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RESEARCH ARTICLE

Study on the correlation of bisphenol A exposure, pro-inflammatory gene expression, and C-reactive protein with potential cardiovascular disease symptoms in young adults

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Abstract

Bisphenol A (BPA) is a plasticizer used in the manufacture of polycarbonate and epoxy resins. It was found that higher urinary BPA levels are more likely to be associated with coronary artery disease (CVD). In recent years, the increasing incidence of CVD among young people is observed, which may be related with inflammation rather than the traditional triple-H risk factors. BPA is an endocrine-disrupting chemical, and can induce oxidative stress and chronic inflammation since its estrogenic effect. Inflammatory responses could come from the stimulation of IκB kinases (IKKs) by estrogen receptors (ERs). Therefore, this study investigated the association of BPA exposure with the gene expression of pro-inflammatory response (ERs and IKKs), an inflammation biomarker of CVD (C-reactive protein, CRP), and physiologic index potency of CVD development symptoms in young adults. This study divided BPA exposure levels into high and low groups based on the median plasma BPA level (4.34 ng/mL), and found that the high BPA group obviously had higher BMI, blood pressure, plasma CRP levels, and gene expression of ERβ and IKKβ. BMI and gene expression of IKKβ were also positively correlated with plasma CRP secretion. Furthermore, the study subjects with potential CVD development symptoms had the increased levels of BPA (OR 2.10, 95% CI 0.83–5.39), CRP (OR 2.61, 95% CI 1.03–10.6) and IKKβ (OR 4.29, 95% CI 1.51–15.6). These results indicated that exposure to BPA is potentially associated with expression of pro-inflammatory genes related to CRP secretion, which may promote the risk of CVD development symptoms in young adults. This study highlighted the possible connection between BPA exposure and CVD development but the mechanism between them needs to be further explored.

Keywords Bisphenol A · Pro-inflammatory genes · C-reactive protein · Cardiovascular disease · Young adults

Introduction

Bisphenol A (BPA) is one of the most widely ubiquitous endocrine-disrupting chemicals (EDCs) with the potency to bind to estrogen receptors (ERs) and disturb the homeostasis of hormones (Nomiri et al. 2019). BPA is used extensively in epoxy-resin-lined cans, beverage containers, and the polycarbonate plastics included in many consumer products (Konieczna et al. 2015). People might intake BPA through food, drinking water, dental sealants, dermal exposure, and

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household dusts (Park et al. 2016). Numerous studies have suggested that human exposure to BPA is widespread, as is evidenced by its presence in urine, blood, plasma, fetal tissues, and amniotic fluid (Geens et al. 2012). Exposure to BPA has been associated with numbers of chronic diseases such as cardiovascular disease (CVD), hypertension, and diabetes because of its ability to induce the estrogenic-like, obesogenic or diabetogenic effects (Magliano and Lyons 2013), oxidative stress, inflammation, and epigenetic change (Han and Hong 2016). A meta-analysis of several studies from the National Health and Nutrition Examination Survey (NHANES) showed that an apparent odds ratio (OR) of CVD increases by BPA concentration in urine (Rancière et al. 2015). The MaGiCAD study in UK indicated that the urinary BPA concentration was significantly higher in patients with severe coronary artery disease (OR 1.43 per 5.96 ng/mL increase in BPA concentrations, 95% CI 1.03 to 1.98) (Melzer et al. 2012a). A longitudinal study also reported an association between higher BPA exposure and CAD incidence during 10.8-year follow-up (OR 1.11, 95% CI 1.00–1.23) (Melzer et al. 2012b). The above studies indicated that people with higher levels of BPA are more likely to have heart diseases.

Inflammation is essential to the initiation and progression of CVD (Pepys and Hirschfield 2003). Previous studies have found that the circulating levels of several inflammatory markers increase in subjects at risk of CVD (Lakind et al. 2014; Savastano et al. 2015). C-reactive protein (CRP), an indicator of degree of inflammation, has been used as a strong predictor of the incidence of myocardial infarction, stroke, peripheral artery disease, and sudden death in prospective epidemiological studies (Windgassen et al. 2011; Meguro et al. 2012). In a cohort study of 3,888 Americans, coronary heart disease (CHD) patients have higher CRP levels than healthy persons (Tehrani et al. 2013). Nuclear factor kappa B (NF-κB) is a transcription factor that controls various aspects of the immune and inflammatory responses (Baker et al. 2011). Endogenous CRP is induced by binding to a specific κB site at −2652 of NF-κB, and synergistically enhanced by co-expression of NF-κB heterodimer p50/p65 with CCAAT-enhancer-binding proteins (C/EBPβ) or signal transducer and activator of transcription 3 (STAT3) (Agrawal et al. 2003). CRP induced NF-κB activation by intracellular calcium and reactive oxygen species (ROS), which indicated that these factors are related to heart diseases (Chang et al. 2005; Zhao et al. 2019). CRP can activate Toll-like receptor (TLR4)/interferon regulatory factor-3/NF-κB pathway and induce the secretion of pro-inflammatory factor interleukin (IL)-6 to promote inflammation in rat vascular smooth muscle cells (VSMC) (Włodarczyk et al. 2017). It was also demonstrated that CRP promotes inflammation through cross-talk between TLR4/NF-κB/transforming growth factor-β1 (TGF-β1) signaling pathway in mouse cardiac myocyte HL-1 cells, highlighting the association between inflammation and the pathogenesis of atrial fibrillation (Sun et al. 2019). In addition, IκB kinases (IKKs) are upstream regulators of NF-κB in the inflammatory response for initiation and progression of cardiac disorders (Maier et al. 2012). NF-κB is activated when IκB (NF-κB inhibitor) is phosphorylated by IKKs. In ER signal pathways, ERs stimulate IKKs to activate NF-κB thereby regulating the downstream gene expression leading to inflammation (Guisasola et al. 2018). In clinical trials, the interaction of ERs and NF-κB has been used to treat cancer and inflammatory and autoimmune diseases (Kalaitzidis and Gilmore 2005).

BPA exposure is known to cause inflammatory response (Rogers et al. 2013). BPA can bind to nuclear receptors such as ERs, G protein-coupled receptors (GPR30), and androgen receptors (AR) to destroy signal transduction potentially contributing to human diseases (Cimmino et al. 2020). ERs are involved in genomic and non-genomic signaling transduction to regulate gene expression in cytosol and membrane (Day et al. 2013; Wehbe et al. 2020). BPA exhibits estrogenic activity through binding ERα/β (Kurosawa et al. 2002; Okazaki et al. 2017) and non-classic ER GPR30 for mediating non-genomic response (Prossnitz et al. 2008; Lee et al. 2013). BPA increases GRP30 and the production of specific inflammatory proteins (e.g., IL-6, IL-8) in human mammary adipocytes and in stromal-vascular fraction cells (Cimmino et al. 2019). Low-dose BPA (0.1 and 1 nM) induces intestinal inflammation and alters intestinal permeability by GPR30 activation and phosphorylation of NF-κB and extracellular regulated protein kinases (ERK)1/2 in human colon adenocarcinoma Caco2 cells (Nanayakkara et al. 2020). The combination of AR and NF-κB leads to transcriptome reprogramming in human prostate adenocarcinoma LNCaP cells with pro-inflammatory signals (Malinen et al. 2017). BPA can affect the expression of IKKs and NF-κB. BPA has been shown to up-regulate the IKKβ gene in the uterus of immature rats (Yang et al. 2015), and markedly activates ERK/NF-κB signal cascade and affects the expression of cytokines (Liu et al. 2014). In an animal study, a chronic low-dose exposure to BPA (50 μg/kg body weight/day for 12 weeks) accelerates atherosclerosis progress, and tends to endothelial dysfunction and vascular inflammation (Kim et al. 2014). A population-based study in Korea pointed that high BPA levels in urine are significantly related to high plasma CRP after adjusting for factors related to obesity and insulin resistance (Choi et al. 2017). BPA exposure has an effect on oxidative stress to increase the levels of CRP in postmenopausal women (Yang et al. 2009). These findings elucidated a possible association between chronic BPA exposure, inflammatory response, and CVD development.

In recent years, cardiovascular disease epidemics among the young population (20–45 years old) have particularly increased (Andersson and Vasan 2018). This is because young people have developed more and more unhealthy risk factors,

including obesity, poor diet, and lack of exercise. The Young Hearts Project 2000 (a study of 2,017 12–15 years old) presents a positive association between CRP and the incidence of CVD in teenagers (Wijnstok et al. 2010). Inflammation is one of the main risk factors concerned for CVD incidence in young people as well as age, gender, smoking, obesity, hypertension, high cholesterol, high blood sugar, and family history. Regarding BPA can mimic estrogen properties, it might induce the perturbation of IKK/NF-κB singling pathways through the interaction of estrogen-like activity with ER, and lead to CRP secretion in response to inflammation and CVD occurrence. Therefore, this study conducted a cross-sectional study of young adults to investigate the distribution of plasma BPA and CRP, and to explore the correlation between BPA exposure and the potential effects of ER and IKK gene expression on CRP secretion and CVD development. A directed acyclic graph (DAG) illustrated the research progress based on the purpose of this study that aimed to investigate the potential CVD occurrence underlying BPA-induced inflammation (Fig. 1).

Materials and methods

Study population

This study declared in the internet and poster to recruit 90 health volunteers (aged 20–45 years old) who are college students, faculties, and employees. Participants with cold or fever symptoms were excluded to avoid excessive CRP levels in the body. All subjects participating in this study signed a written informed consent form, and completed blood collection and a self-report questionnaire including the information of physical characteristics, dietary habits, and disease history. The research procedure of this study was reviewed and approved by the Research Ethics Committee of China Medical University.

Determination of BPA level in plasma

The 10 mL blood samples of study subjects were collected in glass EDTA tubes. Whole blood was centrifuged and stored at −80 °C until analysis. After thawing at room temperature, plasma (1 mL) was added 100 μL of 500 ng/mL (50 ng) internal standard (BPA-d_{16}), 5 mL methanol, and 200 g C18 powder. The mixture was shaken for 30 min and centrifuged at 4,000 rpm for 10 min. The supernatant was dried to concentrate in 0.5 mL acetonitrile and then subjected to LC/MSMS, consisting of an Ultra Performance Liquid Chromatograph (UPLC) system (Waters, Milford, MA) and a tandem mass spectrometer (Quattro premier XE; Waters) equipped with Acquity UPLC BEH® C18 column (2.1 mm × 100 mm; 1.7 μm; Waters). The mobile phase was 0.005 M ammonium acetate mixing 0.1% formic acid solution, and the gradient elution was run at a flow rate of 0.3 mL/min. Samples were ionized in negative ESI mode. The capillary voltage and

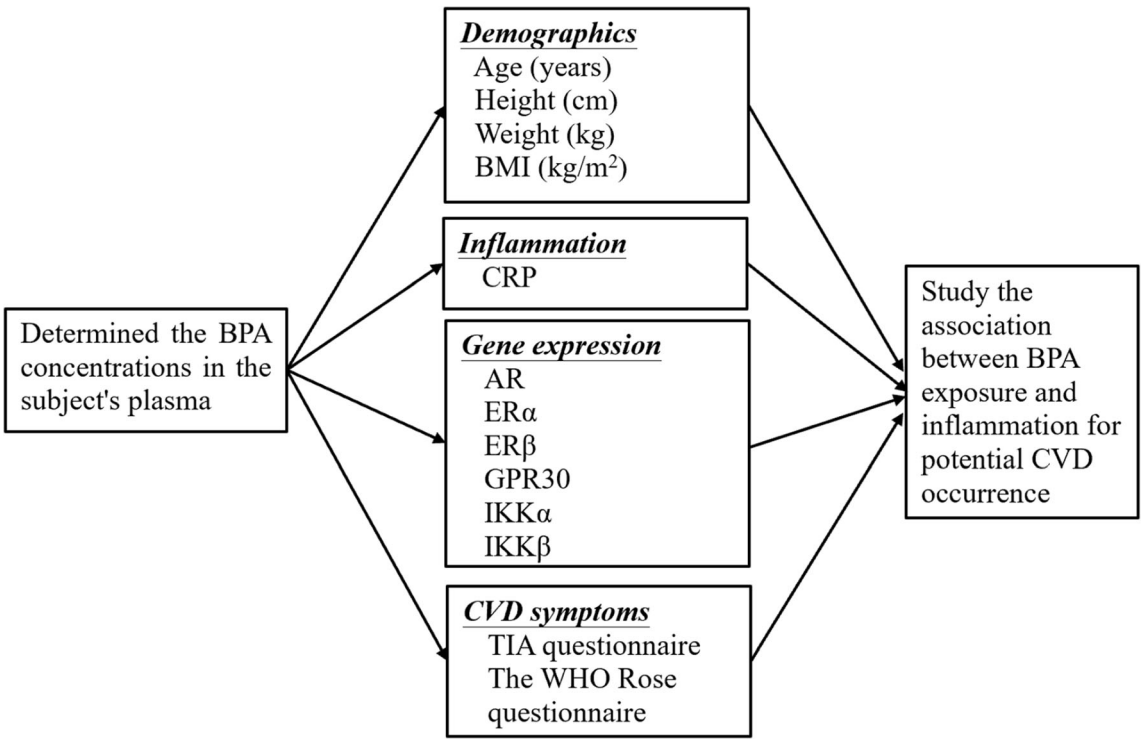

Fig. 1 The research progress in this study. The DAG addressed the process of this study that investigated the potential CVD occurrence underlying BPA-induced inflammation

source temperature were set at 3 kV and 120°C, respectively. The qualitative and quantitative analysis of standard (a) BPA and (b) BPA-d16 at the concentration of 100 ng/mL respectively dissolved in methanol and in plasma is shown in Fig. S1. The recovery rate was 88–115% at the detention time of 2.12 min, and the limitation of detection (LOD) and the limitation of quantitation (LOQ) of BPA were 0.1 ng/mL and 0.05 ng/mL, respectively.

Enzyme-linked immunosorbent assay for CRP determination

The concentrations of plasma CRP were determined using Quantikine® human immunoassay kits (R&D Systems Inc, Minneapolis, MN) according to the manufacturer's manual. The optical density of each well was determined at 450 nm using a VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA). The levels of CRP were deduced from the absorbance value by extrapolation from a standard curve generated with 4-parameter logistic curve fit. The minimum detectable dose of CRP is typically less than 0.005 ng/mL.

Reverse transcription polymerase chain reaction and quantitative real-time PCR for gene expression determination

After RNA samples were extracted by GENEzol TriRNA Pure Kit (Geneaid Biotech Ltd., Taiwan), cDNA was synthesized from total RNA by a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster, CA). The 2 μg RNA was placed in a 0.2 mL PCR tube with 1.0 μL MultiScribeTM reverse transcriptase (50 unit/μL), 2.0 μL 10X RT random primers, 0.8 μL 20× concentrated dNTP mix, 2.0 μL 10× concentrated RT buffer and RNase free water (DEPC water). The mixture was amplified by a ABI 2720 thermal cycler (Applied Biosystems) with one cycle each of 20 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s. One microgram cDNA was amplified by 7300 Real-Time PCR system (Applied Biosystems) with 40 cycles of denaturing (95 °C, 15 s), annealing (55 °C, 30 s) and extension (72 °C, 45 s) using 2× power SYBR green PCR master mix (Applied Biosystems). Quantitative analysis of PCR products was carried out by a sequence detector according to the manufacturer's instruction. The optical density (OD) of DNA transmitted light from SYBR green was measured at 530 nm during the extension phase, and collected and analyzed with SDS 1.0 software. The threshold cycle (Ct) value denotes the cycle number at which the fluorescence generated within a reaction across the threshold, thus the Ct value makes the point during the reaction in which a sufficient number of amplicons were accumulated. The relative level of mRNA expression of AR, ERα, ERβ, GPR30, IKKα, and IKKβ is a ratio of OD to β-actin (internal control, an endogenous housekeeping gene).

Statistical analysis

Statistical analysis was conducted by SPSS17. Plasma BPA levels of study subjects were divided by median (4.34 ng/mL) into high and low BPA exposure groups, and plasma CRP levels were divided by median (2.42 ng/mL) of logCRP into high and low CRP level groups. Student's t test was used to analyze statistical differences between physiological index (age, height, weight, body mass index (BMI), systolic and diastolic blood pressure), plasma BPA levels, CRP concentration, and gene expression between the high and low BPA groups as well as between the high CRP group and low CRP groups. The multiple logistic regression was used to evaluate the OR of the potential impact of BPA exposure on physiological index and gene expression, OR of the potential impact of CRP secretion on physiological indicators and gene expression, and OR of potential CVD development. Symptoms are attributed to the above variables. One-way analysis of variance (ANOVA) was performed to compare the OR of gene expression based on BPA exposure tertiles to test content response. All statistical significances were determined with a two-tailed $^{*}p < 0.05$ and $^{\#}p < 0.1$.

Results

The levels of BPA in the plasma samples of 90 young adults were all detectable. Figure 2(a) shows that the Gaussian distribution (Kolmogorov–Smirnov test for normality test, $p = 0.097$) of BPA levels in the plasma samples was 4.47 ± 2.0 ng/mL (mean) and 4.34 ng/mL (median), respectively. The BPA level in this study was slightly higher than some other studies mentioned in Fig. 2(b).

All of 90 study subjects recruited in this study received a questionnaire survey. According to the median BPA level, the subjects were further divided into the high BPA group and low BPA group. The sample size of each group ($n = 45$) can ensure that when the difference of mean and the variance of BPA levels between the two groups were 3.3 and 1.12, the 2-sided test with alpha = 0.5 has 80% power to detect the difference between the two groups. Table 1 presents the demographic characteristics, and the levels of BPA, CRP, and gene expression in 63 male and 27 female young adults. The average (± SD) age, height, weight, and BMI of young adults were 25.1 ± 4.8 years old, 167.1 ± 8.6 cm, 62.4 ± 11.9 kg and 22.3 ± 3.3 kg/m^2. The means of plasma BPA and CRP were 4.5 ± 2.0 ng/mL and 678.0 ± 918.1 ng/mL, respectively. The relative gene expression of AR, ERα, ERβ, GPR30, IKKα, and IKKβ were 0.52 ± 0.48 × 10^{-3}, 1.16 ± 0.69 × 10^{-3}, 0.42 ± 0.31 × 10^{-3}, 0.94 ± 0.92 × 10^{-3}, 2.90 ± 0.84 × 10^{-3}, and 1.76 ± 0.51 × 10^{-3} in average. The high BPA group had significantly higher levels of BMI, diastolic blood pressure (DBP), and CRP than the low BPA group. The gene expression of ERβ

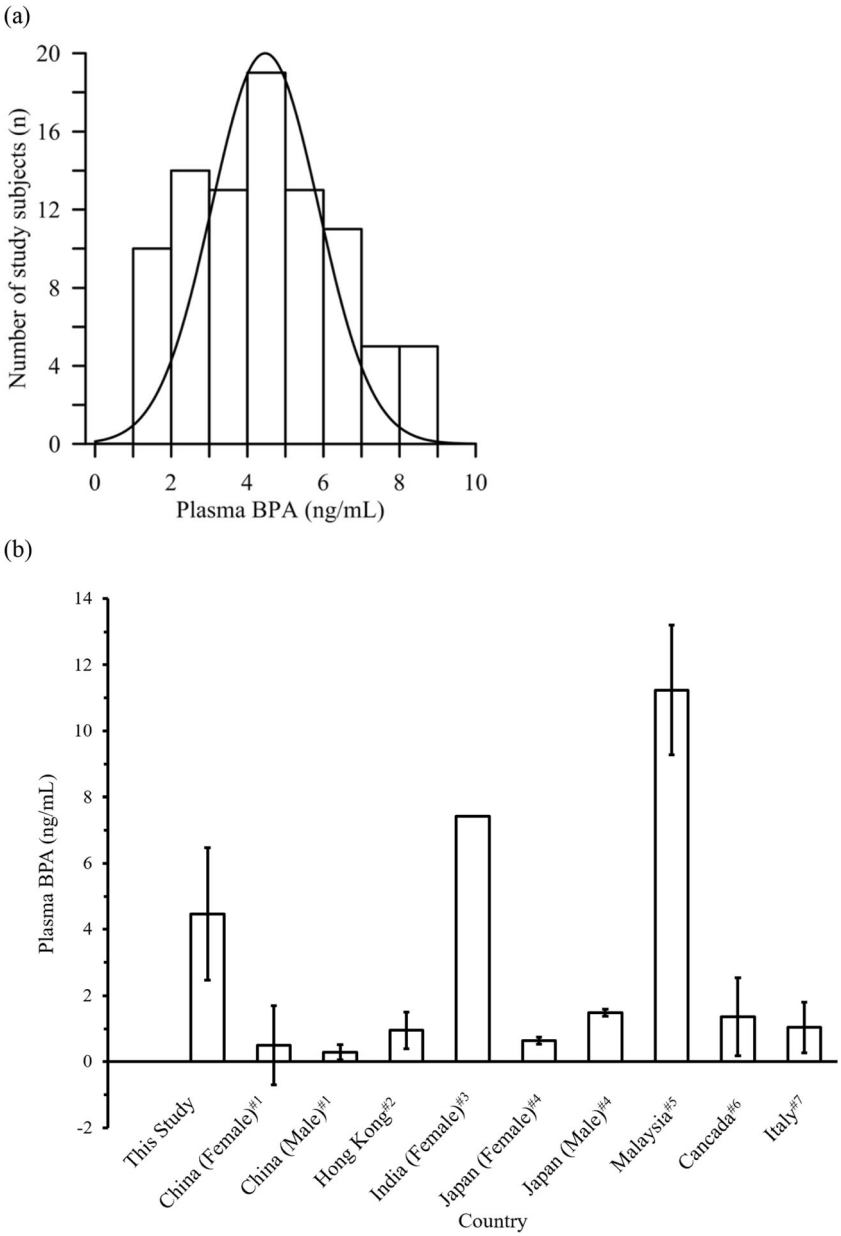

Fig. 2 BPA concentration in plasma samples. (**a**) Distribution of plasma BPA levels of 90 young adults in this study. (**b**) The levels of plasma BPA in this study and other countries. [#1](China; Female, *n*=47; Male, *n*=34; Jin et al. 2018); [#2](Hong Kong; *n*=157; Wan et al. 2013); [#3](India; *n*=53; Shekhar et al. 2017); [#4](Japan; Female, *n*=14; Male, *n*=11; Schönfelder et al. 2002); [#5](Malaysia; *n*= 150, Wiraagni et al. 2020); [#6](Belgium; *n*= 44, Huygh et al. 2015); [#7](Italy; *n*= 76, Savastano et al. 2015).

and IKKβ was also higher in the high BPA group than in the low BPA group.

In Fig. 3 the multivariable adjusted ORs of gene expression of AR, ERα, ERβ, GPR30, IKKα, and IKKβ were separately plotted in the three groups (1st, 2nd, and 3rd) according to the plasma BPA levels at 0–33rd, 33rd–66th, and 66th–100th percentiles. The adjusted ORs of ERβ (OR 4.03, 95% CI 1.37–11.8) and IKKβ (OR 4.75, 95% CI 1.58–14.2) were significant in the 66th–100th percentile of BPA exposure. The adjusted ORs of ERα and GPR30 were not significant, but slightly increased in a dose–response manner of BPA exposure.

The CRP levels are listed according to demographic characteristics and gene expression in Table 2. The values of body weight (66.2 ± 10.9 vs. 60.6 ± 9.6 kg), BMI (23.6 ± 2.9 vs. 21.7 ± 2.3), plasma BPA levels (5.4 ± 2.0 vs. 4.4 ± 1.6 ng/mL), and IKKβ expression (1.97 ± 0.53 vs. 1.68 ± 0.68) were significantly higher in the high CRP group than in the low CRP group, respectively.

The ORs of physiologic index, BPA exposure, and gene expression contributed to plasma CRP secretion are shown in Table 3. Higher values of BMI (OR 2.74, 95% CI 1.03–7.27) and gene expression of IKKβ (OR 3.45, 95% CI 1.15–10.4) significantly promoted the increase of plasma CRP in young adults. In multivariate analysis, the adjusted ORs of plasma CRP secretion were even attributed to BMI (OR 3.25, 95% CI 1.07–11.9) and gene expression of IKKβ (OR 2.23, 95% CI 1.07–4.59), respectively.

Table 1 BPA levels of demographic characteristics and gene expression. Values were presented as mean ± SD

Variables	All (n = 90)	Group[a]					
		High BPA (n = 45)		Low BPA (n = 45)		p value	
Demographics							
Age (years)	25.1	4.8	25.3	5.3	25.2	4.8	0.917
Height (cm)	167.1	8.6	166.6	8.5	167.5	8.7	0.634
Weight (kg)	62.4	11.9	64.3	13.9	60.6	9.4	0.097
BMI (kg/m^2)	22.3	3.3	23.0	3.8	21.6	2.6	0.033*
SBP (mmHg)	119.6	15.4	121.2	13.9	116.0	12.9	0.072
DBP (mmHg)	73.4	10.1	75.8	11.5	71.0	8.1	0.025*
Plasma BPA (ng/mL)	4.5	2.0	6.1	1.3	2.8	0.9	<0.001*
Plasma CRP (ng/mL)	678.0	918.1	881.0	1083.7	425.9	582.8	0.041*
Gene expression (relative to β-actin value)							
AR (10^{-3})	0.52	0.48	0.51	0.39	0.53	0.55	0.812
ERα (10^{-3})	1.16	0.69	1.20	0.71	1.12	0.67	0.581
ERβ (10^{-3})	0.42	0.31	0.50	0.32	0.34	0.27	0.017*
GPR30 (10^{-3})	0.94	0.92	0.96	0.94	0.92	0.90	0.826
IKKα (10^{-3})	2.90	0.84	2.85	0.79	2.95	0.89	0.602
IKKβ (10^{-2})	1.76	0.51	1.83	0.60	1.57	0.57	0.049*

BMI body mass index, *SBP*: systolic blood pressure, *DBP* diastolic blood pressure

[a] Divided by the median of plasma BPA levels (4.34 ng/mL)

p value from Student's t test: *$p < 0.05$

This study referred the transient ischemic attack (TIA) questionnaire (Toole et al. 1996) and the WHO Rose angina questionnaire to investigate the sudden onset of CVD symptoms in the study subjects. All subjects in this study reported that they had no history of CVD, but some of them indicated that they had sudden symptoms of CVD according to the TIA questionnaire (7 items) and the WHO questionnaire (8 items) in Table 4. This study further classified 43 of the 90 subjects (47.8%) as the potential CVD development symptom group because they answered to have at least 2 symptom items of CVD in these two questionnaires. The level of BPA in the potential CVD development symptom group (4.7 ± 0.95 ng/mL, n=43) was slightly higher than in the less CVD symptom group (3.8 ± 0.86 ng/mL, n=47, p = 0.291) (Fig. 4). In Table 5, after covariables were adjusted, the study subjects with CVD symptoms had the higher BPA exposure level (OR 2.10, 95% CI 0.83–5.39) and CRP secretion (OR 2.61, 95% CI 1.03–10.6) and IKKβ expression (OR 4.29, 95% CI 1.51–15.6).

Discussion

This study investigated the level of BPA exposure in young adults and its correlation with gene expression of pro-inflammatory response and CVD symptoms. We found that the high BPA exposure group in this study had higher BMI, DBP, CRP secretion, and gene expression of ERβ and IKKβ. In the multivariate analysis, the ORs of CRP secretion or CVD development symptoms were in the increased manner with BPA exposure even without statistically significant. In addition, the gene expression of IKKβ related to CRP generation of pro-inflammatory response presented significantly associated with BPA exposure, CRP secretion, and CVD development symptoms. Therefore, exposure to BPA exposure may lead to early pro-inflammatory response, which would perform one of potential risks of CVD development symptoms.

Blood BPA levels vary among countries. The mean value of young adults in this study of adult plasma BPA concentration was 4.5 ng/mL. Our previous study determined the plasma BPA level of pregnant women (n = 90) indicating a geometric mean of 2.51 ng/mL (Chou et al. 2011). Elsewhere, the average level of plasma BPA is measured at 4.4 ng/mL in 372 Americans (mean age 35 years) (Clayton et al. 2011), and 3.88 and 2.32 ng/mL respectively in 208 (age 21–30 years) and 423 (age 31–40 years) workers from 10 factories of chemical or machinery industries and their spouses and children in China (He et al. 2009). According to the questionnaire survey in this study (data not shown), the plasma BPA levels (mean ± SD (n)) were different in dining behaviors, such as common food container use (plastic *vs.* non-plastic 4.9 ± 2.1 (50) *vs.* 3.9 ± 1.7 (40) ng/mL, p = 0.012), dining-out place (in store *vs.* takeaway 4.0 ± 2.2 (44) *vs.* 4.9 ± 1.7 (46) ng/mL, p = 0.033), and frequency of takeaway dinner (≤ 3 times/week *vs.* > 3 times/week 3.9 ± 2.0 (45) *vs.* 5.0 ± 1.9 (45) ng/mL, p

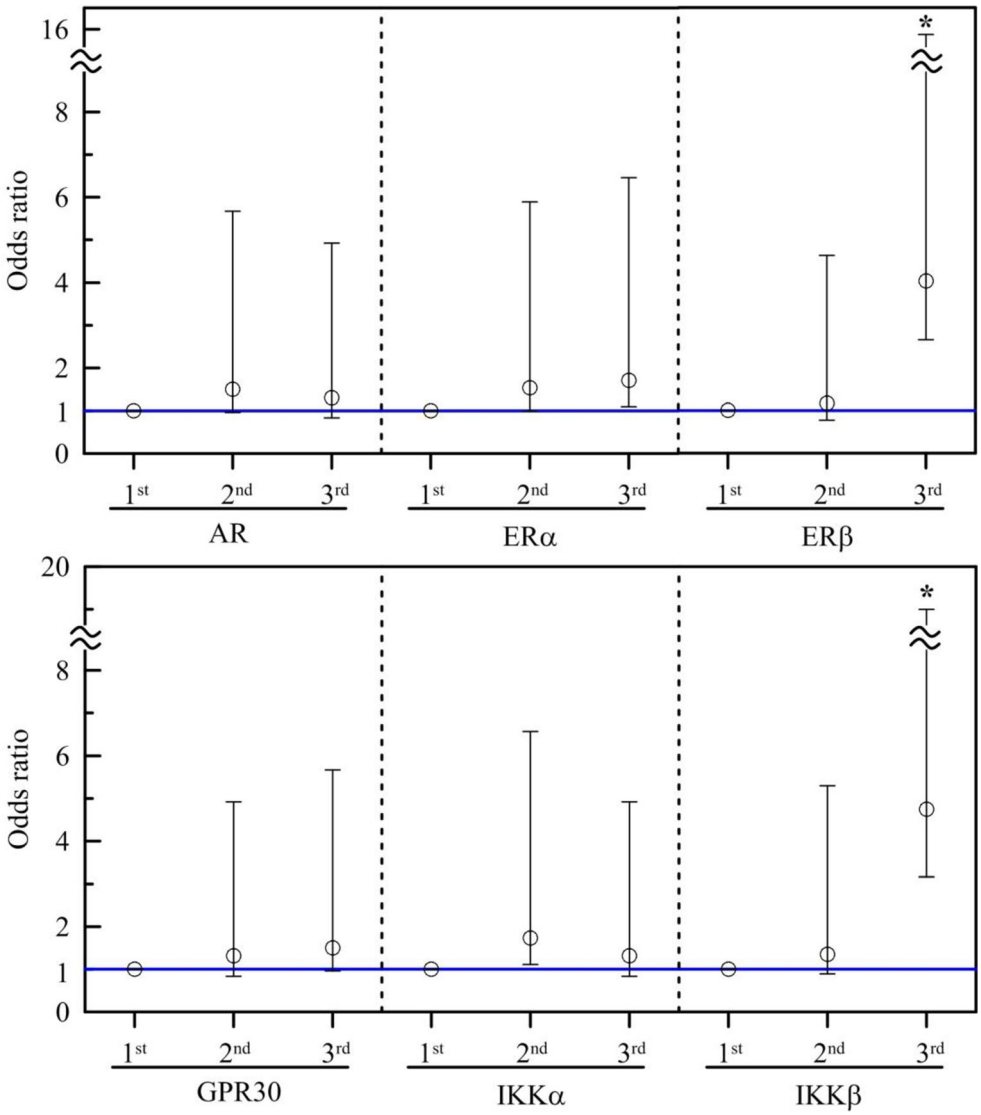

Fig. 3 Adjusted ORs of gene expression by BPA exposure tertiles. The ORs (95% CI) of gene expression of AR, ERα, ERβ, GPR30, IKKα, and IKKβ were respectively calculated in logistical regression analysis. First, the levels of gene expression of these six genes were individually divided by the BPA exposure tertiles (concentration from low to high: the 1st percentile: 0–33%, the 2nd percentile: 33–66%, the 3rd percentile: 66–100%) into three groups. Then, through the adjustment of gender, age, BMI, systolic blood pressure (SBP), DBP, and CRP, these six genes were independently analyzed in logistical regression analysis. Each of the three groups in the same gene was compared with the reference group (the 1st percentile) to perform the OR of each group, and the ORs of three group were further tested by ANOVA.

= 0.010). These findings suggested that dining-out (takeaway) would lead more opportunities to use plastic containers or bags for BPA exposure.

In this study, the high plasma BPA group had significantly higher DBP and BMI values (Table 1). Similarly, a cross-sectional study of 3,967 Americans showed that the multivariate-adjusted OR of BPA levels for BMI-based obesity is 1.69 (95% CI 1.30–2.20) in quartile 4 compared to quartile 1 (Shankar and Teppala 2012). BPA by itself can trigger 3 T3-L1 fibroblasts to differentiate into adipocytes (Wu et al. 2020). Therefore, BPA exposure was related to the increased values of BMI and blood pressure, which may perform as a risk factor for CVD development.

In this study, the gene expression of IKKβ and ERβ was used as pro-inflammatory genetic biomarkers, and we found that they were significantly higher expression in high BPA exposure group than those in the low BPA exposure group (Table 1). In addition, the ORs of BPA exposure level significant contributed to gene expression of ERβ and IKKβ with high concentration (Fig. 3). These results are congruent with those found in 96 adults (age 32–76 years) from the InCHIANTI population in the U.S.A., the high BPA group (0.8 IU) has 65% higher geometric mean ERβ expression than the low BPA group (1.32 IU) (Melzer et al. 2011). ERβ can mediate diverse physiological functions, including anti-proliferation, apoptosis, and association with heart failure (Yakimchuk et al. 2013).

Extracellular signals can activate more secondary messengers via non-genomic pathways. BPA can activate secondary messengers (e.g., cAMP-dependent protein kinase) through estrognic-like activity (binding to estrogenic receptors), and indirectly induce IKKs to regulate activation of NF-κB singling pathway. An *in vitro* study showed that BPA can activate NF-κB, and increase IL-6 and TNFα production through IKKβ phosphorylation (Chou et al. 2011). IKKβ activates NF-κB to promote the transcription of genes encoding

Table 2 CRP levels of demographic characteristics and gene expression. Values were presents as mean ± SD

Variables	All (n = 90)	Group[a]					
		High CRP (n = 45)		Low CRP (n = 45)		p value	
Characteristics							
Age (years)	25.1	4.8	26.1	6.5	24.9	3.7	0.136
Height (cm)	167.1	8.6	167.4	8.1	166.9	8.1	0.791
Weight (kg)	62.4	11.9	66.2	10.9	60.6	9.6	0.026*
BMI (kg/m^2)	22.3	3.3	23.6	2.9	21.7	2.3	0.004*
SBP (mmHg)	119.6	15.4	12.4	17.1	117.5	13.3	0.062
DBP (mmHg)	73.4	10.1	75.2	11.6	71.6	7.7	0.128
Plasma BPA (ng/mL)	4.5	2.0	5.4	2.0	4.4	1.6	0.043*
Plasma CRP (ng/mL)	678.0	918.1	429.8	204.6	105.3	61.9	<0.001*
Gene expression (relative to β–actin value)							
AR (10^{-3})	0.52	0.48	0.57	0.51	0.50	0.39	0.595
ERα (10^{-3})	1.16	0.69	1.14	0.57	1.34	0.94	0.359
ERβ (10^{-3})	0.42	0.31	0.48	0.37	0.43	0.31	0.590
GPR30 (10^{-3})	0.94	0.92	1.02	0.93	0.94	0.98	0.752
IKKα (10^{-3})	2.90	0.84	2.96	1.31	3.07	1.37	0.745
IKKβ (10^{-2})	1.76	0.51	1.97	0.53	1.68	0.68	0.064#

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

[a] Divided by the median of logCRP (2.42 ng/mL)

p value from Student's t test: *$p < 0.05$ and #$p < 0.1$

chemokines and cytokines involved in inflammation (Liu et al. 2017). By activating IKKβ, BPA can promote inflammatory response. As to potential pro-inflammatory effect, BPA markedly up-regulates IL-6 and TNF-α mRNA expression in nerve BV-2 cells in a dose-dependent manner (Zhu et al. 2015), and IL-6 and TNF-α can increase the liver secretion of CRP. This study found that the high BPA group had higher gene expression of ERβ and IKKβ. It suggested that BPA might induce CRP secretion in response to inflammation by ERβ binding and IKKβ stimulation to activate NF-κB and IL-6.

CRP is a well-known inflammation marker, and used to assess the risk of future CVD. In this study, the high BPA group had the significantly higher level of CRP than the low BPA group (Table 1), and the high CRP group had the significantly higher plasma BPA levels than the low CRP group (Table 2). The increase of BPA exposure, BMI, and IKKβ potentially elevated the odds ratio of higher plasma CRP levels (Table 3). The gene expression of IKKβ related to CRP generation of pro-inflammatory response presented significantly associated with BPA exposure (Fig. 3), CRP secretion (Table 3), and CVD development symptoms (Table 5). Furthermore, we found that the levels of BPA, CRP, and IKKβ may contribute to the odds ratio of potential CVD development symptoms (Table 5). Although some studies have failed to find a positive relationship between BPA and CVD risk (Olsén et al. 2012; Wang et al. 2012), a few studies noted a possible positive relationship between BPA and cardiovascular risk (Melzer et al. 2012a; Melzer et al. 2012b). There might be adverse effect of BPA on inducing inflammation response for CRP secretion underlying potential CVD development via binding ERβ to activate IKKβ expression. CRP can induce inflammatory response through enhanced angiotensin II (Wang et al. 2019). Above findings indicated that BPA exposure may activate ERβ and IKKβ gene expression for CRP secretion, which was correlated to early pro-inflammatory response and adverse CVD development symptoms.

Table 3 Odds ratios of plasma CRP secretion attributed to physiologic index, BPA level, and gene expression

Variables (X_n)	Crude OR (95% CI)	p	Adjusted OR (95% CI)[d]	p
Characteristics[a]				
BMI	2.74 (1.03–7.27)	0.043*	3.25 (1.07–11.9)	0.039*
BPA exposure[b]	2.03 (0.75–5.51)	0.165	1.82 (0.58–5.36)	0.283
Gene expression[c]				
ERβ	1.69 (0.59–4.82)	0.329	2.74 (0.78–9.82)	0.132
IKKβ	3.45 (1.15–10.4)	0.027*	2.23 (1.07–4.59)	0.046*

Dependent variable (Y): Plasma CRP levels were divided into two CRP groups (high and low) by logCRP median (2.42 ng/mL).

Independent variables (X_n): BMI, BPA exposure, ERβ, IKKβ

The low-level group of each variable was used as a reference.

[a] Divided by median

[b] Divided by median of plasma BPA levels (4.34 ng/mL)

[c] Divided by 66th percentile of gene expression

[d] Adjusted by gender, age, BMI, BPA exposure, ERβ, and IKKβ.

p value from logistic regression: *$p < 0.05$

Table 4 Questions of TIA questionnaire and Rose questionnaire

Questions	*n* (%)	
	Yes	No
TIA questionnaire		
Were you ever told by a physician that you had a mini-stroke or TIA?	0 (0)	100 (100)
Have you ever had sudden painless weakness on one side of your body?	10 (11)	80 (89)
Have you ever had sudden numbness or a dead feeling on one side of your body?	45 (50)	45 (50)
Have you ever had sudden painless loss of vision in one or both eyes?	6 (57)	84 (93)
Have you ever had suddenly lost one half of your vision?	3 (43)	87 (97)
Have you ever had suddenly lost the ability to understand what people were saying?	28 (31)	62 (69)
Have you ever had suddenly lost the ability to express yourself verbally or in writing?	11 (12)	79 (88)
Rose questionnaire		
Have you ever had any pain or discomfort in your chest?	36 (40)	54 (60)
Do you get the pain or discomfort when you walk uphill or hurry?	8 (29)	82 (91)
Do you get the pain when you walk at an ordinary pace on the level?	2 (22)	88 (98)
Do you get the pain in the center of the chest or left chest or left arm?	0 (0)	100 (100)
Do you slow down if you get the pain while working?	0 (0)	100 (100)
Does the pain go way if you stand still or if you take a tablet under the tongue?	0 (0)	100 (100)
Does the pain go away in less than 10 min?	0 (0)	100 (100)
Have you ever have you ever had severe chest pain across the front of your chest lasting for half an hour or more?	0 (0)	100 (100)

Previous studies have reported an association between the levels of BPA and CRP. Urinary BPA (range 0.28–112 ng/mL) and serum CRP (200–87600 ng/mL) were found to be significantly correlated (Naji 2017). A Korean study showed that healthy volunteers with urinary BPA levels higher than ≥ 75th percentile (≥ 2.057 ng/mL)

Table 5 Odds ratio of CVD development symptoms attributed to physiologic index, plasma BPA and CRP levels, and gene expression

Variables (X_n)	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)[e]	*p*
Characteristics[a]				
BMI	2.05 (0.88–4.74)	0.095[#]	2.81 (0.57–13.9)	0.205
SBP	1.91 (0.82–4.47)	0.134	2.75 (0.37–20.4)	0.323
DBP	1.49 (0.64–3.45)	0.358	1.52 (0.27–8.46)	0.636
BPA exposure[b]	2.93 (0.76–4.90)	0.086[#]	2.10 (0.83–5.39)	0.093[#]
CRP level[c]	2.73 (0.90–8.30)	0.076[#]	2.61 (1.03–10.6)	0.046*
Gene expression[d]				
ERβ	1.10 (0.40-3.02)	0.853	1.61 (0.29-8.82)	0.585
IKKβ	4.08 (1.42-11.7)	0.009*	4.29 (1.51-15.6)	0.015*

Dependent variable of CVD development symptom group (Y): Study subjects having more than two symptom items in the Rose questionnaire or the transient ischemic attack (TIA) questionnaire were divided into the potential CVD development symptom group. Others belonged to the non-CVD symptom group.

Independent variables (X_n): BMI, SBP, DBP, BPA exposure, CRP level, ERβ, and IKKβ

The low-level or non-group of each variable was used as a reference.

[a] Divided by median

[b] Divided by median of plasma BPA levels (4.34 ng/mL)

[c] Divided by median of logCRP (2.42 ng/mL)

[d] Divided by 66th percentile of gene expression

[e] Adjusted by gender, age, BMI, SBP, DBP, BPA exposure, CRP level, ERβ, and IKKβ

p value from logistic regression: *$p < 0.05$ and [#] $p < 0.1$

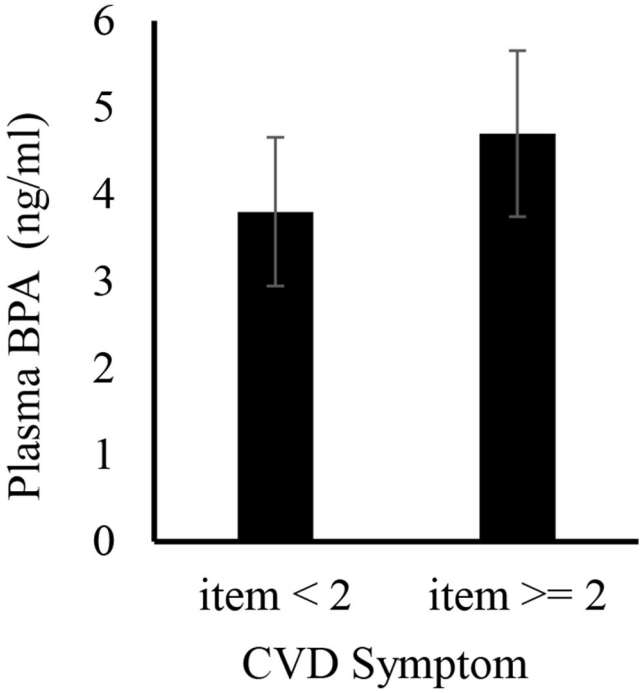

Fig. 4 The levels of plasma BPA by CVD symptom items. The number of items with CVD symptoms was answered by study subjects based on the TIA questionnaire and the WHO questionnaire.

have significant OR for high CRP (≥ 2000 ng/mL) after adjusting for confounding factors (OR 2.85, 95% CI 1.16–6.97) (Choi et al. 2017). The levels of CRP and BPA in serum present a linear relationship, and significantly higher in the COPD patients (means of control and COPD: CRP 3640 and 5060 ng/mL, BPA 0.57 and 3.04 ng/mL) (Erden et al. 2014). The results of this study may underestimate the impacts of BPA on CRP secretion. This study conducted a cross-sectional study in general population without obvious diseases. The 82% of the 90 participants had the plasma CRP concentration below 1,000 ng/mL (mean 678.0 ± 918.1 ng/mL) even though the average plasma BPA level was 4.5±2.0 ng/mL. In clinical practice, the low value of CRP concentrations indicates no correlation with CVD risk. However, this study can still observe differences in CRP levels and potential CVD development symptoms between the high BPA group and the low BPA group. We assumed if study subjects have higher CRP values and/or BPA levels (e.g., occupational exposure), the impact of BPA exposure on CRP secretion would be more obvious. Therefore, it is worth noting that participants who frequently meal out (or takeaway) or often use plastic containers to hold food had a higher BPA content. These results implied that the use of plastic materials may increase the exposure to BPA, and the adverse health effect of BPA exposure may be related to early pro-inflammation, which may be potential for CVD development.

Conclusion

This study indicated that BPA exposure may elevate early pro-inflammatory gene expressions potentially for CRP secretion, which would be correlated with inflammatory response and the risk of CVD development symptoms. In this study, participants who often meal out (or takeaway) may use plastic materials to hold food and increase BPA exposure. Therefore, it is necessary to reduce the plastics used in food containers from the adverse health effects of BPA exposure.

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Author contribution HRC, JLC, and CYC coordinated in the research design. CMT, JHL, and DPY conducted the experiments. WCC, JHL, and CYC performed the data analysis, and WCC, JLC, and YCH assisted in illustrating the graphs. CMT, WCC, YCH, and CYC wrote and edited the draft of the manuscript.

Data availability The dataset and materials of the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards and approved by the Research Ethics Committee of China Medical University.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent to Publish All authors read and approved the final manuscript for publication, and declared no conflicts of interest.

References

Agrawal A, Cha-Molstad H, Samols D, Kushner I (2003) Overexpressed nuclear factor-kappaB can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBPbeta and signal transducer and activator of transcription-3. Immunology 108:539–547

Andersson C, Vasan RS (2018) Epidemiology of cardiovascular disease in young individuals. Nat Rev Cardiol 15:230–240

Baker RG, Hayden MS, Ghosh S (2011) NF-κB, inflammation, and metabolic disease. Cell Metab 13:11–22

Chang JW, Kim CS, Kim SB, Park SK, Park JS, Lee SK (2005) C-reactive protein induces NF-kappaB activation through intracellular calcium and ROS in human mesangial cells. Nephron Exp Nephrol 101:e165–e172

Choi YJ, Ha KH, Kim DJ (2017) Exposure to bisphenol A is directly associated with inflammation in healthy Korean adults. Environ Sci Pollut Res Int 24:284–290

Chou WC, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY (2011) Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ Health 10:94

Cimmino I, Oriente F, D'Esposito V, Liguoro D, Liguoro P, Ambrosio MR, Cabaro S et al (2019) Low-dose Bisphenol-A regulates inflammatory cytokines through GPR30 in mammary adipose cells. J Mol Endocrinol 63:273–283

Cimmino I, Fiory F, Perruolo G, Miele C, Beguinot F, Formisano P, Oriente F (2020) Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease. Int J Mol Sci 21:5761

Clayton EM, Todd M, Dowd JB, Aiello AE (2011) The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ Health Perspect 119:390–396

Day NL, Floyd CL, D'Alessandro TL, Hubbard WJ, Chaudry IH (2013) 17β-estradiol confers protection after traumatic brain injury in the rat and involves activation of G protein-coupled estrogen receptor 1. J Neurotrauma 30:1531–1541

Erden ES, Motor S, Ustun I, Demirkose M, Yuksel R, Okur R, Oktar S, Yakar Y, Sungur S, Gokce C (2014) Investigation of Bisphenol A as an endocrine disruptor, total thiol, malondialdehyde, and C-reactive protein levels in chronic obstructive pulmonary disease. