Curcumin protects against bisphenol A -induced hepatic steatosis by modulating gut microbiota and liver fat synthesis in CD-1 mice

Dan Feng (fengdan3@mail.sysu.edu.cn)
Sun Yat-sen University

Xin Jiang
Sun Yat-sen University

Ting Hong
Sun Yat-sen University

Jie Yang
Sun Yat-sen University

Youming He
Sun Yat-sen University

Zhuo Cao
Sun Yat-sen University

Research Article

Keywords: Curcumin, Bisphenol A, Hepatic steatosis, Gut microbiota, lipogenic genes, Fat synthesis

Posted Date: April 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1511675/v1

License: 🌐 This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

**Background:** Our previous studies showed that low-dose bisphenol A (BPA) exposure induced gut microbiota disorder and hepatic steatosis. Curcumin is a polyphenol phytochemical with lipid-lowering and prebiotic effects. This study aimed to investigate the preventive effect of curcumin on BPA-induced hepatic steatosis and the possible mechanism.

**Methods:** Male CD-1 mice were fed with BPA-contaminated diet with or without curcumin for 24 weeks. Thereafter, blood samples were collected to measured blood lipids, transaminase and lipopolysaccharide (LPS). Gut microbiota and the expression level of liver lipogenic gene were detected.

**Results:** Curcumin supplementation alleviated BPA-induced dyslipidemia and hepatic steatosis by reducing the levels of serum triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol and liver TG, TC content. Moreover, analysis of 16S rRNA sequencing indicated that curcumin reversed BPA-induced gut microbiota disorder, the abundance of *Akkermansia* and the ratio of *Bacteroidetes/Firmicutes*, which are both related to beneficial lipid metabolism, were considerably increased after curcumin treatment, whereas the abundance of harmful *Rikenella* was decreased compared with BPA-exposed mice. Besides, curcumin significantly decreased serum LPS levels and down-regulated the expression of hepatic lipogenic genes, including liver X receptor alpha, sterol regulatory element binding protein-1c, acetyl-CoA carboxylase 1 (ACC1) and ACC2, induced by BPA.

**Conclusions:** These findings indicated that curcumin protected against BPA-induced hepatic steatosis through modulating intestinal flora disorder, reducing serum LPS levels and subsequently inhibiting liver fat biosynthesis, suggesting the potential application of curcumin in prevention of BPA-induced metabolic liver diseases.

1. Background

Non-alcoholic fatty liver disease (NAFLD) is a world-wide popular metabolic liver disease and causes heavy disease burden[1]. The earliest and core feature of NAFLD is hepatic steatosis, which is characterized by fat accumulation in the liver [2]. As the prevalence of NAFLD soars worldwide in recent years, it is urgent to improve the medical and dietary strategies to prevent NAFLD or reverse the pathological progression in early phase.

Bisphenol A (BPA), a persistent organic pollutant with endocrine disrupting effect, is widely used in the manufacture of food packaging and storage containers due to its excellent physical and chemical properties. The main exposure pathway of BPA in human is oral intake, because BPA can migrate from containers to food and beverages [3]. Although the tolerable daily intake (TDI) of BPA is 50 µg/kg/day, several experimental and epidemiological investigations have indicated that low-dose BPA exposure disrupts multiple biological processes e.g. reproduction, development and metabolism[4]. Our previous experiments also showed that 50 µg/kg/day of BPA exposure induced significant liver fat accumulation and liver steatosis in mice[5, 6].
Intestinal microorganisms have a significant impact on intestinal function. Research evidence has demonstrated that BPA exposure can lead to intestinal microbial disorder[6, 7] The imbalance of intestinal microbiota has been reported to be involved in many metabolic diseases, including NAFLD[8], therefore, some scholars put forward the concept of gut-liver axis[9]. Intestinal flora disorder can cause lipopolysaccharide (LPS) translocation, resulting in the increase of serum LPS level, and then LPS enters the liver and induces hepatic lipogenesis[10]. Liver lipid synthesis is mainly regulated by sterol regulatory element binding proteins and metabolic enzymes. Increased LPS can activate hepatic liver X receptor alpha(LXRα) and enhance sterol regulatory element binding protein-1c(SREBP-1c) expression[11], which in turn activates the metabolic enzyme acetyl-CoA carboxylase 1 (ACC1) and ACC2, leading to increased lipid synthesis and fat accumulation in the liver[12, 13].

