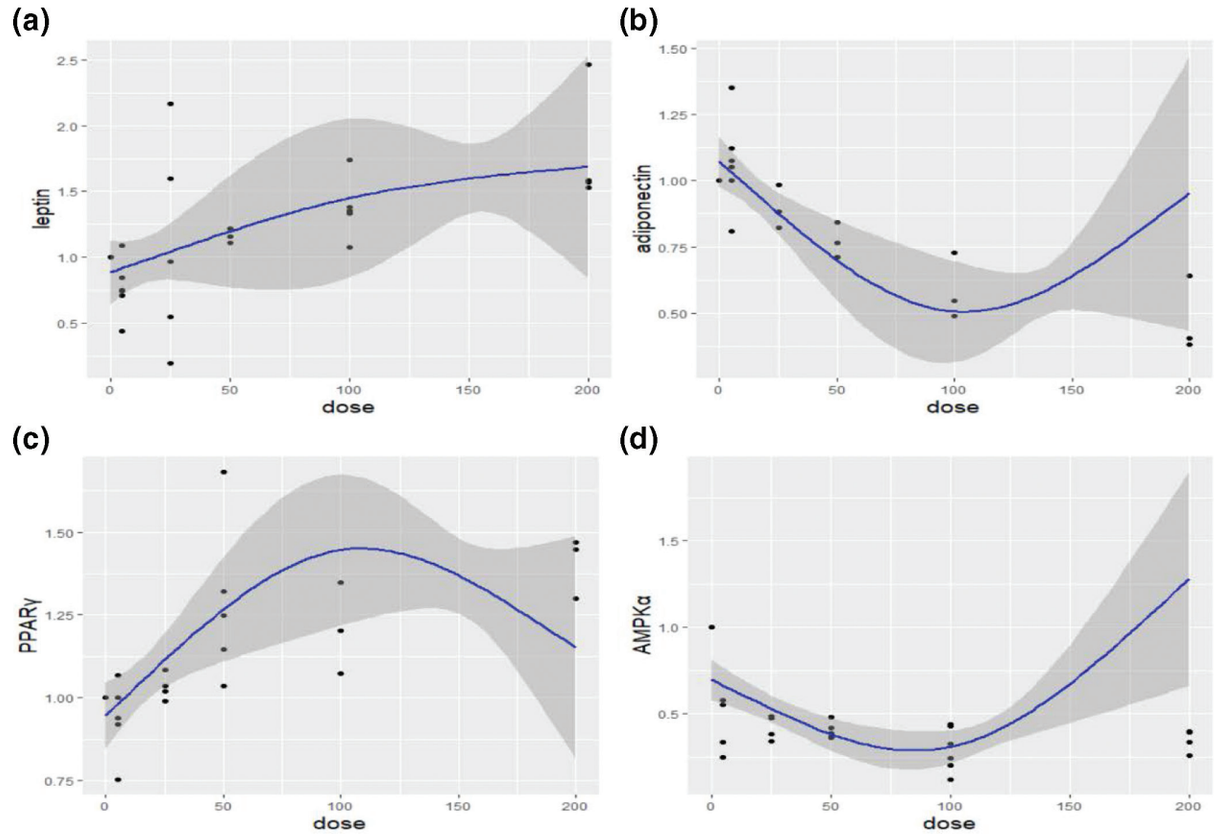

Fig. 4 Dose–response relationship between BDE-153 exposure and the mRNA expression of leptin (A), adiponectin (B), PPARγ (C), and AMPKα (D). BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether; PPAR-γ = proliferators activated receptors gamma.

$$Y = \beta 0 + \beta 1 X_1 + \beta 2 (X_1 - A) X_2 = \ 0.000998X + 0.707679$$

Adjusted R^2 = 0.5303

The A is the dose node.

The regression equation of AMPKα was: $Y = \beta 0 + \beta 1 X_1 + \beta 2 (X_1 - A) X_2$

(1) When $X_1 \leq 5.583$, $X_2 = 0$

$$Y = \beta 0 + \beta 1 X_1 = -0.10832X + 1$$

(2) When $X_1 > 5.583$, $X_2 = 1$,

$$Y = \beta 0 + \beta 1 X_1 + \beta 2 (X_1 - A) X_2 = -0.00036X + 0.39726$$

Adjusted R^2 = 0.8242

The A is the dose node.

3.7. Effect of BDE-153 on the protein expression levels of leptin, adiponectin, AMPKα, and p-AMPKα in the liver of C57BL/6 mice

Western blot results of PPARγ, adiponectin, leptin, p-AMPKα, and AMPKα genes in liver tissues of mice are shown in Fig. 5. After BDE-153 exposure, the expression levels of leptin protein in liver tissues were downregulated in mice in the low-dose group, and increased in the other BDE-153 groups. Leptin protein expression was upregulated in liver tissues in the high-dose group compared with that in the control group. Furthermore, leptin expression was upregulated in the high- and medium-dose groups compared with the low-dose group, and upregulated in the high-dose group compared with medium-low-dose group ($p < 0.05$). After BDE-153 exposure, adiponectin protein expression level was decreased in liver tissues in all groups compared with the control group. It was downregulated in the medium-dose group compared with the low-dose group, and downregulated in the high- and medium–high-dose groups compared with the medium-dose group ($p < 0.05$). After BDE-153 exposure, PPARγ protein expression was downregulated in liver tissues in the low and medium-low-dose groups. However, it was upregulated in the high, medium–high, and medium-dose groups. PPARγ protein expression levels in the high, medium–high, and medium-dose groups were higher than those in the control, low, and medium-low-dose groups ($p < 0.05$, Table 6).

After BDE-153 exposure, the protein expression level of AMPKα was downregulated in the liver tissues of all groups of mice compared with the control group, and downregulated in the high and medium–high-dose groups compared with the low, medium-low, and medium-dose groups ($p < 0.05$). After BDE-153 exposure, p-AMPKα protein expression levels were downregulated in liver tissues of mice, except in the medium-dose group. p-AMPKα protein expression levels in the high and medium–high-dose groups were downregulated in liver tissues compared with those in the control and medium-dose groups ($p < 0.05$). Compared with the low-dose group, p-AMPKα protein expression was downregulated in the high-dose group, and upregulated in the

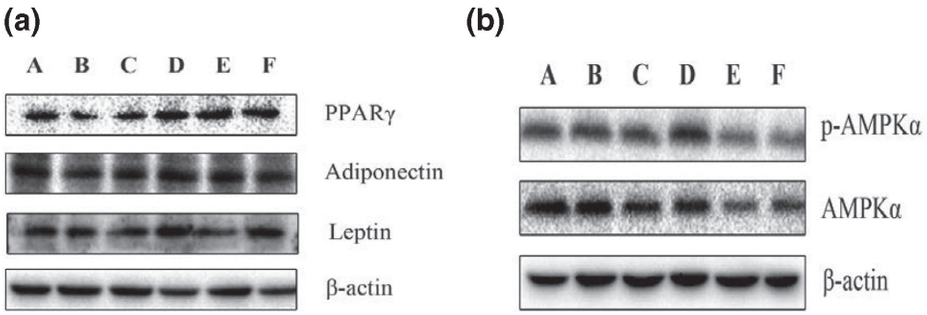

Fig. 5 Western blot results of PPARγ (a), adiponectin (a), Leptin (a), p-AMPKα (b), and AMPKα (b) genes in liver tissues of mice. A: Control group; B: low-dose group; C: medium-low-dose group; D: medium-dose group; E: medium–high-dose group; F: high-dose group. PPAR-γ = proliferators activated receptors gamma.

Table 6

Comparison of protein expression of leptin, adiponectin, PPARγ, AMPKα, and *p*-AMPKα among the groups of mice after BDE-153 exposure ($\bar{x} \pm s$)

Group	Leptin	Adiponectin	PPARγ	AMPKα	*p*-AMPKα
Control	0.56 ± 0.14	0.99 ± 0.14	0.44 ± 0.12	1.91 ± 0.31	0.75 ± 0.12
Low dose	0.50 ± 0.16	0.74 ± 0.10[a]	0.33 ± 0.06	1.60 ± 0.30[a]	0.64 ± 0.11
Medium-low dose	0.58 ± 0.15	0.81 ± 0.14[a]	0.39 ± 0.07	1.41 ± 0.27[a]	0.63 ± 0.18
Medium dose	0.69 ± 0.07[b]	0.92 ± 0.10[b]	0.60 ± 0.17[a,b,c]	1.44 ± 0.10[a]	0.80 ± 0.07[b,c]
Medium–high dose	0.58 ± 0.12	0.75 ± 0.07[a,d]	0.63 ± 0.11[a,b,c]	1.00 ± 0.28[a,b,c,d]	0.50 ± 0.16[a,d]
High dose	0.75 ± 0.10[a,b,c]	0.70 ± 0.16[a,d]	0.65 ± 0.11[a,b,c]	1.09 ± 0.22[a,b,c,d]	0.35 ± 0.11[a,b,c,d]

BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether; PPAR-γ = proliferators activated receptors gamma.
[a]$p < 0.05$ compared with the control group.
[b]$p < 0.05$ compared with the low-dose group.
[c]$p < 0.05$ compared with the medium-low-dose group.
[d]$p < 0.05$ compared with the medium-dose group.

middle dose group ($p < 0.05$). Compared with the medium-low-dose group, p-AMPKα protein expression was downregulated in the high-dose group, and upregulated in the medium-dose group ($p < 0.05$, Table 6).

BDE-153 exposure dose had a weak nonlinear dose–response relationship with protein expression of leptin (Fig. 6A), a weak nonlinear dose–response relationship with protein expression of adiponectin (Fig. 6B), a strong nonlinear inverted "U" dose–response relationship with protein expression of PPARγ (Fig. 6C), and a strong nonlinear "U" dose–response relationship with protein expression of AMPKα (Fig. 6D).

The regression equation of PPARγ is:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - A) X_2$$

(1) When $X_1 \le 64.239$, $X_2 = 0$

$$Y = \beta_0 + \beta_1 X_1 = 0.004012X + 0.360153$$

(2) When $X_1 > 64.239$, $X_2 = 1$,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - A) X_2 = 0.000243X + 0.60227$$

Adjusted $R^2 = 0.46$

The A is the dose node.

The regression equation of AMPKα is:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - A) X_2$$

(1) When $X_1 \le 97.32$, $X_2 = 0$

$$Y = \beta_0 + \beta_1 X_1 = -0.007709X + 1.748246$$

(2) When $X_1 > 97.32$, $X_2 = 1$,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 (X_1 - A) X_2 = 0.000928X + 0.90769316$$

Adjusted $R^2 = 0.5132$

The A is the dose node.

4. DISCUSSION

In this study, it was found that mice gained weight with longer exposure to BDE-153 and a small decrease in weight was observed with increasing BDE-153 dose. This is in contrast with the results of Gao et al.[18] In the study of Gao et al[18] different doses of BDE-47 to Sprague-Dawley female rats were administered by continuous gavage for 1 month until weaning their offspring. Results showed weight gain in male offspring in the exposure groups. Up to now, there are limited experimental studies on the association of exposure to PBDEs with weight, and the existing studies show inconsistent conclusions. Differences may be explained by the type of PBDEs, dose, exposure time, mode of intervention, subjects, and limited number and sample sizes of studies. Therefore, further studies with large sample sizes are still needed to explore the relationship between the PBDEs and weight.

Studies on lipid metabolism have found that either too high or too low HDL-C concentration increases the risk of hyperlipidemia.[19] Increased T-CHO levels are considered a major risk factor for cardiovascular disease. TG is another important risk factor, and LDL-C is the primary target of lipid-lowering

