Archives of Toxicology
https://doi.org/10.1007/s00204-018-2177-0

REPRODUCTIVE TOXICOLOGY

In utero and lactational exposure to BDE-47 promotes obesity development in mouse offspring fed a high-fat diet: impaired lipid metabolism and intestinal dysbiosis

Dezhen Wang[1] · Jin Yan[1] · Miaomiao Teng[1] · Sen Yan[1] · Zhiqiang Zhou[1] · Wentao Zhu[1]

Received: 12 November 2017 / Accepted: 26 February 2018

Abstract
In this study, we investigated the effects of in utero and lactational exposure to BDE-47 on the progression of obesity and metabolic dysfunction in a diet-induced obesity model. Pregnant ICR mice were treated via oral gavage with low doses of BDE-47 (0, 0.002, and 0.2 mg/kg body weight) from gestational day 6 to postnatal day 21. After weaning, male offspring were fed an AIN93-based normal diet (ND) or high-fat diet (HFD: 60% calories from fat) for 14 weeks. We examined body weight, liver weight, histopathology, blood biochemistry, gene expression, and serum metabolic changes. A combination of 16S rRNA gene sequencing and ^{1}H NMR-based metabolomics was conducted to examine the effects of BDE-47 on the gut microbiome. Results showed that in utero and lactational exposure to BDE-47 caused a worsening of HFD-induced obesity, hepatic steatosis, and injury; impaired glucose homeostasis and metabolic dysfunction, and mRNA levels of genes involved in lipid metabolism were significantly altered in the BDE-47-treated HFD group. The gut microbiome were perturbed by BDE-47, causing diversity reduction, compositional alteration, and metabolic changes. These changes were more pronounced for BDE-47-treated HFD mice. All these results indicate that early life exposure to low doses of BDE-47 can promote obesity and the development of metabolic dysfunction.

Keywords BDE-47 · Obesity · NAFLD · Gut microbiome · Metabolomics

Introduction

Polybrominated diphenyl ethers (PBDEs), which were once widely used as flame retardants in textiles, electronics, plastics, and furniture, were restricted or banned in some countries in the mid-2000s because of these compounds' persistence, bioaccumulation, and adverse health effects in humans and wildlife (EPA 2007; Meeker et al. 2009). After being withdrawn from use in products, PBDE levels are decreasing in human samples in some areas (Guo et al. 2016), but levels are still high enough to potentially induce significant reproductive and developmental toxicity (Suvorov and Takser 2010). Moreover, the use of products containing PBDEs or the re-processing of e-waste could contribute to the release of environmentally relevant compounds. Consistent with this, residents in the vicinity of e-waste recycling facilities can experience high exposures to these compounds (Song and Li 2014). Therefore, it is necessary to evaluate PBDE-induced health effects on the local populations.

Among the 209 PBDE structures, BDE-47 is the most abundant in human samples, and BDE-47 concentrations in serum samples from Jiading, Shanghai were estimated as 0.978–5.380 ng/g lipid weight (Xu et al. 2017), while three cohorts of Northern California women showed a geometric mean of BDE-47 serum concentrations as high as 24.6 ng/g in 2014, having reached a plateau between 2011/12 and 2014 (Parry et al. 2018). BDE-47 exposure has been associated with liver toxicity (Dunnick and Nyska 2009; Suvorov and Takser 2010), neurotoxicity (Dingemans et al. 2007; Koenig et al. 2012), thyroid toxicity (Talsness et al. 2008),

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00204-018-2177-0) contains supplementary material, which is available to authorized users.

✉ Wentao Zhu
wentaozhu@cau.edu.cn

[1] Beijing Advanced Innovation Center for Food Nutrition and Human Health, Department of Applied Chemistry, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, People's Republic of China

Published online: 09 March 2018

and reproductive toxicity (Huang et al. 2015; Talsness et al. 2008). Recent studies have further linked BDE-47 exposure to an increase in the incidence of obesity and diabetes in animal models. For example, perinatal exposure to low doses of BDE-47 has been shown to increase body weight, plasma IGF-1, and glucose uptake in male adult rats (Suvorov et al. 2009), while perinatal exposure to BDE-47 caused a decrease in the weight of mice offspring after weaning day. Body weight changes in adulthood have rarely been reported in the previous studies (Ta et al. 2011). More importantly, BDE-47's biological effects and obesity-related mechanisms have not been clarified. Therefore, in this study, we evaluated the effects of fetal exposure to low doses of BDE-47 on obesity and related metabolic dysfunction in adult mice.

The prevalence of obesity worldwide has dramatically increased over past 50 years. Many factors such as age, genetics, and dietary habits have been identified as major risks, but they cannot sufficiently explain the rapid increase in obesity and obesity-related diseases in recent years. There is growing evidence that environmental toxins may play vital roles in obesity and obesity-related metabolic syndrome pathogenesis and development. Among obesity-related mechanisms, impaired lipid metabolism is thought to be a key cause. Gut microbiota, which play important roles in metabolic processing, nutrient digestion, immune development, and epithelial homeostasis (Nicholson et al. 2012), can also be an important factor in the development of obesity and metabolic syndromes (Devaraj et al. 2013; Upadhyaya and Banerjee 2015). Because the homeostasis of gut microbiota develops in early life and the composition of the gut microbiome is highly diverse, it is vulnerable to external factors, such as environmental toxins, diet, and antibiotics at this stage. Therefore, the harmful effects of environmental chemicals on human health may be exacerbated by gut microbiome alteration or disruptions. Several studies have demonstrated positive interactions between environmental chemicals and gut microbiota. For example, 2,3,7,8-tetrachlorodibenzofuran (TCDF) exposure altered the gut microbiota by shifting the ratio of *Firmicutes* to *Bacteroidetes*, which further led to altered bile acid metabolism, inflammation, and host metabolic disorders (Zhang et al. 2015). Diazinon exposure induced perturbations of the gut microbiome and its functions that were identified as potential mechanisms contributing to diazinon neurotoxicity (Gao et al. 2017). Early low-dose cadmium exposure that caused diversity reduction and composition alterations of gut microbiota led to fat accumulation in adult male mice (Ba et al. 2017). Here, to elucidate the role of gut microbiota in BDE-47-induced metabolic orders, we examined the impact of BDE-47 exposure on the gut microbiome composition and on metabolic profiles.

In this study, we evaluated hepatic steatosis and injury, glucose tolerance, and metabolic profiles in adult mice after gestational and lactational exposure to low doses of BDE-47. Genes involved in lipid metabolism were quantified by a quantitative polymerase chain reaction (qPCR); the effects of BDE-47 on the gut microbiome were examined using a combination of 16S rRNA gene sequencing and nuclear magnetic resonance (NMR) spectroscopy-based fecal metabolomics.

Materials and methods

Animals and exposure

Primigravida pregnant ICR mice were purchased from Peking University Health Science Center and were housed individually under controlled environmental condition (22 °C, a 12:12 h light:dark cycle) with free access to food and water. The pregnant mice were provided with a rodent growth and breeding feed (Keao Xieli Feed Co., Ltd, Beijing, China) containing the constant phytoestrogens in it, excluding the absence of phytoestrogens feed during fetal development on obesity development of mice offspring (Ruhlen et al. 2008), and all animals were exposed to the same levels of phytoestrogens according to the equivalent food intake among groups. From gestational day 6 to postnatal day 21, pregnant mice (six pregnant mice per group) were orally gavaged with corn oil (control group, vehicle) or BDE-47 (0.002 mg/kg (low, L) or 0.2 mg/kg (high, H) BW) corn oil solutions. Each dam was culled to eight pups at postnatal day 2. The low dose was within the relevant human concentration and equivalent to that used in a previous low-dose exposure study in rats (Suvorov and Takser 2010). After weaning at day 21, male offspring were divided into two groups (six mice per group and one mice from each dam) by providing them with an AIN 93 based normal diet (ND) or a western high-fat diet (HFD: 60% calories from fat, Trophic Animal Feed High-tech Co. Ltd., Beijing, China) for 14 weeks. Body weights were measured weekly in the morning, and blood was collected at weaning, at 14 weeks after fasting overnight and at 16 weeks at the end of this study. The experimental design is shown in Fig. 1. The male offspring were selected, because many previous studies had demonstrated that male mice were more sensitive to environmental toxin-induced obesity and glucose intolerance than females (Alonso-Magdalena et al. 2010; Ba et al. 2017), which was probably due to the presence of physiological estrogens that protected female mice against metabolic diseases (Liu and Mauvais-Jarvis 2010). All animals were treated humanely and with regard to the alleviation of suffering according to the independent Animal Ethical Committee of China Agricultural University.

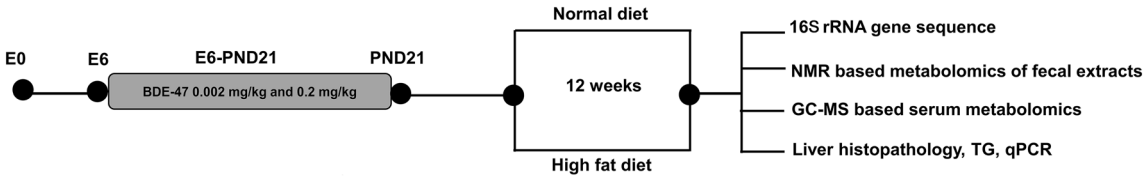


Fig. 1 Experimental design. From gestational day 6 to postnatal day 21, pregnant mice were orally gavaged with corn oil (control group, vehicle) or BDE-47 (0.002 mg/kg (low, L) or 0.2 mg/kg (high, H) BW) corn oil solutions. After weaning at day 21, male offspring were divided into two groups by providing them with an AIN 93 based normal diet (ND) or a western high-fat diet (HFD: 60% calories from fat) for 14 weeks ($n = 6$)

Blood measurements

Blood glucose levels were determined using a glucose meter (Beijing Yicheng JSP-7, China). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using their respective assay kits from Nanjing Jiancheng Bioengineering Institute. Serum thyroxin (T4) levels were determined by enzyme-linked immunosorbent assay (ELISA) kits (Hengyuan Biological Technology Co., Ltd., Shanghai, China).

Glucose tolerance test

A glucose tolerance test (GTT) was performed to assess glucose homeostasis by evaluating the effectiveness of the clearance of the glucose load administrated by oral gavage. At 14 weeks, mice were orally administrated D-glucose (2 g/kg) in sterilized 0.9% NaCl after overnight fasting, and glucose were measured before and 15, 30, 45, 75, and 105 min after glucose loading.

Liver triglyceride, total cholesterol, and histopathology analysis

Lipids were extracted with 900 µl chloroform:methanol ($v/v = 2:1$) from 100 mg liver using MM 400 (RETSCH, Germany). Liver triglyceride (TG) and cholesterol (T-Cho) were quantified by TG and total T-Cho assay kits (Nanjing Jiancheng Bioengineering Institute, China), respectively, according to the manufacturer's instructions. Liver sections from 16-week-old mice were frozen using the optimal cutting temperature or fixed in 10% neutral buffered formalin for 24 h, and then embedded in paraffin for histological examination. Tissue sections were stained with either Oil Red O or hematoxylin–eosin (H&E) and examined under light microscopy at 200× magnification.

RNA extraction and qPCR analysis

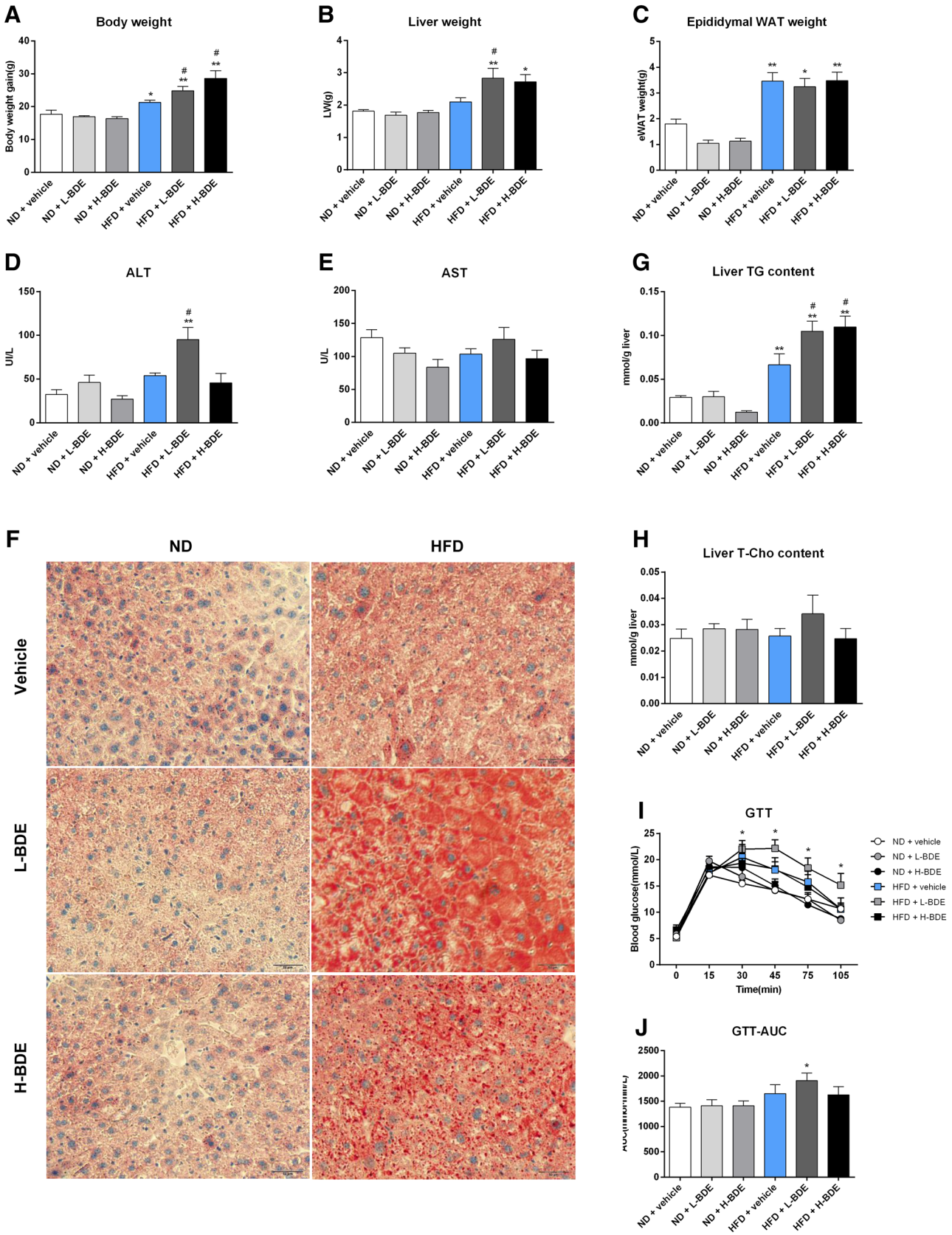
Total RNA was extracted from the liver of six treatment groups (ND + vehicle, ND + L-BDE, ND + H-BDE, HFD + vehicle, HFD + L-BDE, and HFD + H-BDE, five mice/group) with TRIzol A + reagent (Qiagen, Beijing, China) according to the manufacturer's instructions. Then, RNA (1.5 µg) was reverse-transcribed to cDNA using a FastQuant RT Kit (with gDNase) (Qiagen, China) after the removal of genomic DNA. The qPCR was performed on Bio-Rad CFX 96 PCR system with SuperReal PreMix Plus (SYBR Green) (Qiagen, China). The relative mRNA levels were normalized with the delta–delta cycle threshold (C_t) method, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene, and the expression levels in the ND + vehicle group were set to 1. PCR primers (Table S1) were ordered from Sangon Biotech, Shanghai, China.

16S rRNA gene sequencing

Fecal pellet bacteria were extracted using a DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For 16S rRNA gene sequencing, 16S rDNA was amplified with universal 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′) primers to target the V3–4 region of 16S genes, followed by purification with an AxyPrep DNA Purification kit (Axygen Biosciences, Union City, USA), and quantification by a QuantiFluor-ST Fluoremeter (Promega, Wisconsin, USA) and PicoGreen dsDNA Quantitation Reagent (Invitrogen, Carlsbad, USA) to produce an even concentration. Sequencing was performed on an Illumina Miseq platform (Illumina, San Diego, USA). Data analysis was performed using QIIME software (version 1.8, http://qiime.org/) and UCLUST (version 1.2.22, http://www.drive5.com/uclust/downloads1_2_22q.html) for operational taxonomic unit (OTU) picking and diversity analysis.

^{1}H NMR-based metabolomics analysis of fecal extracts

Feces from 16-week-old mice were subjected to an NMR-based metabolomics analysis. Briefly, 50 mg fecal pellets were directly extracted by precooled phosphate buffer with


A
Body weight
Body weight gain(g)
B
Liver weight
LW(g)
C
Epididymal WAT weight
eWAT weight(g)
D
ALT
U/L
E
AST
U/L
G
Liver TG content
mmol/g liver
F
ND
HFD
Vehicle
L-BDE
H-BDE
H
Liver T-Cho content
mmol/g liver
I
GTT
Blood glucose(mmol/L)
Time(min)
ND + vehicle
ND + L-BDE
ND + H-BDE
HFD + vehicle
HFD + L-BDE
HFD + H-BDE
J
GTT-AUC

◂**Fig. 2** Effects of BDE-47 exposure on a high-fat diet (HFD)-induced obesity, hepatic steatosis, injury, and glucose homeostasis. **a–c** Body weight (BW) gain, liver weight, and epididymal white adipose tissue (WAT) weight. **d, e** Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. **f** Representative images of Oil Red O staining of hepatic sections from different groups. **g, h** Liver triglycerides (TG) and total cholesterol (T-Cho) content measured at 16 weeks. **i** Glucose tolerance test (GTT). **j** Area under curve (AUC) of GTT. $^{*}P<0.05$, $^{**}P<0.01$ compared with the normal diet (ND) + vehicle group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the HFD + vehicle group, $n=6$

homogenization using an MM 400 (Retsch GmbH, Germany). After centrifugation at 10,800×*g* for 10 min, 550 µl of each supernatant was transferred into a 5 mm NMR tube. The ^{1}H NMR analysis of fecal extracts was recorded on a Bruker Avance III 600 spectrometer (Bruker, Billerica, MA, USA) at 298 K. Typical one-dimensional NMR spectra were acquired for each of the samples using the first increment of the nuclear Overhauser effect spectroscopy pulse sequence (NOESYPR1D) according to a previous study (Gronwald et al. 2008). All NMR spectra were processed using the software MestRenova 6.1.0. Free induction decays were multiplied by an exponential function of 1 Hz prior to Fourier transformation. Phase and baseline corrections were then manually performed and the spectral region δ0.5–9.0 was integrated into regions with an equal width of 0.01 ppm after excluding regions of water resonances. The remaining integrated areas were normalized to the total spectral area to facilitate comparisons among the samples.

Gas chromatography–mass spectrometry (GC–MS)-based serum metabolomics analysis

Serum from 16-week-old mice was subjected to a GC–MS-based metabolomics analysis. Briefly, 50 µl of serum was extracted with 200 µl of precooled methanol, followed by centrifugation at 10,000×*g* for 10 min. The resultant upper phase (150 µl) was transferred into a GC–MS vial and evaporated to dryness under a stream of nitrogen, followed by derivatization with 50 µl of *O*-methoxylamine (20 mg/ml in pyridine) and then 50 µl of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (1% TCMS silylation reagent). Analyses were performed using a Trace GC gas chromatograph coupled to a Quantum XLS mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) running in full scan mode. The resultant data were processed with XCMS to extract peak intensities. Features with significant changes were identified from thein National Institute of Standards and Technology (NIST) Standard Reference Database.

Multivariate statistical analysis

Binning data from the NMR spectroscopy or peak intensities from GC–MS were imported into SIMCA + 13.0 for multivariate statistical analysis. Principal component analysis (PCA) was first performed to reveal the intrinsic clusters and exclude potential outliers. Partial least squares-discriminant analysis (PLS-DA) was then conducted on the refined data. Variable Importance in Projections (VIPs) based on loading plots was used to determine metabolites that best defined group separations. The discriminated metabolites were also assessed using non-parametric tests (Kruskal–Wallis) or parametric tests [analysis of variance (ANOVA)] via SPSS 19.0 (IBM, USA). Metabolites with VIP > 1 and $P<0.05$ were selected as potential discriminatory features.

Statistical analysis

All the experimental values are presented as the mean ± SEM. Graphical illustrations were performed with GraphPad Prism version 6.0 (GraphPad), and statistical analyses were performed using SPSS 19.0 (IBM, USA). The significance of variables among the different groups was determined by one-way ANOVA and a Tukey's post-hoc test ($P<0.05$). The normality of the variables was confirmed by the Shapiro–Wilk test and homogeneity of variance by the Levene's test. A non-parametric Kruskal–Wallis was used when data did not meet the parametric assumptions. *P* values < 0.05 were considered to be significant.

Results

Effects of BDE-47 exposure on litter size and body weight

BDE-47 exposure has no adverse effects on the birth date and litter size; however, decreased body weights were observed at day 21 (Figure S1A), along with a decreased level of T4 in the high-dose BDE-47 exposure group (Figure S1B).

Effects of BDE-47 exposure on HFD-induced hepatic steatosis, injury, and glucose homeostasis

The decreased body weight observed during puberty prompted us to investigate whether this body weight change continued later in life and affected metabolism. Therefore, we recorded body weight changes from 4 to 16 weeks in male mice exposed to BDE-47 after mice were fed either an ND or HFD for 12 weeks starting at the age of 4 weeks. After 12 weeks of diet intervention, body weight, body composition, hepatic steatosis, hepatic injury, and glucose

homeostasis were investigated. The HFD led to a significant increase in body weight, liver weight, and epididymal fat weight when compared with the ND, and this increase was further enhanced by H-BDE and L-BDE in the HFD-fed mice, whereas BDE had no effect on these factors in mice that were fed an ND (Fig. 2a–c). Hepatic injury was roughly assessed by the serum levels of ALT and AST. Serum ALT activities were significantly higher in the HFD + L-BDE group than in the ND + vehicle group, while serum activities of AST were not different between either groups (Fig. 2d, e). The histological analysis of liver samples showed the presence of steatosis in HFD-fed mice. In addition, BDE-47 exposure significantly worsened steatosis and led to severe microvesicular fatty changes and hepatocyte ballooning (Fig. 2f). The worsened steatosis caused by BDE-47 was demonstrated by the quantification of TG and T-Cho (Fig. 2g, h), which indicated an additive effect of BDE-47 exposure. We further determined the effect of BDE-47 exposure on glucose homeostasis. The fasting glucose levels were significantly increased by the HFD but not by BDE-47 (Supplemental Material, Figure S2). However, impaired glucose tolerance was only observed in the HFD + L-BDE group (Fig. 2i), accompanied by the elevation of the total area under the curve (AUC) for blood levels in GTT (Fig. 2j). These observations suggest that in utero and lactation exposure to BDE-47 has no effects on obesity, NAFLD, and glucose tolerance in ND-fed mice, whereas BDE-47 exposure leads to obesity, a worsening of hepatic steatosis, and injury and impaired glucose tolerance when combined with a HFD intervention.

Effects of BDE-47 exposure on expression of genes involved in lipid metabolism, inflammation, and xenobiotic metabolism

To elucidate the mechanisms involved in the aggravation of hepatic steatosis and injury in HFD + BDE groups, the levels of several genes that are markers of lipid metabolism, and involved, respectively, in fatty acid synthesis (*Fasn, Scd1, Srebf1*, and *Acaca*), fatty acid uptake and lipid storage (*Pparγ* and *Cd36*), β-oxidation (*Pparα* and *Cpt1α*), and TG synthesis (*Dgat1, Dgat2*, and *Mogat*) were quantified by qPCR (Fig. 3a). When combined with an HFD intervention, BDE-47 exposure significantly increased the expression of *Fasn*, an enzyme which encodes an enzyme crucial for palmitate synthesis. The expression of *Scd1*, which encodes an endoplasmic reticulum enzyme that catalyzes the rate-limiting step in the formation of monounsaturated fatty acids, was decreased in HFD-fed mice, with no additive effects of BDE-47. Expressions of *Pparα* and its target gene *Cpt1α*, which regulate fatty acid catabolism and mitochondrial β-oxidation, were significantly increased by BDE-47 exposure in ND-fed mice, whereas co-treatment BDE-47 with an HFD counteracted the increase in *Pparα* and *Cpt1α*. Compared with the ND + BDE and HFD + vehicle groups, HFD + H-BDE resulted in a further increase in expression of the nuclear receptor gene *Pparg* and fatty acid transporter *Cd36*, which encodes proteins involved in fatty acid uptake and lipid storage. Similarly, expression of *Mogat1*, encoding an upstream enzyme regulating the monoacylglycerol pathway, was increased in the HFD + BDE groups compared with the ND groups and HFD + vehicle group. In contrast, expressions of *Dgat1* and *Dagat2*, encoding two enzymes involved in de novo TG synthesis, displayed a non-significant decrease in HFD-fed mice, but there was an increase in the ND + H-BDE group (for *Dagat2*). These results suggested that BDE-47 exposure led to a dramatic increase in the accumulation of TG by activating the monoacylglycerol pathway rather than de novo TG synthesis. In addition, BDE-47 exposure could promote fatty acid uptake while inhibiting fatty acid catabolism, which may result in an increase in fatty acid levels and the inflammation response when combined with an HFD intervention.

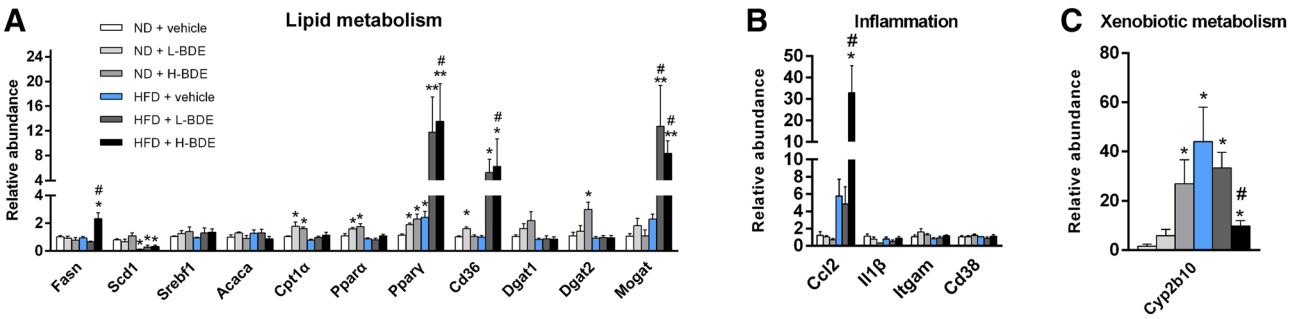


Fig. 3 Effects of co-exposure to a high-fat diet (HFD) and BDE-47 on the hepatic mRNA levels of the markers of lipid metabolism, inflammation, and xenobiotic metabolism. The mRNA levels of hepatic genes were measured by a quantitative polymerase chain reaction (qPCR). Mean expression in the normal diet (ND) + vehicle group was set at 100%. **a** Markers of lipid metabolism (fatty acid synthesis, fatty acid β-oxidation, fatty acid uptake, and triglyceride (TG) synthesis). **b** Markers of inflammation. **c** Marker of xenobiotic metabolism. $^{*}P<0.05$, $^{**}P<0.01$ compared with the ND + vehicle group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the HFD + vehicle group, $n=6$

We further quantified the expression levels of the genes *Ccl2, Ilβ, Itgam*, and *Cd38*, as markers of inflammation (Fig. 3b). Except for gene *Ccl2*, the expression of which was significantly increased by the combination of HFD and BDE-47, expressions of the other three genes were not influenced by either BDE-47 or HFD. The expression levels of the target gene of the constitutive androstanol receptor (CAR), *Cyp2b10* were increased by either BDE-47 or HFD alone, whereas in combination, they led to a relative decrease in the expression of *Cyp2b10* (Fig. 3c).

Effects of BDE-47 exposure on gut microbiome composition and its metabolic profiles

We examined the changes in the gut microbiome following prenatal and postnatal exposure to BDE-47 in a diet-induced obesity model. The effects of BDE-47 on gut microbiome were determined by a 16S rRNA sequence-based analysis of fecal pellets. PCA and non-metric multidimensional scaling (NMDS) of the 16S rRNA sequencing results indicated that BDE-47 exposure induced remarkable changes in the overall gut microbiota community structure and composition in both ND and HFD-fed BDE-exposed mice compared to ND-fed vehicle-exposed mice (Fig. 4a, b). Shannon index, chao1, observed_species, and PD_whole_tree revealed a significant reduction in the alpha diversity of the microbial community in the HFD + BDE groups (Fig. 4c), implying a cumulative effect of HFD and BDE-47. Consistent with the change in alpha diversity, most perturbed gut microbiota at the phylum level were observed in the HFD + L-BDE or HFD + H-BDE groups. Specifically, the abundances of *Bacteroidetes* and *Proteobacteria* were decreased and that of *Actinobacteria* was increased as a result of BDE-47 exposure in mice fed an HFD compared to the ND + vehicle group (Fig. 4d). At the genus level, a total of 6, 7, 13, and 10 bacterial genera were altered in the ND + L-BDE, ND + H-BDE, HFD + L-BDE, and HFD + H-BDE groups, respectively, compared with the ND + vehicle group (Fig. 4e–k, Table S2). Moreover, the taxonomic classification of distinctive bacterial genera was significantly different between BDE-exposed mice fed an ND and an HFD. The predominant perturbed bacteria among the microbiota are presented in Table S2. As shown in Table S2, in ND-fed mice, BDE-L caused an increase in the relative abundances of *Parasutterella* and *Gemella*, but caused a decrease in the abundances of *Christensenellaceae_R-7_group, Atopostipes, Family_XIII_UCG-001*, and *Bacillus*, BDE-H caused an increase in the abundances of *Candidatus_Saccharimonas, Ruminococcaceae_UCG-013, Staphylococcus, Gemella, Eubacterium_nodatum_group, Corynebacterium_1*, and *Paenalcaligenes* when compared with the ND + vehicle group. In HFD-fed mice, the vehicle group caused a decrease in the abundances of *Turicibacter, Akkermansia*, and *Family_XIII_UCG-001*, and an increase in the abundance of *Dorea*. Both BDE-L and BDE-H caused a decrease in the abundances of *Turicibacter* and *Anaerotruncus* compared to the ND + vehicle group. In addition, BDE-L caused a decrease in the abundances of *Bacteroides, Ruminiclostridium_9, Helicobacter, Alloprevotella, Oscillibacter, Christensenellaceae_R-7_group, Ruminiclostridium_5, Odoribacter, Ruminococcaceae_UCG-010*, and *Rikenella*, and caused an increase in the abundance of *Staphylococcus* compared to the ND + vehicle group. However, BDE-H caused a decrease in the abundances of *Ruminococcaceae_UCG-014, Ruminococcaceae_UCG-009, Candidatus_Saccharimonas, Ruminiclostridium_5*, and *Family_XIII_UCG-001*, and an increase in the abundances of *Dorea, Lactococcus*, and *Eubacterium_nodatum_group* compared to the ND + vehicle group.

To determine the metabolic changes in gut microbiota caused by BDE-47 exposure, ^{1}H NMR-based metabolomics was coupled with a multivariate statistical analysis. Representative 600 MHz ^{1}H NMR spectra from fecal extracts are shown in Figure S3. A clear separation between the ND and HFD groups and between the BDE and vehicle groups was observed on the score plots of 3D PCA (Figure S4) and PLS-DA (Fig. 5a). This separation was similar to the 16S rRNA gene sequencing results described previously. Pairwise comparisons were then performed to obtain the metabolic variations associated with HFD or BDE-47 exposure. As shown in Fig. 5b, a total of 14 metabolites were altered by BDE-47 exposure alone, HFD alone, or BDE-47 and HFD in combination. In ND-fed mice, BDE-47 exposure caused an increase in bile acids, succinate, taurine, glycine, glucose, arabinose, and galactose levels, but a decrease in methionine levels compared with the ND + vehicle group. In HFD-fed mice, BDE-47 exposure caused an increase in bile acids, choline, and α-ketoglutarate levels, but caused a decrease or negated an increase in glucose, arabinose, and galactose levels compared with the ND + vehicle group. In particular, choline and α-ketoglutarate metabolites were disrupted only in the HFD + BDE groups, and the effects of BDE-47, either alone or in combination with HFD on a glucose, arabinose, and galactose were entirely the opposite.

Effects of BDE-47 exposure on host metabolic profiles

The 3D PCA (Figure S5) and PLS-DA (Fig. 5c) score plots showed that the metabolic profiles of HFD-fed mice were significantly different from those of ND-fed mice. Moreover, mice in the HFD groups were more susceptible to BDE-47 induced toxicity than mice in the ND groups, as seen by the clear separation of the HFD + BDE and HFD + vehicle groups compared with the ND separation results (Fig. 5c). Figure 5d shows the metabolites

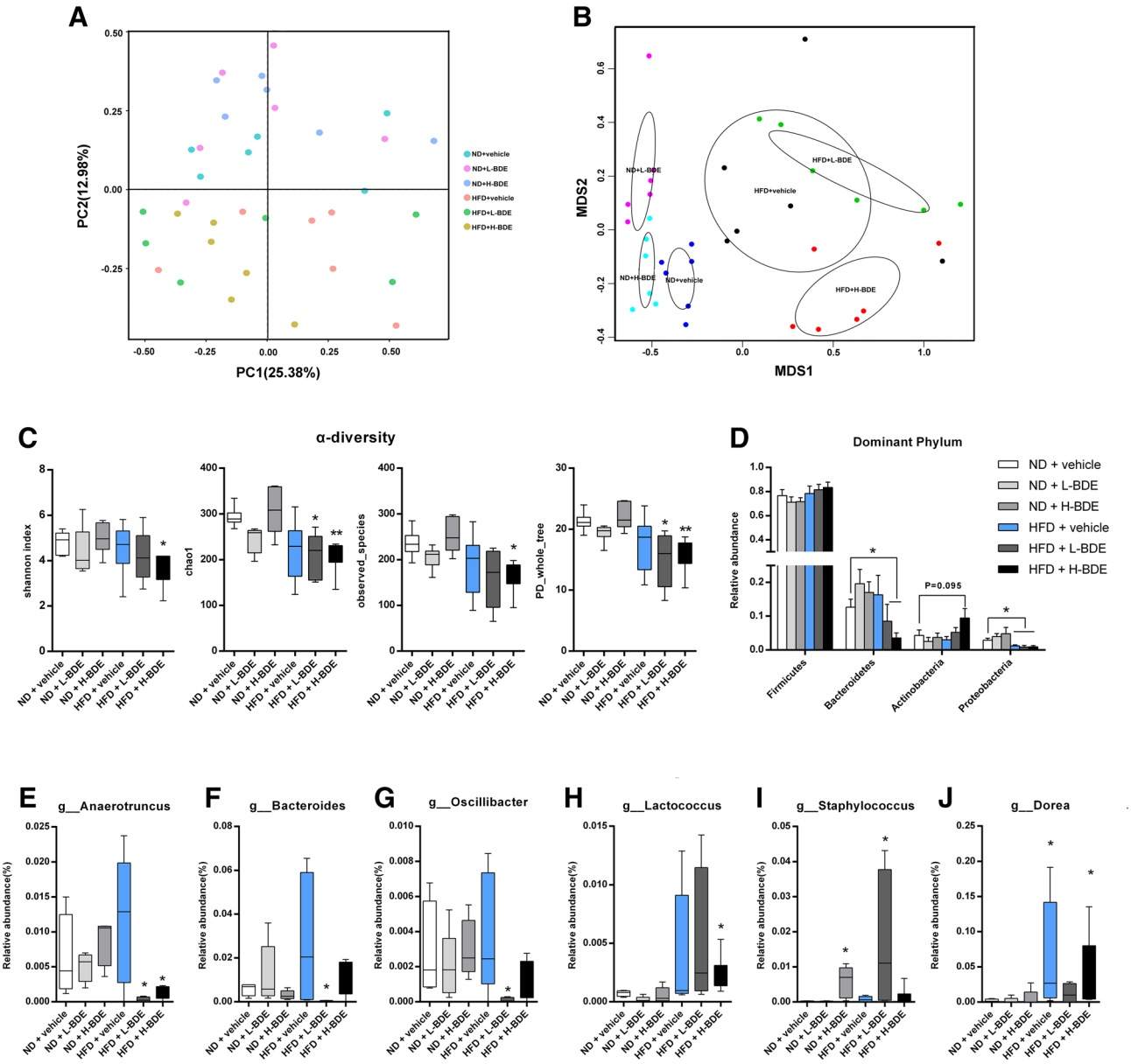


Fig. 4 Effects of co-exposure to a high-fat diet (HFD) and BDE-47 on gut microbiome composition. **a** Principal component analysis (PCA) and **b** non-metric multidimensional scaling (NMDS) of the total population of the gut microbiome of fecal pellets. **c** Alpha diversity of the microbial community indicated by the Shannon index, Chao1, observed_species, and goods_coverage. **d** 16S rRNA gene sequencing analysis at the phylum level of the fecal pellets. **e**–**k** 16S rRNA gene sequencing analysis at the genus level of the fecal pellets. $^{*}P<0.05$, $^{**}P<0.01$ compared with the normal diet (ND) + vehicle group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the HFD + vehicle group, $n=6$

perturbed by BDE-47 or HFD. Among these 12 metabolites, seven metabolites, pyruvate, lactate, phosphoric acid, glutamine, ornithine, 3-hydoxybutyric acid, and isoleucine were only significantly different in the HFD + BDE-47 groups compared with the ND + vehicle group. These observations were consistent with the 16S rRNA and ^{1}H NMR analyses, as described previously.

Discussion

Recent animal studies have identified a strong relationship between obesity and exposure to persistent organic pollutants (POPs) in diet-induced obesity models, especially for tetrachlorodibenzo-*p*-dioxin, tetrachlorodibenzofuran, and dioxin-like polychlorinated biphenyls (PCBs). Although

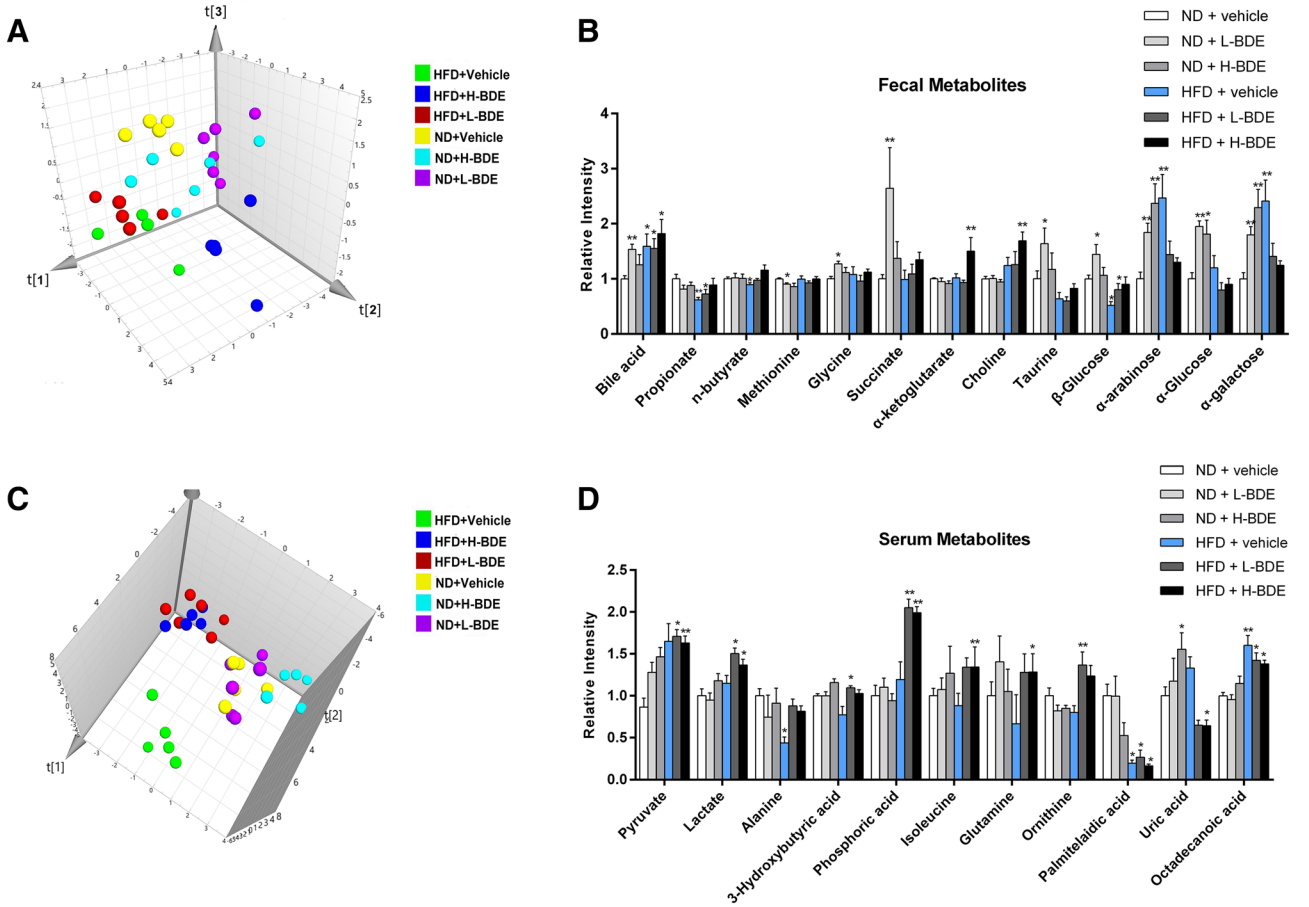


Fig. 5 Effects of the co-exposure to a high-fat diet (HFD) and BDE-47 on the metabolic profiles of the gut microbiome and host. **a** 3D partial least squares-discriminant analysis (PLS-DA) score plot from the nuclear magnetic resonance (NMR) spectra of aqueous fecal extracts. **b** Relative abundance of fecal metabolites of different groups. The relative amount of each metabolite in the normal diet (ND) + vehicle group was set to 1. **c** PLS-DA score plot from a gas chromatography–mass spectrometry (GC–MS) analysis of serum samples. **d** Relative abundance of serum metabolites of different groups. The relative amount of each metabolite in the ND + vehicle group was set to 1. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the ND + vehicle group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the HFD + vehicle group, $n = 6$

many studies have been conducted to examine their interrelationships, few have considered the effects of environmental toxins exposure during early life stages. In this study, we investigated the effects of in utero and *lactation* exposure to low doses of BDE-47 on metabolic functions in male offspring. Result showed that BDE-47 exposure caused a worsening of HFD-induced obesity and NAFLD via lipid metabolism and gut microbiome regulation.