Existing studies have shown that some plant active substances in the diet can effectively regulate the host's intestinal flora and improve metabolism[14, 15]. Curcumin is a polyphenol phytochemical substance extracted from the rhizome of turmeric and is very common in daily diet, such as mustard, curry and so on [16]. After years of research and exploration, this polyphenol has been proved to possess anti-inflammatory, antioxidant, lipid-lowering and hypoglycemic effects [17–19], and has the potential to treat diabetes, obesity, NAFLD and other diseases[20–22]. In addition, curcumin has been reported to possess prebiotic effects and regulate the gut flora involved in fatty acid metabolism[23]. Recent studies have also shown that curcumin can prevent the damages caused by BPA[24–26]. However, whether curcumin can protect against BPA-induced hepatic steatosis through modulating gut microbiota and liver fat biosynthesis remains to be elucidated.

Based on previous studies, here we hypothesized that curcumin can protect against BPA-induced intestinal flora disorder, elevated LPS levels and abnormal lipid metabolism, and then reduce liver fat synthesis and prevent hepatic steatosis.

2. Materials And Methods

2.1. Animals and experimental design

A total of 24 male CD-1 mice at 5-weeks of ages were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were fed normal diet in polypropylene cages with polypropylene water bottles for one week, and then were randomly distributed in 3 groups (n = 8) according to the following diets: Control group (MD12062 normal diet, Additional file 1), BPA group (0.5mg/kg BPA was incorporated in normal diet), and curcumin group (0.5mg/kg BPA and 0.1% w/w curcumin were added to normal diet). The daily diet of mice accounted for 10% of their body weight, which was equivalent to a daily intake of 50 µg/kg BPA. The body weight and food intake of mice were monitored every week. Three days before the end of the experiment, the fecal materials of mice were taken and frozen at -80°C for further analysis. After fasting for 12 h, the mice were anesthetized and euthanized. The orbital vein blood was gathered and centrifuged to collect serum. The tissues of liver and
small intestine were collected, weighed and fixed for the following experiments, and the remaining tissues were frozen at -80°C.

All procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University and were conducted in accordance with the National Research Council Guide for Care and Use of Laboratory Animals.

All reagents and chemicals involved in the test were provided in Additional file 2.

2.2. Biochemical analysis

The activity of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the levels of serum total triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and LPS were determined by different assay kits, respectively.

Hepatic TG and TC contents were measured as previously described [6]. In brief, the frozen liver tissue (50 mg) was homogenized in 1 ml lysate at 4°C, and then was stood for 10 minutes at 25°C. After heating at 70°C for 10 min and centrifuging at 2000 rpm for 5 min, the supernatant was gathered and analyzed for liver TG and TC concentration by different assay kits, respectively. The liver TG and TC contents were expressed as nmol of TG or TC per mg of protein.

2.3. Histological examination

The liver tissue in the middle part of left lobe was excised, fixed in 4% paraformaldehyde for 24 h at room temperature, paraffin embedded, cut into 5µm thickness sections and stained with haematoxylin-eosin (H&E) using standard techniques. 10 µm thickness of frozen sections from formalin-fixed liver tissue were stained with Oil Red O for assaying hepatic lipid accumulation.

The image of Oil Red O staining was captured by optical microscope at 200× magnification, and the areas of total image and Oil Red O staining image were measured by Image J software (NIH, USA) to quantify the lipid droplets in liver.

2.4. Real-time quantitative polymerase chain reaction (RT-qPCR) assay

Total RNA from liver tissue was isolated with TRIzol reagent and reverse-transcribed into cDNA using Takara Prime Script™ RT reagent Kit. RT-qPCR was performed with Applied Biosystems 7500 using the SYBR Green PCR master mix kit. The necessary primers were synthesized by Sangon Biotech. The expression level of each gene was analyzed by \( 2^{-\Delta\Delta Ct} \) method and normalized with β-actin. Primer sequences and detailed process were presented respectively in Additional file 3 and Additional file 4.

2.5. Western blotting assay
Western blotting was performed as previously described[6]. In brief, total protein lysates of liver and small intestine were separated by 10% SDS-PAGE and transferred onto PVDF membrane. The membranes were firstly incubated with 1:500 diluted primary polyclonal antibodies (anti-β-actin, anti-SREBP-1c, anti-LXRα, anti-ACC1, anti-ACC2) at 4°C for 12h, and then incubated with goat anti-rabbit IgG-HRP secondary antibody at room temperature for 2 h. The bands were identified using chemiluminescence detection system and the intensities of bands were quantified by the Image J software. β-actin was used as internal control for equal protein loading. Details were presented in Additional file 5.

2.6. Gut microbiota analysis

Fecal bacterial DNA was extracted, amplified and purified. The V4 region of 16S rRNA gene of intestinal flora was sequenced by Illumina and analyzed by QIIME and R software. The indices of alpha diversity including observed species, shannon index and simpson index, were used to determine the microbial diversity in single sample. Beta diversity, which reflects the difference of biodiversity among samples, was obtained by comparing the microbial community composition among different samples. The association between intestinal flora diversity and biochemical parameters was further analyzed by Spearman correlation analysis. The detailed operation was described in Additional file 6.

2.7. Statistical analysis

All values were expressed as mean ± SEM, and assessed by One-way ANOVA or non-parametric test. SNK-q test or Kruskal-Wallis non-parametric test were further adopted for pairwise comparisons among three group. Data was analyzed using the SPSS 21.0 software. The graphs were produced by Priam 8.0 software. When $P<0.05$, The results were considered statistically significant.

3. Results

3.1. Basic indices of CD-1 mice

After 24 weeks dietary BPA exposure, the liver weight and the ratio of liver to body weight in BPA-exposed mice were higher than those in control mice ($P<0.05$). Also, curcumin-treated mice exhibited lower liver weight and liver to body weight ratio than those in BPA-exposed mice ($P<0.05$). However, no significant difference of animal daily food intake and weight was observed among three group mice (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic parameters and biochemical markers measured for mice.</td>
</tr>
</tbody>
</table>
### Table 1: Basic and liver parameters measured for mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>BPA</th>
<th>BPA+curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily food intake (g)</td>
<td>4.41±0.46</td>
<td>4.41±0.21</td>
<td>4.39±0.51</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>39.9±2.79</td>
<td>41.5±7.56</td>
<td>39.1±3.80</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.44±0.10</td>
<td>1.79±0.11*</td>
<td>1.65±0.23#</td>
</tr>
<tr>
<td>Liver/body weight (g/g)</td>
<td>0.03±0.00</td>
<td>0.05±0.00*</td>
<td>0.04±0.00#</td>
</tr>
<tr>
<td>Liver TC (nmol/mg)</td>
<td>47.32±4.70</td>
<td>53.92±1.85*</td>
<td>38.48±4.43#</td>
</tr>
<tr>
<td>Liver TG (nmol/mg)</td>
<td>16.63±4.92</td>
<td>81.98±10.63*</td>
<td>50.33±9.90#</td>
</tr>
<tr>
<td>Liver lipid drops area (%)</td>
<td>0.90±0.41</td>
<td>14.45±0.73*</td>
<td>2.79±0.53#</td>
</tr>
</tbody>
</table>

Basic and liver parameters measured for mice.

Results are mean ± SEM, n = 8. *P < 0.05 compared to control group, #P < 0.05 compared to BPA group. TC, total cholesterol; TG, total triglycerides.

### 3.2. Curcumin improved dyslipidemia in CD-1 mice

In BPA-exposed mice, the levels of serum TG, TC and LDL-C were significantly increased and the levels of serum HDL-C were reduced compared to control mice (P<0.05). Elevated serum LPS levels and AST and ALT activity were observed in BPA-exposed mice (P<0.05). It is noteworthy that these blood biochemical changes were significantly attenuated by curcumin supplementation (Fig. 1A-F, P<0.05).

### 3.3. Curcumin reversed liver histological changes and hepatic steatosis induced by BPA

H&E staining showed that the hepatocytes of control mice were normal and tightly arranged. On the contrary, the hepatocytes of BPA-exposed mice showed disordered hepatic plate arrangement and abnormal morphology with many small vacuoles. While the liver histological changes caused by BPA were effectively reversed by the addition of curcumin, and the liver cells tended to be normal (Fig. 2A).

Compared with control mice, Oil red O staining exhibited a large amount of fat droplets accumulated in the hepatocytes of BPA-exposed mice. However, curcumin intervention significantly reduced the accumulation of fat droplets caused by BPA (Fig. 2B, P<0.05). Lipid quantitative analysis further showed that the contents of liver TG and TC in curcumin-treated mice were also significantly reduced (Table 1).

### 3.4. Curcumin inhibited the expression of liver lipogenic genes induced by BPA
Excessive fat synthesis can lead to fat accumulation in the liver, which in turn induces hepatic steatosis. In order to understand the mechanism underlying BPA-induced hepatic steatosis and the preventive effect of curcumin, we measured the mRNA and protein expression of liver lipogenic genes LXRα, SREBP-1c, ACC1 and ACC2. Obviously, the mRNA expression levels of liver LXRα, SREBP-1c, ACC1 and ACC2 were significantly up-regulated after BPA exposure ($P<0.05$). It should be noted that curcumin treatment effectively inhibited the mRNA expression of these lipogenic genes induced by BPA (Fig. 3A). Western blotting analysis yielded consistent results, supporting this finding (Fig. 3BC).

**3.5. Curcumin modified the changes of gut microbial composition induced by BPA**

The gut microbial diversity of three group mice was determined by the alpha diversity analysis. Compared with control mice, the decreased microbial diversity in BPA-exposed mice was reflected by the lower observed species and shannon index and higher simpson index. However, curcumin supplementation significantly improved the adverse effects of BPA on the flora, and the microbial diversity was restored (Fig. 4A-C). NMDS showed that the microbial composition of BPA-exposed mice was quite different from that of control mice, while the microbial composition of curcumin-treated mice was close to control mice (Fig. 4D). At the same time, consistent results were observed in the analysis of sample distance heat map (Fig. 4E).

**3.6. Curcumin modified the changes of gut microbial abundance induced by BPA**

The relative abundance analysis of the gut microbial taxa showed that *Bacteroidetes* and *Firmicutes* were the two main flora of intestinal microbes, accounting for more than 50% at the phylum level. The relative abundance of *Bacteroidetes* and *Firmicutes* were both increased in BPA-exposed mice, but the ratio of *Bacteroidetes* to *Firmicutes* was significantly decreased. Interesting, curcumin treatment effectively reversed these changes induced by BPA (Fig. 5A, Fig. 5C-E, $P<0.05$). The relative abundance of *Verrucomicrobia* in BPA-exposed mice was extremely low, but it was markedly increased in curcumin-treated mice, even was slightly higher than that in control mice (Fig. 5A, Fig. 5F, $P<0.05$). At the genus level, the changes of *Akkermansia* were consistent with *Verrucomicrobia*, while the relative abundance of *Rikenella* was similar in curcumin-treated mice and control mice, and was significantly increased in BPA-exposed mice (Fig. 5B, Fig. 5GH, $P<0.05$).

**3.7. The associations of gut microbiota with biochemical parameters and lipogenic genes**

Spearman's correlations analysis was used to evaluate the potential associations between gut microbiota and biochemical biomarkers and lipogenic genes. The results showed that *Bacteroidetes* was positively correlated with liver weight, serum TG, LDL-C and LPS levels, ALT and AST activity and liver LXRα, SREBP-1c and ACC1 expression. *Firmicutes* was positively correlated with serum TG levels, ALT and AST activity, liver TC content and LXRα, SREBP-1c and ACC1 expression; the ratio of *Bacteroidetes* to *Firmicutes* was...
negatively associated with liver weight, serum TG, TC, LDL-C and LPS levels, ALT and AST activity, liver TC content and LXRα, SREBP-1c, ACC1 and ACC2 expression; *Verrucomicrobia* and *Akkermansia* were negatively associated with serum TG, TC, LDL-C and LPS levels, ALT and AST activity, liver TC content and LXRα, SREBP-1c and ACC2 expression; *Rikenella* was positively correlated with ALT and AST activity, liver TC content and SREBP-1c and LXRα expression (Fig. 6AB).

4. Discussion

Our earlier studies found that dietary BPA exposure at TDI level induced hepatic steatosis in CD-1 mice through causing intestinal flora disorder and consequently activating the gut-liver axis. Based on our previous research, we further explored the protective effect of curcumin on BPA-mediated hepatic steatosis. Our current results indicated that curcumin supplementation effectively inhibited BPA-induced hepatic steatosis through improving intestinal flora disorder, and then reducing serum LPS levels and down-regulating the expression of lipogenic genes in the liver. Our study provided a new intervention approach for curcumin as an effective dietary nutrient to prevent BPA-mediated liver steatosis.

As a traditional medicine materials and common food spice, curcumin is often used to prevent and treat various diseases. Many studies have demonstrated that curcumin possesses anti-inflammatory, antioxidant and cholesterol-lowering effects[17–19], and has therapeutic potential for metabolic diseases such as type 2 diabetes[20], cardiovascular diseases[27] and NAFLD[21, 22]. For example, our previous study showed that curcumin reduced serum and hepatic TG and TC levels, and attenuated liver lipid deposition in mice[19]. We also found that supplementation with curcumin inhibited intestinal cholesterol absorption and lowered serum cholesterol levels in HFD-fed hamsters, and prevented HFD-induced atherosclerosis in apolipoprotein E knockout mice[17, 18]. A double-blind, placebo-controlled clinical trial showed that short-term administration of curcumin improved serum TG and HDL-C levels, liver transaminases and steatosis index in overweight subjects with impaired fasting plasma glucose[28]. In addition, administration of curcumin has been shown to regulate the abundance of gut microbial communities including *Bacteroidetes* and *Rikenellaceae* in HFD-fed rats[29]. With the deepening of research, studies found that curcumin can resist the damage caused by BPA. Tandon et al. demonstrated that curcumin protected against BPA-induced neurotoxicity and behavioural deficits through up-regulating the Notch signaling pathway in rats[26]. Curcumin had also been found to alleviate BPA-induced insulin resistance via inhibition of the JNK/p38 signaling pathway in HepG2 cells[25]. Consistent with previous research, our current study revealed that curcumin supplementation substantially attenuated BPA-induced lipid metabolism disorder and hepatic steatosis in CD-1 mice.

As a complex ecosystem, intestinal microbiota is closely associated with energy and lipid metabolism, and play a critical role in the onset and development of NAFLD. In this study, we observed an altered microbial composition induced by BPA. BPA exposure for up to 6 months shifted the structure of gut microbiota in CD-1 mice, including the microbial richness, diversity, and composition, which was markedly reversed by curcumin treatment. *Bacteroidetes* and *Firmicutes* are the leading genus of intestinal flora, and are considered to be related to lipid absorption and metabolism. Animal experiment indicated that
the gut microbiota in obese male C57 BL/6 mice exhibited a lower *Bacteroidetes*/*Firmicutes* ratio compared with normal-weight individuals[30]. Acupuncture protocol was found to improve obesity-related dyslipidemia through increasing the ratio of *Bacteroidetes*/*Firmicutes* and promoting the recovery of *Akkermansia* abundance in the gut microbiome[31]. Likewise, in this study, we observed that the ratio of *Bacteroidetes*/*Firmicutes* was remarkably reduced in BPA-exposed mice and was significantly elevated in curcumin-treated mice, indicating curcumin supplementation reversed the decreased *Bacteroidetes*/*Firmicutes* ratio induced by BPA.

Moreover, we also found that BPA had a strong inhibitory effect on the flora of *Verrucomicrobia* and *Akkermansia*, while curcumin was shown to significantly increase the abundance of these flora. *Akkermansia* is the only representative of the *Verrucomicrobia* phylum[32] and is considered as a promising probiotic owing to its health-promoting properties. Animal study demonstrated that *Akkermansia* administration reduced major adipose tissues weight, adipogenesis and serum TC levels, and improved the liver function, metabolic dysregulation and obesity in HFD-fed mice[33]. *Akkermansia* was also found to up-regulate the expression of hepatic LDL receptor and alleviate hyperlipidemia in CREHB gene deleted mice [34]. Additionally, *Rikenella*, a harmful bacteria belonging to *Bacteroidetes* phylum, was reported to be enriched in diabetic db/db mice[35], hyperlipidemia mice[36] and diet-induced obese mice [37]. We also found that the relative abundance of *Rikenella* is much higher in BPA-exposed mice than that in control mice, however, the growth of *Rikenella* was restrained in curcumin-treated mice. Collectively, curcumin effectively modulated the changes of intestinal flora composition induced by BPA, suppressing the growth of harmful flora and promoting the growth of beneficial flora and then improving dyslipidemia.

The imbalance of intestinal flora can lead to the increase of LPS. It was reported that the relative abundance of *Bacteroidetes* and the concentration of fecal and plasma LPS in preeclampsia patients were higher than those in healthy control group[38]. Furthermore, oral administration of *Akkermansia* to C57BL/6 mice reduced blood LPS concentration and prevented obesity and abnormal glucose metabolism [39]. Fecal microbiota transplantation of *Akkermansia* reduced serum and colon LPS levels in male C57BL/6J mice[40]. Similarly, our current study showed that the relative abundance of *Bacteroidetes* and *Firmicutes* was increased and the relative abundance of *Akkermansia* was decreased in BPA-exposed mice, which was correlated with the increased serum LPS levels. LPS can pass through the intestinal mucosa with the help of chylomicrons and be transported to the liver through lipoproteins[41]. The increase of LPS can stimulate lipogenic gene expression in the liver. Research demonstrated that LPS induced liver LXRα expression in rats, leading to the lipid metabolism disorder[11]. Liver lipogenic gene SREBP-1c was also found to be activated by LPS. Consistent with the activation of hepatic SREBP-1c, liver ACC1 and ACC2 were markedly up-regulated in LPS-treated mice [12, 13].

The liver is the central organ of lipid metabolism and hepatic lipid biosynthesis is transcriptionally regulated by LXRα, SREBP-1c, ACC1 and ACC2. LXRα is a transcription factor that can stimulate SREBP-1c expression by binding to SREBP-1c promoter and lead to liver steatosis[42]. SREBP-1c is a key lipogenic transcription factor that can activate ACC, and is dedicated to fatty acid uptake and triglyceride
synthesis[43]. ACC is the first enzyme in liver de novo lipogenesis (DNL) pathway. There are two isozymes of ACC1 and ACC2 in mammals. Combined inhibition of ACC1 and ACC2 results in DNL reduction, leading to decreased TG in liver and substantially improve hepatic steatosis[44]. The activation of hepatic lipogenic pathway is a critical metabolic change required for hepatic steatosis formation. Our present study found that dietary BPA exposure induced obvious liver fat accumulation and hepatic steatosis, which was accompanied by increased serum LPS levels and up-regulation of hepatic LXRα, SREBP-1c, ACC1, ACC2. In accordance with our results, Marmugi et al. also found that low doses of BPA exposure induced gene expression related to lipid synthesis including LXRα, SREBP-1c and ACC1 in adult mice liver[45]. However, curcumin treatment significantly suppressed the expression of hepatic LXRα, SREBP-1c, ACC1 and ACC2 and reduced liver fat accumulation induced by BPA.

5. Conclusion

In conclusion, our study demonstrates a protective effect of curcumin on BPA-induced hepatic steatosis, which is related to curcumin’s improving intestinal flora disorder, reducing serum LPS levels and down-regulating the expression of hepatic lipogenic genes induced by BPA. These results suggest the potential application of curcumin as a prebiotic to prevent BPA-induced metabolic liver diseases.

Abbreviations
ACC1  Acetyl-CoA carboxylase 1
ACC2  Acetyl-CoA carboxylase 2
ALT  Alanine aminotransferase
ANOVA  Analysis of variance
AST  Aspartate transaminase
B/F  Bacteroidetes to Firmicutes
BPA  Bisphenol A
DNL  De novo lipogenesis
H&E  Hematoxylin and eosin
HDL-C  High-density lipoprotein cholesterol
HFD  High-fat diet
LDL-C  Low-density lipoprotein cholesterol
LPS  Lipopolysaccharide
LXRα  Liver X receptor alpha
NAFLD  Non-alcoholic fatty liver disease
POP  Persistent organic pollutant
PVDF  Polyvinylidene difluoride
QIIME  Quantitative insights into microbial ecology
RT-qPCR  Real-time quantitative polymerase chain reaction
SDS-PAGE  Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SNK-q test  Student-Newman-Keuls
SREBP-1c  Sterol regulatory element binding protein-1c
TBS-T  Tris-buffered saline containing 0.1% Tween-20
TC  Total cholesterol
TDI  Tolerable daily intake
TG  Triglyceride
Xin Jiang: Writing - original draft, Methodology, Investigation, Formal analysis. Ting Hong: Methodology, Investigation, Validation, Formal analysis. Jie Yang: Investigation. Youming He: Investigation, Formal analysis. Zhuo Cao: Investigation. Dan Feng: Conceptualization, Writing - review & editing, Methodology, Investigation, Project administration and Funding acquisition. All authors reviewed and approved the final manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (81973019); the Natural Science Foundation of Guangdong Province (2020A1515011167, 2022A1515011610).

Availability of data and materials

The datasets analyzed are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to publish the present results.

Competing interests

The authors declare that they have no competing financial interests.

Acknowledgements

Not applicable.

References

1. Paik JM, Kabbara K, Eberly KE, Younossi Y, Henry L, Younossi ZM: Global Burden of Non-alcoholic Fatty Liver Disease (NAFLD) and chronic liver disease (CLD) Among Adolescents and Young Adults. *Hepatology*2021 https://doi.org/10.1002/hep.32228


Figures
Figure 1

Bar plot of serum biochemical markers in CD-1 mice. (A,B,C,D) Serum TG, TC, LDL-C and HDL-C levels; (E) Serum LPS levels; (F) Serum ALT and AST activity. Data are expressed as mean ± SEM (n=8), *P<0.05 vs. Control group,

#P<0.05 vs. BPA group.
Figure 2

Curcumin reversed liver histological changes and hepatic steatosis induced by BPA in CD-1 mice. (A) Representative pictures of H&E staining showing the liver morphological changes (200×magnifications, 400×magnifications); (B) Representative pictures of Oil Red O staining showing the liver fat accumulation (200×magnifications, 400×magnifications).
Figure 3

Curcumin inhibited the expression of liver lipogenic genes induced by BPA in CD-1 mice. (A) The mRNA expression levels of LXRα, SREBP-1c, ACC1 and ACC2 in the liver; (B) The protein expression levels of LXRα, SREBP-1c, ACC1 and ACC2 in the liver; (C) The quantitative analysis of 3-times-repetitions of Western blotting assay. Data are expressed as mean ± SEM (n=6), *P<0.05 vs. Control group,

#P<0.05 vs. BPA group.
Figure 4
Curcumin modified the changes of gut microbial composition induced by BPA in CD-1 mice. (A, B, C) The observed species, Shannon indices and Simpson indices showing the difference in gut microbial diversity; (D) NMDS showing the difference in gut microbial composition; (E) Heatmap showing the sample distance of three groups. Data are expressed as mean ± SEM (n=6), *\( P<0.05 \) vs. Control group, #\( P<0.05 \) vs. BPA group.

Figure 5
Curcumin modified the changes of gut microbial abundance induced by BPA
in CD-1 mice. (A) Bar plot analysis of gut microbial abundance at phylum level; (B) Bar plot analysis of gut microbial abundance at genus level; (C,D) Percentages of Bacteroidetes and Firmicutes in intestinal microbiota; (E) Ratio of Bacteroidetes to Firmicutes; (F, G, H) Percentages of Verrucomicrobia, Akkermansia and Rikenella in intestinal microbiota. Data are expressed as mean ± SEM (n=6), *P<0.05 vs. Control group, #P<0.05 vs. BPA group.

Figure 6
Spearman's correlations of intestinal microbiota with biochemical parameters and lipogenic genes. (A) The associations of intestinal microbiota at the phylum level with biochemical parameters and lipogenic genes. (B) The associations of intestinal microbiota at the genus level with biochemical parameters and lipogenic genes. The red indicates positive association and the blue represents negative association. The intensity of the colors indicates correlation degree between intestinal microbiota abundances and parameters, n=6 per group, *$P<0.05$ meaning significant correlation.

**Supplementary Files**

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

- Supplementarymaterial.docx