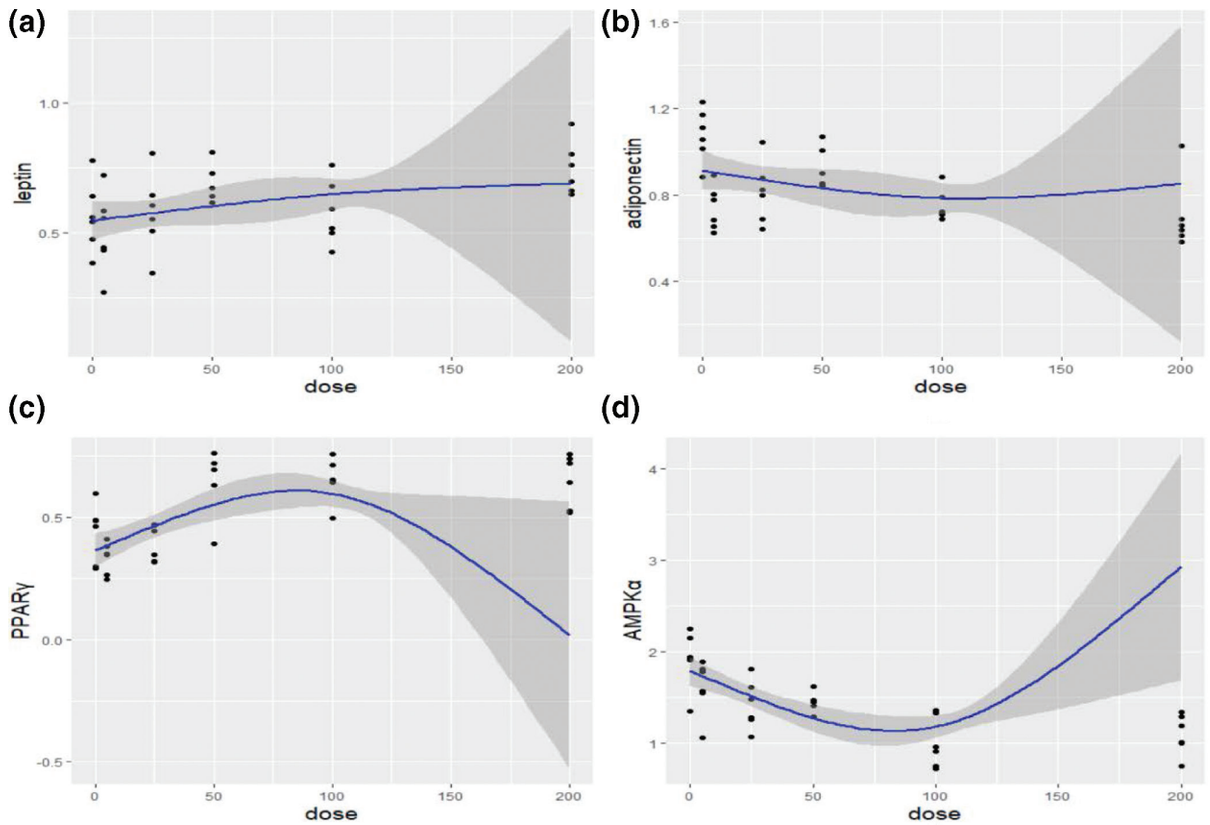

Fig. 6 Dose–response relationship between BDE-153 exposure and the protein expression of leptin (A), adiponectin (B), PPARγ (C), and AMPKα (D). BDE-153 = 2,2′,4,4′,5,5′-Hexabromodiphenyl ether; PPAR-γ = proliferators activated receptors gamma.

therapy.[20] Liver and adipose tissue play crucial roles in glycolipid metabolism. The results of this study showed that the serum TG, T-CHO, and LDL-C levels were significantly increased, and the HDL-C concentration was significantly reduced in mice after BDE-153 exposure. This is consistent with the results of the study by Huang,[21] in which 1,3-DCP was administered to wild-type C57BL/6 mice for 1 month. The concentrations of TG, T-CHO, and LDL-C in serum were significantly increased, and the concentration of HDL-C was significantly decreased. These results indicate that BDE-153 exposure causes hyperlipidemia and lipid metabolism disorder in mice. Combined with the changes of liver appearance and morphology, these results further indicate that BDE-153 induces the disorder of lipid metabolism in mice, and may lead to fatty liver.

When blood glucose levels increase in vivo, islet β cells secrete insulin, thereby maintaining stable blood glucose levels. When insulin resistance occurs, the body's sensitivity to insulin is reduced. To maintain the normal blood sugar level, the body compensates by high secretion of insulin. When insulin secretion cannot meet the needs of the body, abnormal increased blood sugar levels will lead to diabetes.[22] When determining the glucose tolerance status in T2DM, the glucose tolerance experiment is one of the most commonly used experiment in both clinical and animal experimental studies. In mice experiments, after fasting, glucose is injected resulting in increased blood glucose levels over time, which will return to normal within 2 hours. However, in the case of insulin resistance the time that blood glucose concentration has returned to basal levels is slightly prolonged.[23]

Furthermore, changes in islet function often are associated with islet morphology and structural lesions. Immunohistochemical (IHC) analysis of the pancreas in mice in this study showed a dose-dependent increase in the rate of positive staining for insulin, indicating that the number of islet β cells increased with increasing BDE-153 dose. This is consistent with the findings of increased weight and organ coefficient in the pancreas after BDE-153 exposure. The intraperitoneal glucose tolerance test (IPGTT) results showed no significant differences in fasting blood glucose between groups. After 30 minutes of glucose injection, the high and medium-low-dose groups had lower blood glucose than the control group, indicating that blood glucose increased slower in the BDE-153 groups after intraperitoneal injection of glucose than in the control group. The blood glucose of mice treated with BDE-153 was decreased compared with the control group, possibly due to the increased sensitivity to insulin or excessive insulin synthesis and release.[24] Decreased glucose levels were only observed at 30 minutes after glucose injection in BDE-153 groups when compared with the control group, while there were no differences in glucose levels at other time points. Combined with the result of increased weight of the pancreas and increased number of islet β cells, we hypothesize that the reduced glucose levels in mice were caused by increased insulin release. Although serum insulin levels were reduced in the middle dose group, the medium-low and medium–high-dose groups tended to have higher insulin levels compared with the control group. When observation would be prolonged, mice may develop hyperinsulinemia, or even insulin resistance. This suggests that BDE-153 not only affects the morphology of the mouse pancreas and islets, but also leads to its impaired function, causing aberrant insulin secretion.