One important finding of this study indicated that exposure to BDE-47 interfered with metabolic adaptation to the HFD. In contrast to the distinct response caused by either BDE-47 exposure or the HFD alone, their combination induced a unique alteration in gene expression and the metabolic profile. The unique alterations of gene expression in fatty liver implied that BDE-47 could interact with the HFD to influence the lipid metabolism. The previous studies have shown that environmental obesogens can mimic natural lipophilic hormones and mediate their effects via members of the nuclear receptor transcription factor superfamily (Hugo et al. 2008). For example, peroxisome proliferator activated receptors (PPARs), which are essential for adipogenesis and lipid metabolism and have been reported to play critical roles in the development of obesity and NAFLD, may be a relevant factor contributing to BDE-47 induced toxicity (Gervois et al. 2004; Lalloyer et al. 2011; Sanyal et al. 2010). The up-regulation of *Pparγ* and down-regulation of *Pparα* observed in this study, which has also been reported in many previous studies, such as PCBs and TCDD (Duval et al. 2017; Wahlang et al. 2013), indicated an important role for *Ppar* in the accumulation of fatty acids and elevation in lipid storage. The expression of *Pparα* and its target gene *Cpt1α*, increased in BDE-47 exposed ND-fed mice as a result of a metabolic adaptation to attenuate the harmful effects of

BDE-47, while an increased in *Pparα* and *Cpt1α* mRNA levels was counteracted in HFD-fed mice as a result of a compensatory decrease caused by the HFD, which could lead to more severe outcomes (Wahlang et al. 2013). However, BDE-47 caused an increase in the mRNA expression of *Pparγ* and its target gene *Cd36*, and this effect was enhanced by the HFD, thus reflecting the concurrent stimulation of fatty acid uptake and lipid storage (*Pparg*) by BDE-47 and the HFD. The up-regulation of *Fasn* and down-regulation of *Scd1*, which indicated the activation of fatty acid synthesis, but inhibition of unsaturated fatty acids, were consistent with the changes in levels of palmitelaidic acid (decrease) and octadecanoic acid (increase) obtained from measurements of serum metabolic changes. Although responsible for TG synthesis, the *Dgat1* and *Dgat2* expressions activated by BDE-47 in ND-fed mice were not significantly different in HFD-fed mice compared with ND-fed vehicle-exposed mice. A similar decrease in *Dgat2* expression has also been reported following TCDD exposure in obese mice (Duval et al. 2017) and in a human hepatic cell line (Ambolet-Camoit et al. 2015), which may be due to a compensatory decrease in DGAT expression by an elevation of *Mogat1* expression (Shi and Cheng 2009). Furthermore, *Dgat2* knock-down indicates its potential protective role against the aggravation of hepatic lesions (Yamaguchi et al. 2007); therefore, the relative decrease in *Dgat2* induced by the combination of BDE-47 exposure and the HFD could lead to poorly regulated TG storage and lipotoxicity.

CAR, which is responsible for the induction of the enzymes that metabolize BDE-47(Sueyoshi et al. 2014) and the regulation of lipid and xenobiotic metabolism, may be another possible mechanism involved in BDE-47 induced obesity and NAFLD. More importantly, CAR is thought to have a protective role during metabolic stress. For example, treatment by the CAR agonist, TCPOBOP, can attenuate diet-induced obesity, diabetes, and hepatic steatosis in animal models (Dong et al. 2009; Gao et al. 2009). CAR activation has also been reported to be beneficial in reducing steatohepatitis in the MCD mouse model of diet-induced non-alcoholic steatohepatitis (NASH) (Baskin-Bey et al. 2007). The previous studies have reported that the consumption of a HFD interfered with PCBs-activated CAR/pregnane X receptor (PXR) (Wahlang et al. 2014); therefore, a similar response may be elicited by BDE-47 exposure in combination with the HFD (decrease), which could attenuate the protective effects of the receptor against BDE-47 toxicity. Consistent with this hypothesis, CAR activation (elevation of *Cyp2b10* expressions) by HFD was inhibited by the addition of BDE-47 (HFD + H-BDE), implying that the inhibition of CAR was one reason for the accumulating effects of BDE-47 and the HFD. Moreover, the activation of CAR in the HFD groups can explain to some extent the inactivation of the inflammation-related genes *Itgam, Cd38* and *Il1β*.

Another important finding of this study showed that BDE-47 exposure in combination with an HFD reduced the diversity and altered the community structure of the gut microbiota, and in particular changed the bacteria composition involved in obesity-related metabolic pathways in the HFD + BDE groups. At the phylum level, BDE-47 treatment significantly shifted the microbial community from *Bacteroidetes* (decrease) towards *Firmicutes* (increase) in the HFD + BDE groups, which was consistent with the results of both obese human (Ley et al. 2005) and mice models (Mujico et al. 2013). *Firmicutes* and *Bacteroidetes* are two predominant members of the gut microbiota, and the ratio of *Bacteroidetes* to *Firmicutes* has been shown to be inversely associated with liver pathology (De Minicis et al. 2014). At the genus level, BDE exposure induced different alterations in gut microbiotal composition in ND-fed and HFD-fed mice, and obesity-related bacteria were more significantly disrupted in the HFD + L-BDE or HFD + H-BDE groups. For example, shifts in *Bacteroides* have been associated with weight loss and the amelioration of HFD-induced metabolic syndromes (Gauffin Cano et al. 2012; Nadal et al. 2012), whereas the abundance of *Bacteroides* was only decreased in the HFD + L-BDE group in the present study. The abundances of other probiotic bacteria, such as *Anaerotruncus, Oscillibacter*, and *Christensenellaceae*, which suspected to protect against HFD-induced obesity or NAFLD (Lam et al. 2012; Stenman et al. 2016; Togo et al. 2016), were also decreased in the HFD + BDE groups. In addition, some bacteria such as *Dorea* that are positively correlated with obesity or NAFLD, were up-regulated in the HFD + BDE groups (Million et al. 2012; Raman et al. 2013). Interestingly, we observed the down-regulation of most genera in the HFD + BDE groups, while up-regulation was observed in the ND + H-BDE group. The up-regulation of the gut microbial community in the ND + H-BDE group may reflect a metabolic adaption to ameliorate the toxicity of BDE-47, while an HFD counteracted this effect, leading to increased BDE-47 toxicity compared with mice fed an ND. A similar pattern of change has been observed in the gene expressions of *Pparα* and *Cpt1α*, implying a possible connection between the bacteria and the genes.

To understand how all of the effects of BDE-47 on obesity, NAFLD, and gut microbiome are interrelated and connected, we performed a Pearson correlation analysis (Table S3). Body weight changes were positively correlated with liver weight ($r = 0.681$, $P < 0.001$), TG content ($r = 0.734$, $P < 0.001$) and ALT activity ($r = 0.621$, $P < 0.001$), indicating that NAFLD worsened by BDE-47 exposure were obesity-related. Moreover, body weight, liver weight, and liver TG content were significantly correlated with *Fasn, Scd1, Pparα, Pparγ*, or Mogat1 gene

expressions ($|r| = 0.39–0.553$, $P < 0.05$), implying that perturbed lipid homeostasis was the mechanism accounting for the NALFD. Gut microbiome alterations, including *Turicibacter, Anaerotruncus, Ruminococcaceae_UCG014, Helicobacter, Alloprevotella, Lactococcus, Parasutterella, Ruminiclostridium5, Odoribacter, Ruminococcaceae_UCG013, Ruminococcaceae_UCG010, Staphylococcus, Rikenella, Family_XIII_UCG001, Gemella*, and *Corynebacterium_1*, correlated with body weight, liver weight, or TG content ($|r| = 0.371–0.661$, $P < 0.05$), implying the potential role of gut microbiome in the development of obesity and NAFLD. Furthermore, we found that some bacteria, including several of the obesity or NAFLD relevant bacteria (*Alloprevotella, Lactococcus, Parasutterella, Ruminiclostridium5, Ruminococcaceae_UCG013*, and *Gemella*) were significantly correlated with the mRNA levels of genes *Scd1, Pparα, Pparγ*, and *Mogat1* ($|r| = 0.373–0.668$, $P < 0.05$), indicating that a perturbed gut microbiome contributed to impaired lipid metabolism. In addition, based on the differences between alteration of gut microbiome correlated with NAFLD and genes, we speculated that BDE-47 may directly interact with the genes involved in lipid metabolism in the liver as several of ways contribute to NAFLD development. All in all, we concluded that the mechanism for BDE-47 induced worsening NAFLD as probably mediated through lipid metabolism and gut microbiome regulation. As shown in Fig. 6, BDE-47 exposure in combination with an HFD led to alterations in the gut microbiota composition and its metabolic profiles, which then disrupted genes expressions involved in lipid metabolism (impaired lipid metabolism can also be caused directly by BDE-47 in liver), and the impaired lipid metabolism then contributed to the development of NAFLD and obesity.

Based on the results of the Pearson correlation analysis, we may conclude the mechanism for BDE-47 induced worsening of obesity and NAFLD. However, the dose-dependent effects are still unclear. In this study, some adverse effects of BDE exposure, such as ALT activity and GTT, were more prominent in the BDE-L group, but not in the BDE-H group of HFD-fed mice, while some adverse effects such as *Fasn* and *Ccl2* mRNA expression were only observed in the BDE-H group of HFD-fed mice. Differences in which gut microbiome were perturbed may be one possible reason. The Pearson's correlation analysis indicated that the gut microbiome was a possible factor promoting the development of obesity and NAFLD. However, the numbers and types of altered bacteria in the HFD + L-BDE and HFD + H-BDE groups were different. More disrupted genus of bacteria were observed in the L-BDE group (13) than in the H-BDE group (10), and among the obesity or NAFLD correlated bacteria, the number of such genus for the the L-BDE group reached nine, while the H-BDE group only reached six. These differences may reflect the different capacity of two treatments to influence the gut microbiome. Another possible mechanism may be linked to the insulin signal pathway. Although we did not determine the insulin concentrations or the activity of the insulin signal pathway, differences between GTT may reflect insulin resistance or impaired pancreatic function (Kramer et al. 2014; Ohashi et al. 2015), and the insulin signal pathway can also regulate the hepatic glucose and lipid metabolism. Specific factors contributing to dose-specific effects should be determined in

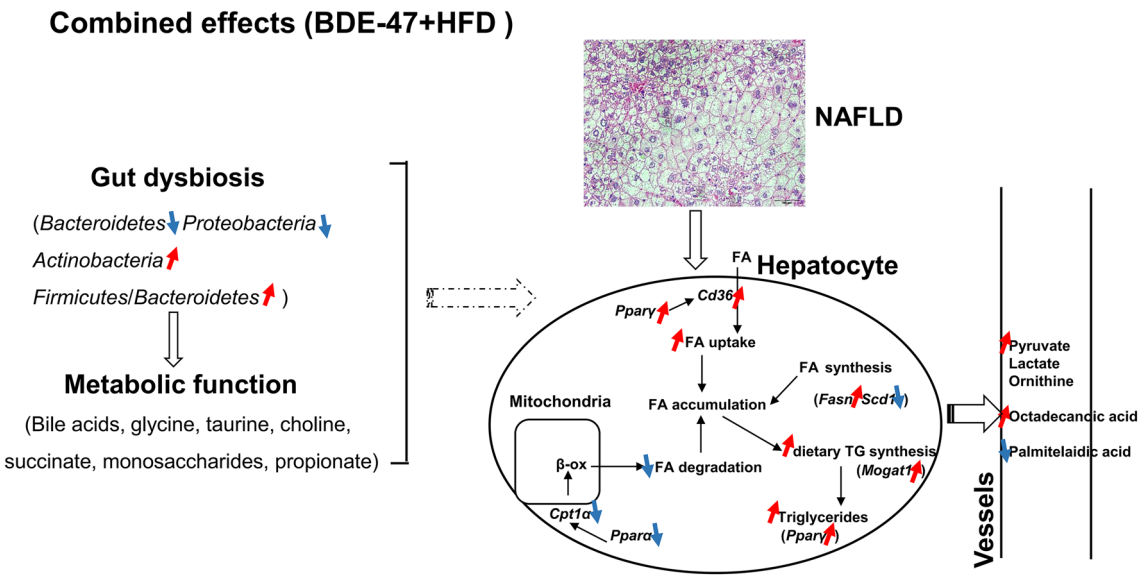


Fig. 6 Schematic of the combined effects of a high-fat diet (HFD) and BDE-47 on the gut microbiome, liver gene expression, serum metabolic profiles, and various endpoints. Significant increased or decreased endpoints were compared with the normal diet (ND) + vehicle group

further studies. Dose-specific effects have been reported in many previous studies, especially during early life exposure. For example, in utero exposure to arsenic increased body weight at 6 months in a low-dose group (10 ppb) but not a high-dose group (42.5 ppm), although serum leptin levels, which were positively associated with obesity, were only significantly increased by high-dose of arsenic (Rodriguez et al. 2016). Low-dose developmental bisphenol A exposure at 0.5 μg/kg (but not 50 μg/kg) body weight caused an increase in plasma TG levels in male mice offspring, while in females, elevations in TG levels were observed at 50 μg/kg but not at 0.5 μg/kg body weight (Lejonklou et al. 2017). Maternal exposure to BPA also induced dose-specific effects on islets in male offspring, and exposure level of 10 μg/kg BPA reduced β-cell mass and increased β-cell death, while 10 mg/kg BPA exposure impaired mitochondrial function (Bansal et al. 2017). Further studies should be conducted to determine whether the dose-specific effects occurred after exposure to BDE-47 during adulthood or not.

This study used a low BDE-47 dose (2 μg/kg body weight), which is within the same range as levels measured in the blood of the population of Shanghai (Xu et al. 2017) but lower than in Northern California residents (Parry et al. 2018). Such low doses were nevertheless found to modulate body weight, hepatic lipid metabolism, and gut microbiome. More importantly, the sum of PBDE concentrations in human serum sample was higher than BDE-47, and considering the similar structures among PBDEs congeners, more severe health effects may occur in human beings exposed to PBDEs. BDE-47 exposure in ND-fed mice was found to have no effect on obesity or NAFLD, but in HFD-fed mice, more obvious phenotype were observed under BDE exposure. These results highlight the importance of a balanced diet (low calorie) and safe food (no PBDEs contamination).

This study reports new findings regarding impaired glucose homeostasis, indices of NAFLD, and increased body weight in male offspring in combination with an HFD intervention after in utero and lactational exposure to low doses of BDE-47. It also improves our understanding of the molecular mechanisms that underline the worsening hepatic steatosis and obesity induced by an HFD, including impaired lipid metabolism and gut dysbiosis. Further studies should be conducted to solve several problems: (1) the effects of BDE-47 exposure during fetal development on females to know if the effects were sexually dimorphic; (2) fecal microbiota transplantation to build strong connection between gut dysbiosis and obesity and NAFLD; (3) to identify particular strains that are targeted by BDE-47 exposure to develop probiotics strategies for eliminating health hazards; and (4) more dose-dependent studies to delineate the mechanism of the dose-specific effects and determine whether or not the dose-specific effects only occur after fetal exposure. These results demonstrate that early life exposure to environmental pollutants can result in susceptibility to disease later in life and suggest that more studies are necessary to investigate this connection.

Acknowledgements We gratefully acknowledge the financial support from National Key Research and Development Program of China (2016YFD0200202), the National Natural Science Foundation of China (21337005); and the Young Elite Scientists Sponsorship Program by CAST.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

References

Alonso-Magdalena P, Vieira E, Soriano S et al (2010) Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect 118(9):1243–1250. https://doi.org/10.1289/ehp.1001993

Ambolet-Camoit A, Ottolenghi C, Leblanc A et al (2015) Two persistent organic pollutants which act through different xenosensors (alpha-endosulfan and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin) interact in a mixture and downregulate multiple genes involved in human hepatocyte lipid and glucose metabolism. Biochimie 116:79–91. https://doi.org/10.1016/j.biochi.2015.07.003

Ba Q, Li M, Chen P et al (2017) Sex-dependent effects of cadmium exposure in early life on gut microbiota and fat accumulation in mice. Environ Health Perspect 125(3):437–446. https://doi.org/10.1289/EHP360

Bansal A, Rashid C, Xin F et al (2017) Sex- and dose-specific effects of maternal bisphenol A exposure on pancreatic islets of first- and second-generation adult mice offspring. Environ Health Perspect 125(9):097022. https://doi.org/10.1289/EHP1674

Baskin-Bey ES, Anan A, Isomoto H, Bronk SF, Gores GJ (2007) Constitutive androstane receptor agonist, TCPOBOP, attenuates steatohepatitis in the methionine choline-deficient diet-fed mouse. World J Gastroenterol 13(42):5635–5641

De Minicis S, Rychlicki C, Agostinelli L et al (2014) Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. Hepatology 59(5):1738–1749. https://doi.org/10.1002/hep.26695

Devaraj S, Hemarajata P, Versalovic J (2013) The human gut microbiome and body metabolism: implications for obesity and diabetes. Clin Chem 59(4):617–628. https://doi.org/10.1373/clinchem.2012.187617

Dingemans MM, Ramakers GM, Gardoni F et al (2007) Neonatal exposure to brominated flame retardant BDE-47 reduces long-term potentiation and postsynaptic protein levels in mouse hippocampus. Environ Health Perspect 115(6):865–870. https://doi.org/10.1289/ehp.9860

Dong B, Saha PK, Huang W et al (2009) Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. Proc Natl Acad Sci USA 106(44):18831–18836. https://doi.org/10.1073/pnas.0909731106

Dunnick JK, Nyska A (2009) Characterization of liver toxicity in F344/N rats and B6C3F1 mice after exposure to a flame retardant containing lower molecular weight polybrominated diphenyl ethers. Exp Toxicol Pathol 61(1):1–12. https://doi.org/10.1016/j.etp.2008.06.008

Duval C, Teixeira-Clerc F, Leblanc AF et al (2017) Chronic exposure to low doses of dioxin promotes liver fibrosis development in

the C57BL/6J diet-induced obesity mouse model. Environ Health Perspect 125(3):428–436. https://doi.org/10.1289/EHP316

EPA (2007) Report on alternatives to the flame retardant DecaBDE: evaluation of toxicity, availability, affordability, and fire safety issues. A Report to the Governor and the General Assembly US-EPA, Illinois

Gao J, He J, Zhai Y, Wada T, Xie W (2009) The constitutive androstane receptor is an anti-obesity nuclear receptor that improves insulin sensitivity. J Biol Chem 284(38):25984–25992. https://doi.org/10.1074/jbc.M109.016808

Gao B, Bian X, Mahbub R, Lu K (2017) Sex-specific effects of organophosphate diazinon on the gut microbiome and its metabolic functions. Environ Health Perspect 125(2):198–206. https://doi.org/10.1289/EHP202

Gauffin Cano P, Santacruz A, Moya A, Sanz Y (2012) Bacteroides uniformis CECT 7771 ameliorates metabolic and immunological dysfunction in mice with high-fat-diet induced obesity. PLoS One 7(7):e41079. https://doi.org/10.1371/journal.pone.0041079

Gervois P, Kleemann R, Pilon A et al (2004) Global suppression of IL-6-induced acute phase response gene expression after chronic in vivo treatment with the peroxisome proliferator-activated receptor-alpha activator fenofibrate. J Biol Chem 279(16):16154–16160. https://doi.org/10.1074/jbc.M400346200

Gronwald W, Klein MS, Kaspar H et al (2008) Urinary metabolite quantification employing 2D NMR spectroscopy. Anal Chem 80(23):9288–9297. https://doi.org/10.1021/ac801627c

Guo W, Holden A, Smith SC, Gephart R, Petreas M, Park JS (2016) PBDE levels in breast milk are decreasing in California. Chemosphere 150:505–513. https://doi.org/10.1016/j.chemosphere.2015.11.032

Huang S, Cui Y, Guo X et al (2015) 2,2′,4,4′-Tetrabromodiphenyl ether disrupts spermatogenesis, impairs mitochondrial function and induces apoptosis of early leptotene spermatocytes in rats. Reprod Toxicol 51:114–124. https://doi.org/10.1016/j.reprotox.2015.01.009

Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N (2008) Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ Health Perspect 116(12):1642–1647. https://doi.org/10.1289/ehp.11537

Koenig CM, Lango J, Pessah IN, Berman RF (2012) Maternal transfer of BDE-47 to offspring and neurobehavioral development in C57BL/6J mice. Neurotoxicol Teratol 34(6):571–580. https://doi.org/10.1016/j.ntt.2012.09.005

Kramer CK, Swaminathan B, Hanley AJ et al (2014) Each degree of glucose intolerance in pregnancy predicts distinct trajectories of beta-cell function, insulin sensitivity, and glycemia in the first 3 years postpartum. Diabetes Care 37(12):3262–3269. https://doi.org/10.2337/dc14-1529

Lalloyer F, Wouters K, Baron M et al (2011) Peroxisome proliferator-activated receptor-alpha gene level differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice. Arterioscler Thromb Vasc Biol 31(7):1573–1579. https://doi.org/10.1161/ATVBAHA.110.220525

Lam YY, Ha CW, Campbell CR et al (2012) Increased gut permeability and microbiota change associate with mesenteric fat inflammation and metabolic dysfunction in diet-induced obese mice. PLoS One 7(3):e34233. https://doi.org/10.1371/journal.pone.0034233

Lejonklou MH, Dunder L, Bladin E et al (2017) Effects of low-dose developmental bisphenol A exposure on metabolic parameters and gene expression in male and female Fischer 344 rat offspring. Environ Health Perspect 125(6):067018. https://doi.org/10.1289/EHP505

Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102(31):11070–11075. https://doi.org/10.1073/pnas.0504978102

Liu S, Mauvais-Jarvis F (2010) Minireview: estrogenic protection of beta-cell failure in metabolic diseases. Endocrinology 151(3):859–864. https://doi.org/10.1210/en.2009-1107

Meeker JD, Johnson PI, Camann D, Hauser R (2009) Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. Sci Total Environ 407(10):3425–3429. https://doi.org/10.1016/j.scitotenv.2009.01.030

Million M, Maraninchi M, Henry M et al (2012) Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. Int J Obes (Lond) 36(6):817–825. https://doi.org/10.1038/ijo.2011.153

Mujico JR, Baccan GC, Gheorghe A, Diaz LE, Marcos A (2013) Changes in gut microbiota due to supplemented fatty acids in diet-induced obese mice. Br J Nutr 110(4):711–720. https://doi.org/10.1017/S0007114512005612

Nadal I, Santacruz A, Marcos A et al (2012) Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents (vol 33, pg 758, 2009). Int J Obes 36(10):1370. https://doi.org/10.1038/ijo.2012.135

Nicholson JK, Holmes E, Kinross J et al (2012) Host-gut microbiota metabolic interactions. Science 336(6086):1262–1267. https://doi.org/10.1126/science.1223813

Ohashi K, Komada H, Uda S et al (2015) Glucose homeostatic law: insulin clearance predicts the progression of glucose intolerance in humans. PLoS One 10(12):e0143880. https://doi.org/10.1371/journal.pone.0143880

Parry E, Zota AR, Park JS, Woodruff TJ (2018) Polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDE metabolites (OH-PBDEs): a six-year temporal trend in Northern California pregnant women. Chemosphere 195:777–783. https://doi.org/10.1016/j.chemosphere.2017.12.065

Raman M, Ahmed I, Gillevet PM et al (2013) Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 11(7):868–875.e1–3. https://doi.org/10.1016/j.cgh.2013.02.015

Rodriguez KF, Ungewitter EK, Crespo-Mejias Y et al (2016) Effects of in utero exposure to arsenic during the second half of gestation on reproductive end points and metabolic parameters in female CD-1 mice. Environ Health Perspect 124(3):336–343. https://doi.org/10.1289/ehp.1509703

Ruhlen RL, Howdeshell KL, Mao J et al (2008) Low phytoestrogen levels in feed increase fetal serum estradiol resulting in the "fetal estrogenization syndrome" and obesity in CD-1 mice. Environ Health Perspect 116(3):322–328. https://doi.org/10.1289/ehp.10448

Sanyal AJ, Chalasani N, Kowdley KV et al (2010) Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 362(18):1675–1685. https://doi.org/10.1056/NEJMoa0907929

Shi Y, Cheng D (2009) Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. Am J Physiol Endocrinol Metab 297(1):E10-8. https://doi.org/10.1152/ajpendo.90949.2008

Song Q, Li J (2014) A systematic review of the human body burden of e-waste exposure in China. Environ Int 68:82–93. https://doi.org/10.1016/j.envint.2014.03.018

Stenman LK, Burcelin R, Lahtinen S (2016) Establishing a causal link between gut microbes, body weight gain and glucose metabolism in humans - towards treatment with probiotics. Benef Microbes 7(1):11–22. https://doi.org/10.3920/BM2015.0069

Sueyoshi T, Li L, Wang H et al (2014) Flame retardant BDE-47 effectively activates nuclear receptor CAR in human primary hepatocytes. Toxicol Sci 137(2):292–302. https://doi.org/10.1093/toxsci/kft243

Suvorov A, Takser L (2010) Global gene expression analysis in the livers of rat offspring perinatally exposed to low doses of 2,2′,4,4′-tetrabromodiphenyl ether. Environ Health Perspect 118(1):97–102. https://doi.org/10.1289/ehp.0901031

Suvorov A, Battista MC, Takser L (2009) Perinatal exposure to low-dose 2,2′,4,4′-tetrabromodiphenyl ether affects growth in rat offspring: what is the role of IGF-1? Toxicology 260(1–3):126–131. https://doi.org/10.1016/j.tox.2009.03.018

Ta TA, Koenig CM, Golub MS et al (2011) Bioaccumulation and behavioral effects of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in perinatally exposed mice. Neurotoxicol Teratol 33(3):393–404. https://doi.org/10.1016/j.ntt.2011.02.003

Talsness CE, Kuriyama SN, Sterner-Kock A et al (2008) In utero and lactational exposures to low doses of polybrominated diphenyl ether-47 alter the reproductive system and thyroid gland of female rat offspring. Environ Health Perspect 116(3):308–314. https://doi.org/10.1289/ehp.10536

Togo AH, Valero R, Delerce J, Raoult D, Million M (2016) "*Anaerotruncus massiliensis*", a new species identified from human stool from an obese patient after bariatric surgery. New Microbes New Infect 14:56–57. https://doi.org/10.1016/j.nmni.2016.07.015

Upadhyaya S, Banerjee G (2015) Type 2 diabetes and gut microbiome: at the intersection of known and unknown. Gut Microbes 6(2):85–92. https://doi.org/10.1080/19490976.2015.1024918

Wahlang B, Falkner KC, Gregory B et al (2013) Polychlorinated biphenyl 153 is a diet-dependent obesogen that worsens nonalcoholic fatty liver disease in male C57BL6/J mice. J Nutr Biochem 24(9):1587–1595. https://doi.org/10.1016/j.jnutbio.2013.01.009

Wahlang B, Song M, Beier JI et al (2014) Evaluation of Aroclor 1260 exposure in a mouse model of diet-induced obesity and non-alcoholic fatty liver disease. Toxicol Appl Pharmacol 279(3):380–390. https://doi.org/10.1016/j.taap.2014.06.019

Xu B, Wu M, Wang M et al (2017) Polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs in human serum from Shanghai, China: a study on their presence and correlations. Environ Sci Pollut Res Int. https://doi.org/10.1007/s11356-017-0709-4

Yamaguchi K, Yang L, McCall S et al (2007) Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 45(6):1366–1374. https://doi.org/10.1002/hep.21655

Zhang L, Nichols RG, Correll J et al (2015) Persistent organic pollutants modify gut microbiota-host metabolic homeostasis in mice through aryl hydrocarbon receptor activation. Environ Health Perspect 123(7):679–688. https://doi.org/10.1289/ehp.1409055