Bisphenol A induces cholesterol biosynthesis in HepG2 cells via SREBP-2/HMGCR signaling pathway

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ABSTRACT

— Bisphenol A (BPA), an environmental chemical to which humans are commonly exposed, has been shown to increase cholesterol level but the molecular mechanism is not clear. Since cholesterol biosynthesis plays an important role in elevating cholesterol level, the aim of the present study is to explore the effects of BPA on cholesterol biosynthesis in HepG2 cells and its possible mechanisms. HepG2 cells were treated with different concentrations of BPA for 24 hr, the total cholesterol level and the activity of 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) were measured using commercial enzymatic assay kits, and the mRNA and protein expression levels of sterol regulatory element binding protein-2 (SREBP-2) and HMGCR were analyzed by qPCR, Western blotting and immunofluorescence, respectively. After treating HepG2 cells with different concentrations (0.1 nM~10 µM) of BPA for 24 hr, we found that BPA at the environmentally relevant concentrations of 1 nM and 10 nM significantly increased the total cholesterol content, the activity and expression of HMGCR in HepG2 cells, but at 100 nM, 1 µM and 10 µM doses, BPA had no stimulatory effect on cholesterol biosynthesis. The whole dose-response relationship follows non-monotonic dose responses, such as an inverted U-shape. Using human SREBP-2 small interfering RNA, we further discovered that the stimulatory effects of BPA on cholesterol biosynthesis and HMGCR expression could be prevented by blockade of the SREBP-2 pathway. This study provides important implications for understanding the potential lipotoxicity of BPA exposure, and it also indicates that low-dose BPA induces hepatic cholesterol biosynthesis through upregulating the SREBP-2/HMGCR signaling pathway.

Key words: Bisphenol A, Cholesterol biosynthesis, 3-Hydroxy-3-methylglutaryl coenzyme A reductase, Sterol regulatory element binding protein-2, HepG2 cells

INTRODUCTION

High cholesterol levels in vivo constitute an important risk factor for cardiovascular disease (Seo and Choi, 2015). The levels of circulating cholesterol are mainly regulated by cholesterol synthesis, bile acid catabolism, cholesterol intake and excretion through faeces (van der Wulp et al., 2013). Actually, cholesterol biosynthesis in the liver plays a crucial role in elevating cholesterol level, about 2/3 cholesterol in the body is from hepatic cholesterol biosynthesis (Mackay and Jones, 2011; van der Wulp et al., 2013). Reducing hepatic cholesterol biosynthesis can significantly lower plasma cholesterol levels (Oliveira et al., 2016).

Cholesterol biosynthesis in the liver is a multi-step process and is mainly mediated by 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the rate-limiting enzyme that regulates de novo cholesterol synthesis in vivo (Ness, 2015). Activation of HMGCR results in an increased cholesterol synthesis which may contribute to liver cholesterol accumulation and hypercholesterolemia (Soto-Acosta et al., 2013; Wu et al., 2013). HMGCR is also the target of cholesterol-lowering drug, statins, which is widely used for treating hypercholesterolemia (Oliveira et al., 2016). In addition, HMGCR has been shown to be mainly regulated by sterol regulatory element binding protein-2 (SREBP-2). SREBP-2 is a key regulatory factor to regulate cholesterol homeostasis. Especially, it can regulate cholesterol synthetic metabolism, including the gene expression of the related enzymes of cholesterol synthesis (Sato, 2010). In the promoter region of HMGCR, there is a SREBP-2 binding site and HMGCR can be modulat-
ol biosynthesis and explore the underlying mechanisms. HepG2 cells to investigate the effect of BPA on cholesterol metabolism (Han et al., 2017). Therefore, in the present study, we used HepG2 cells to culture. Moreover, HepG2 cells are widely used to study physiological functions and properties similar to normal hepatocytes. Compared to other typical cells, HepG2 cells have a stronger proliferation capacity and are easier to culture. Moreover, HepG2 cells are widely used to study cholesterol metabolism in vitro (Han et al., 2017; Guo et al., 2017). Therefore, in the present study, we used HepG2 cells to investigate the effect of BPA on cholesterol biosynthesis and explore the underlying mechanisms.