White adipose tissue mainly secretes cytokines such as adiponectin, leptin, and resistin, and widely participates in various physiological metabolism under the regulation of PPARγ.

At the organ level, leptin activates PPAR-α in nonadipocytes, thereby promoting adipolysis. In skeletal muscle cells, leptin activates adenylate activated protein kinase (AMPK) and enhances fat metabolism.[25] qPCR results showed that the expression of leptin and PPARγ mRNA was upregulated in all groups and the expression of adiponectin mRNA was downregulated in all groups except the low-dose group after BDE-153 exposure. The results of Western blotting showed that the expression of leptin protein was upregulated except in the low-dose group and adiponectin expression was downregulated in all groups, which was consistent with the results of qPCR. Compared with the control group, the mRNA and protein expression of AMPKα in each exposed group decreased, and the protein expression of p-AMPKα decreased. Our results suggest that BDE-153 may upregulate the mRNA and protein expression of leptin and downregulate the mRNA and protein expression of adiponectin, and then affect the changes of leptin and adiponectin content in circulating blood. The increased expression of leptin indicates that exposure to BDE-153 will have an adverse effect on serum leptin in mice. Clinical studies have shown that high circulating leptin levels are related to the severity of fatty liver.[26] Adiponectin is associated with improved outcomes in T2DM, and studies have found that the lower the level of plasma adiponectin, the higher the risk of T2DM.[27,28] In vivo and vitro studies have shown that the fattening effect of TBT is partly mediated by the methylation of PPARγ itself or the methylation of PPARγ target gene.[29] Studies have reported that EDCs such as tributyltin, BDE-47 and polycyclic aromatic hydrocarbons can act as ligands for PPARγ nuclear receptors and have been proved to have obesity inducing effects.[30] Studies have shown that the expression and activity of AMPK in skeletal muscle tissue of long-term high-fat fed rats decreased, and the activity of AMPK was negatively correlated with TG, FPG, and FFA,[31] which confirmed the close relationship between the decrease of AMPK expression and activity and insulin resistance and glucose and lipid metabolism disorder. Our experiment showed that high dose of BDE-153 exposure increased the mRNA and protein expression levels of PPARγ. BDE-153 reduced the mRNA and protein expression levels of AMPKα and protein expression level of p-AMPKα in exposure groups of mice. Our results suggest that BDE-153 may regulate the expression of cytokines such as leptin and adiponectin by affecting the mRNA and protein expression of PPARγ and AMPKα, and eventually lead to the disorders of lipid metabolism. RCS results showed that the expression of serum leptin increased with the increase of BDE-153 exposure dose. BDE-153 exposure showed an inverted U-shaped association with the expression of PPARγ (a strong effect across low doses but a weakened or no effect at high doses), and showed an U-shaped association with the expression of adiponectin and AMPKα (a strong effect underacross low doses and high doses). The results of the segmented regression model showed that the effect of BDE-153 on PPARγ, AMPKα, and adipocytokines had a nonlinear dose–response relationship. An epidemiological investigation on association of brominated flame retainers with diabetes and metabolic syndrome in the US population found that BDE-153 showed an inverted U-shaped association with diabetes and metabolic syndrome.[32] Wang et al[33] found that there was a nonlinear dose–response relationship between the exposure level of PBDEs during pregnancy and birth weight. Most adipokines influence the metabolic effects of insulin on glucose and fat, for example, tumor necrosis factor, leptin, adiponectin, and resistin.[34] Abnormal secretion of adipokines can affect the biological effects of insulin thereby accelerating the progression of diabetes.[35]

Our results suggest that BDE-153 may interfere with the secretion of adipokines and insulin by affecting the expression of PPARγ and AMPKα, which play key roles in glucose and lipid metabolism, leading to the occurrence of glucose and lipid metabolism disorder. And our study found the bidirectional regulation of BDE-153 on the expression of PPARγ, AMPKα, and adipocytokines. However, studies focusing on the precise mechanism by which BDE-153 cause the disorder of glucose and lipid metabolism are highly warranted, and more research is required to identify the duration, dose, and impact of long-term exposure of BDE-153 to clarify its risk assessment.

ACKNOWLEDGMENTS

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A180.

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