MATERIALS AND METHODS

Chemicals

BPA(4,4’-dihydroxy-2,2-diphenylpropane, 97%, CAS#80057, Sigma-Aldrich, St. Louis, MO, USA), dimethyl sulfoxide (DMSO) and HMGCR assay kit were purchased from Sigma-Aldrich. The Dulbecco’s modified Eagle’s medium/low-glucose (DMEM/low-glucose) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA), penicillin-streptomycin was purchased from Life Technology (Waltham, MA, USA). Anti-SREBP-2 and anti-HMGCR antibodies were purchased from Abcam (Cambridge, UK). SYBR Green-based real-time PCR kit was obtained from Takara (Shiga, Japan). Human SREBP-2 small interfering RNA (SREBP-2 siRNA), control siRNA-A and anti-HMGCR antibody used for immunofluorescence were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). TRizol reagents and Lipofectamine™ 2000 Transfection Reagent was obtained from Invitrogen Life Technology (Carlsbad, CA, USA).

Cell culture and treatment

HepG2 cells (human hepatoma cell line, American Type Culture Collection) were cultured in growth medium containing DMEM/low-glucose medium with 10% fetal bovine serum, 1% penicillin-streptomycin to 80-90% confluence. The medium was removed and the cells were washed with PBS, then the cells were passaged into 6-well plate to continue incubation for 24 hr. BPA was delivered to the cells using DMSO solvent, the final concentrations of BPA added to the culture medium were 0.1 nM, 1 nM, 10 nM, 100 nM, 1 µM and 10 µM, and the amount of DMSO added to the cells was not greater than 0.1% (v/v). Cells treated with the equal amount of DMSO alone were taken as controls.

Cell viability assay

The effect of BPA on cell viability was assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. In brief, HepG2 cells were seeded into 96-well plates (1 × 10^4 cells / well) and allowed to adhere overnight at 37°C in a humidified atmosphere with 5% CO₂. The cells were incubated with different concentrations (0.1 nM~10 µM) of BPA for 24 hr, then 50 µL 1 × MTT was added into per well and hatched in 37°C for 4 hr to make MTT revert to formazan. Next, the supernatant was sucked out, and the precipitated formazan crystals per well were dissolved in 150 µL of DMSO. After gentle mixing, the absorbance of each well was assayed at 570 nm by using an ELx-800 instrument (BioTek, Vermont, VT, USA) and the survival rate of cells was calculated as follows: (optical density of experimental sample/optical density of control) × 100%.
**Total cholesterol assay**

The total cholesterol level in HepG2 cells was measured using a commercial enzymatic assay kit (T-CHO kit) (Jiancheng, Nanjing, China, Product code: A111-1). Briefly, after 24 hr of BPA treatment, the cells were lysed by the cell lysis buffer, and the main components of the cell lysates were 20 mM Tris (pH 7.5), 150 mM NaCl and 1% Triton X-100 without protease, phosphatase and other inhibitors. The cell lysates were loaded on 96-well plate, then 250 μL enzyme reagent was added to each well and incubated with cell lysates for 10 min at 37°C. Optical absorbance of each well was measured at 510 nm. Cholesterol provided in the kit was used as a standard.

**HMGCR activity assay**

The enzymatic activity of HMGCR was evaluated by quantitation of the NADPH extinction using the HMG-CoA reductase assay kit (Sigma Aldrich, Catalog Number CS1090). The assay is based on the spectrophotometric measurement of the decrease in absorbance at 340 nm, which represents the oxidation of NADPH by the catalytic subunit of HMGCR in the presence of the substrate HMG-CoA. Briefly, HepG2 cells (2.5–3.0 × 10⁵) were treated with different concentrations (0.1 nM–10 µM) of BPA for 24 hr, then the cell culture medium was removed and the cells were washed with PBS for three times. The cells were lysed by the cell lysis buffer as described in the total cholesterol assay. 2 μL cell lysates were loaded on 96-well plate and 198 μL enzyme reagent was added to each well in the following order: 1 × assay buffer, reconstituted NADPH, substrate solution (HMG-CoA). Finally, 250 μL siRNA-lipid complex was added and incubated with cells for 24 hr at 37°C, then the transfected cells were analyzed. The NCBI reference sequence of SREBP-2 siRNA is NM_004599.3. SREBP-2 siRNA was the target siRNA of SREBP-2 (Test). Control siRNA-A was a nonrelated scrambled siRNA and was recommended as a negative control (NC) for evaluating RNAi off-target effects. Normal cell was used as blank control (Blank) and transfection reagent was used as mock control (Mock).

**Real-time quantitative PCR**

Methods for RNA extraction and real-time quantitative PCR followed the experimental instructions. The primers used to quantify the mRNA of HMGCR and SREBP-2 are shown in Table 1. RNA was extracted from the cells using TRizol reagents and reverse transcribed into cDNA by mixing with 5 × Prime Script RT Master Mix, and then the cDNA was mixed with the primers and 2 × SYBR Green PCR master mix in 10 μL. Real-time quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR System. Two-step PCR reaction procedures were used, the heat cycle program was as follows: predenaturation for 30 sec at 95°C for one cycle, followed by amplification of cDNA for 40 cycles with melting for 5 sec at 95°C, and annealing and extension for 34 sec at 60°C. The PCR efficiency (%) in the dark for 30 min at 37°C. Finally, the cells were washed 5 min for 3 times and were photographed using Laser Scanning Confocal Microscope (Leica TCS SP5II, Zeiss, Wetzlar, Germany).

**SREBP-2 RNA interference**

HepG2 cells were cultured in 6-well plate with 2.5–3.0 × 10⁵ density per well. For SREBP-2 knockdown, the cells were transfected with human SREBP-2 small interfering RNA (SREBP-2 siRNA) following the manufacturer’s instruction. First, HepG2 cells were seeded into 6-well plate and were incubated at 37°C in a CO₂ incubator to 70-90% confluence. Second, diluted siRNA and diluted Lipofectamine™ 2000 Reagent (1:1 ratio) were mixed and incubated for 5 min at room temperature. Finally, the 250 μL siRNA-lipid complex was added and incubated with cells for 24 hr at 37°C, then the transfected cells were analyzed. The NCBI reference sequence of SREBP-2 siRNA is NM_004599.3. SREBP-2 siRNA was the target siRNA of SREBP-2 (Test). Control siRNA-A was a nonrelated scrambled siRNA and was recommended as a negative control (NC) for evaluating RNAi off-target effects. Normal cell was used as blank control (Blank) and transfection reagent was used as mock control (Mock).

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>SREBP-2-F</td>
<td>5’-CAGCAGCCTTGTGATACCCAGAAATG-3’</td>
</tr>
<tr>
<td>SREBP-2-R</td>
<td>5’-AGGATGTCACCAGGCTTTGGAC-3’</td>
</tr>
<tr>
<td>HMGCR-F</td>
<td>5’-ATAACACGATGCTAGCCATCCGATCGCTG-3’</td>
</tr>
<tr>
<td>HMGCR-R</td>
<td>5’-AAAAATTGTGAAAAGGGCCAGCAATAC-3’</td>
</tr>
<tr>
<td>β-actin-F</td>
<td>5’-CCTTGCCACCCAGCAC-3’</td>
</tr>
<tr>
<td>β-actin-R</td>
<td>5’-GCCGATCCACCGGAGT-3’</td>
</tr>
</tbody>
</table>
for HMGCR and SREBP-2 gene expression was 100%. The relative expression of the gene is calculated by comparing the threshold period (Ct) method, and normalized with β-actin.

**Western blot analysis**

Proteins (30 μg) from whole-cell lysates or nuclear extracts were subjected to 8% SDS-PAGE. After electrophoresis, samples were transferred to a PVDF membrane (Millipore, Boston, MA, USA) and then blocked for 1.5 hr with 5% skim milk. The membrane was rinsed three times consecutively with TBS-T buffer, followed by incubation with 1:1,000 dilutions of primary polyclonal antibodies (anti-β-actin, anti-SREBP-2, anti-HMGCR) at 4°C overnight. After three washes, the membrane was incubated for 1.5 hr with 1:10000 dilutions of goat anti-rabbit IgG-HRP secondary antibody. Development was carried out using Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific Inc., Waltham, MA, USA). β-actin was used as control for equal protein loading.

**Statistical analysis**

IBM SPSS Statistics 22.0 software was used in our statistical analysis. All the data were analyzed using mean ± SEM. Differential effects were analyzed by analysis of variance (ANOVA) followed by the Bonferroni or LSD posttest for multiple comparisons. At \( P < 0.05 \), the difference was considered to be significant.

**RESULTS**

**BPA has no cytotoxicity on HepG2 cells**

The cytotoxicity of BPA was evaluated by MTT assay, cell viability in different concentrations (0.1 nM~10 µM) of BPA was 97.90%, 95.94%, 98.50%, 97.90%, 97.84% and 101.59% compared to the control group (Fig. 1). The difference was not statistically significant.

**BPA promoted cholesterol synthesis in HepG2 cells**

We first examined whether BPA could promote cholesterol synthesis in HepG2 cells. HepG2 cells were incubated with different concentrations (0.1 nM~10 µM) of BPA for 24 hr, and the content of total cholesterol and the activity of HMGCR were then measured. As shown in Fig. 2A and B, BPA significantly increased the total cholesterol content and HMGCR activity in HepG2 cells at the environmentally relevant concentrations of 1 nM and 10 nM, but in the presence of 100 nM, 1 µM and 10 µM BPA, the total cholesterol content and the HMGCR activity had a decreased trend. The whole dose-response relationship appeared like an inverted U-shape.

**BPA promoted HMGCR mRNA and protein expression**

To further study the molecular mechanism by which BPA promoted cholesterol biosynthesis in HepG2 cells, we then analyzed the impact of BPA on HMGCR expression in the HepG2 cells. The mRNA and protein expression levels of HMGCR were evaluated by Real-time quantitative PCR and Western blot after treating the cells with different concentrations (0.1 nM~10 µM) of BPA for 24 hr. BPA treatment markedly increased HMGCR mRNA and protein expression at the environmentally relevant concentrations of 1 nM and 10 nM (Fig. 3A, B). Moreover, as it is well known that after activation, SREBP-2 will be translocated to the nucleus and bind to DNA, we then assayed the nuclear SREBP-2 expression. As shown in Fig. 3C, BPA treatment significantly up-regulated HMGCR expression compared with control cells.

**BPA up-regulated SREBP-2 mRNA and protein expression**

As we know, HMGCR expression can be regulated by SREBP-2 at transcriptional level. Next, the influence of BPA on SREBP-2 expression in the HepG2 cells were analyzed. Compared to the control group, the mRNA and protein expression levels of SREBP-2 were significantly up-regulated by 1 nM and 10 nM BPA treatment (Fig. 4A, B). Moreover, as it is well known that after activation, SREBP-2 will be translocated to the nucleus and bind to DNA, we then assayed the nuclear SREBP-2 expression. As shown in Fig. 4C, BPA treatment markedly increased the protein expression levels of nuclear SREBP-2.
SREBP-2 at the environmentally relevant concentrations of 1 nM and 10 nM.

BPA may impact cholesterol biosynthesis via SREBP-2/HMGCR pathway
Since SREBP-2 regulates the function of HMGCR, so we wanted to know whether SREBP-2 could be involved in up-regulation of HMGCR expression and activity. We examined the effects of the knockdown of SREBP-2 by small interfering RNA transfection on BPA mediated up-regulation of HMGCR expression and activity. When SREBP-2 was blocked by the specific siRNA interference, no stimulatory effects of BPA on HMGCR expression were observed (Fig. 5A). Also, no significant increases in HMGCR activity and total cholesterol content could be detected (Fig. 5B, C).

DISCUSSION
Our study discovered that bisphenol A promoted cholesterol biosynthesis in HepG2 cells at environmentally relevant doses of 1 nM and 10 nM, and the stimulative effect of BPA on cholesterol synthesis is at least, partially mediated by the SREBP-2/HMGCR signaling pathway.

Previous studies in animal and human have demonstrated that BPA exposure increased the cholesterol levels in blood (Marmugi et al., 2014; Moghaddam et al., 2015; Olsén et al., 2012). Furthermore, exposure to a human relevant dose of BPA has also been found to increase hepatic accumulation of cholesterol and the expression levels of key enzymes of cholesterol synthesis in male mice (Ke et al., 2016). However, the molecular mechanisms by which BPA elevates cholesterol and causes hypercholesterolemia are still not clear. The liver is the most important organ of cholesterol synthesis and plays a key role in the process of maintaining the balance of cholesterol (Mackay and Jones, 2011; van der Wulp et al., 2013). Also, the liver is the primary target organ of BPA (Moon et al., 2012). Whether cholesterol synthesis in the liver can be affected by BPA is an important and interesting question to be studied. Our results revealed that BPA at environmentally relevant doses had promotive effects on cholesterol synthesis in the HepG2 cells. The conclusion is supported by the following evidence. First, in our experiments, BPA treatment significantly increase the total cholesterol content in HepG2 cells. Second, the activity of HMGCR is markedly enhanced by BPA treatment. Third, treatment of the HepG2 cells with BPA obviously up-regulated the protein and mRNA expression levels of HMGCR.

The exposure levels of BPA in human serum are indeed very low (ranging from 0.7–20 nM) (Vandenberg et al., 2007; Kim et al., 2014; Welschons et al., 2006). Moreover, exposure to environmentally relevant doses of BPA could affect various biological functions, including reproductive, behavioral, metabolic, and immune systems (Fang et al., 2015; Lakind et al., 2014; Peluso et al., 2014) and have bad effects on human health (Vom Saal et al., 2012; Marmugi et al., 2014). For instance, Vom Saal et al. have revealed that BPA at environmentally relevant doses influences proper body weight by affecting the activity of lipoprotein lipase, aromatase, lipogenesis regulators and the level of fat tissue hormones (pectin, adiponect-
Fig. 3. The effects of BPA on HMGCR mRNA and protein expression in HepG2 cells. A. The mRNA expression of HMGCR in the HepG2 cells. HMGCR mRNA expression was measured by Real-time quantitative PCR as described in Materials and Methods. Expression values were normalized to \( \beta \)-actin. B. The protein expression of HMGCR in the HepG2 cells. The cells were cultured to 80-90% confluence. The cellular protein was extracted and subjected to Western blot analysis for the expression of HMGCR and \( \beta \)-actin. C. Immunofluorescence assay for HMGCR protein expression. HepG2 cells with 10 nM BPA treatment for 24 hr subjected to immunofluorescent staining for HMGCR. The images were captured at 400 × magnifications. Values are expressed as mean ± SEM (n = 3), *\( P < 0.05 \), **\( P < 0.001 \) compared with untreated cells (Control).
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Fig. 4. The effects of BPA on SREBP-2 mRNA and protein expression in HepG2 cells. A. The mRNA expression of SREBP-2 in the HepG2 cells. SREBP-2 mRNA expression was measured by Real-time quantitative PCR as described in Materials and Methods. Expression values were normalized to β-actin. B. The protein expression of SREBP-2 in the HepG2 cells. C. The protein expression of nuclear SREBP-2 in the HepG2 cells. The cells were cultured to 80-90% confluence. The cellular and nuclear protein was extracted and subjected to Western blot analysis for the expression of SREBP-2 and β-actin. Values are expressed as mean ± SEM (n = 3), *P < 0.05, ***P < 0.001 compared with untreated cells (Control).

Bisphenol A (BPA), which may be responsible for the metabolic disorders and obesity caused by BPA (Vom Saal et al., 2012). Similarly, treating HepG2 cells with environmentally relevant concentrations of 0.1 nM, 1 nM and 10 nM BPA, we observed a significantly stimulative effect of BPA on cholesterol synthesis, but at 100 nM, 1 µM and 10 µM doses, we did not observe any promotive effects. The whole dose-response relationship between 0.1 nM and 10 µM appeared like an inverted U-shape, which was consistent with previous studies. Marmugi et al. (2012) have shown that the effects of BPA on the expression of de novo lipogenesis follow a non-monotonic dose-response curve, with more obvious effects at lower doses than at higher dose. Angle et al. (2013) have found that the low but not the high doses of BPA have a significant impact on metabolic disruption in male mice. Overall, the dose-response relationship between the concentrations of BPA and the health damage effects often follows non-monotonic dose responses (NMDRCs), such as “U” or “inverted U” shape (Rochester, 2013;
BPA is a typical endocrine disrupting chemical (EDC) and acts like a hormone. The mechanisms responsible for these NMDRCs may be related to the interactions between the ligand (hormone or EDC) and a hormone receptor, including the effect of hormone concentration on receptor number, cell and tissue specific receptors and co-factors, receptor selectivity, receptor competition, and endocrine negative feedback loops (Vandenberg, 2013). Furthermore, the concentrations of 1 nM and 10 nM BPA were in the range of 0.7~20 nM, which were the human exposure levels of BPA. Based on the in vitro results and human exposure levels of BPA, here we can predict that human exposure to 0.7~20 nM BPA could promote hepatic cholesterol synthesis in vivo.

HMGCR is the rate-limiting enzyme of cholesterol synthesis and plays a key role in maintaining cholesterol homeostasis in the body (Ness, 2015). Enhancing the activity and expression of HMGCR can significantly promote cholesterol synthesis and elevate the cholesterol level in the body (Ness, 2015; Soto-Acosta et al., 2013; Wu et al., 2013). Especially, in the progress of de novo cholesterol synthesis, regulation of HMGCR activity is the primary pathway. Cholesterol synthesis in the liver has been shown to correspond to the activity of the HMGCR (Ness, 2015), abnormal activation of HMGCR can cause hepatic cholesterol accumulation and hypercholester-
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olemia (Soto-Acosta et al., 2013; Wu et al., 2013). For example, dengue virus infection increased intracellular cholesterol level at early times by increasing the enzyme activity of HMGCR (Soto-Acosta et al., 2013). High-fat diets have also been shown to increase cholesterol biosynthesis in the liver through inducing hepatic HMGCR activation, which may contribute to the liver cholesterol accumulation and hypercholesterolemia in high-fat-diet-fed male C57BL/6 mice (Wu et al., 2013). Since HMGCR plays an important role in mediating the processes of cholesterol synthesis and cholesterol homeostasis, we debated that BPA may influence HMGCR function to regulate cholesterol synthesis. In our study, we found that treatment HepG2 cells with different concentration of BPA, the activity of HMGCR as well as the mRNA and protein levels of HMGCR were markedly increased by BPA at the environmentally relevant concentrations. Moreover, the increase in hepatic total cholesterol was in accordance with the increase in HMGCR activity and expression. These results indicate that the mechanism may involve an increase in cholesterol synthesis due to up-regulation of HMGCR activity and expression, which is responsible for the hypercholesterolemia effect of BPA.

SREBP-2 is an important transcription factor for regulating cholesterol homeostasis. SREBP-2 can combine the promoter region of HMGCR to regulate its gene expression and then promote hepatic cholesterol biosynthesis (Howe et al., 2017). Activation of SREBP-2 has also been shown to increase HMGCR activity and expression (Wu et al., 2013). Moreover, the cholesterol synthesis is closely related to the up-regulation of SREBP-2 and HMGCR (Wang et al., 2009). Therefore, here we hypothesized that BPA promoted the expression and activity of HMGCR through the signal transduction pathway mediated by SREBP-2. In order to support this hypothesis, when we used BPA to treat HepG2 cells, we did find that BPA can increase the expression of SREBP-2 at the environmentally relevant concentrations, and such an increase was consistent with the increase in HMGCR expression and activity. However, when SREBP-2 was knocked down by small interfering RNA, no stimulatory effect of BPA on HMGCR expression and activity was observed. Also, no significant increase in total cholesterol content could be detected. Our results confirmed that BPA can enhance HMGCR activity and HMGCR expression through SREBP-2 transcription factor. Therefore, the overall results of our present study support the hypothesis that BPA promotes cholesterol biosynthesis in the HepG2 cells through upregulating the SREBP-2/HMGCR signaling pathway.

In addition to the SREBP-2/HMGCR signaling pathway, it should be pointed out that there may be some other pathways involved in BPA-mediated increase in hepatic total cholesterol, such as LDL receptor (LDLr) pathway. LDLr is the protein responsible for the uptake of extracellular LDL-cholesterol and plays an important role in maintaining the cholesterol metabolism. Additionally, LDLr genes are also regulated by the same transcription factor SREBP-2 (Kartawijaya et al., 2016; Rice et al., 2014). Thus, the role of the SREBP-2-LDLr pathway in the hypercholesterolemic effect of BPA is needed to be further studied. Moreover, the potential targets of BPA also include estrogen receptors, androgen receptors, peroxisome proliferator-activated receptors, and nuclear receptors (MacKay and Abizaid, 2018). For example, BPA concentrations in thermal paper receipts from Brazil, France, and Spain were positively correlated with its estrogenic and anti-androgenic activities, and such effects of BPA were mediated by binding to the nuclear estrogen receptor (ER) and androgen receptor (Molina-Molina et al., 2019). BPA could induce adipogenesis through directly activating peroxisome proliferator-activated receptor gamma (PPARγ) in 3T3-L1 adipocyte (Ahmed and Atlas, 2016), and induce cell proliferation in Ishikawa cells and HEC265 cells via the BPA/ estrogen-related receptor γ (ERRγ) signaling pathway (Yaguchi, 2019). Also, BPA may have direct effects on hypothalamic pro-opiomelanocortin neurons through PPARγ nuclear receptors (Salehi et al., 2019). Whether BPA can influence liver cholesterol metabolism through regulating these receptors needs to be investigated in the future.

In conclusion, BPA at environmentally relevant doses can promote the synthesis of cholesterol, which is a key risk factor of hypercholesterolemia, further leading to a series of related diseases induced by hypercholesterolemia, such as atherosclerosis, diabetes, obesity, etc. The mechanisms may be involved in SREBP-2 activation and subsequent up-regulation of HMGCR expression and activity.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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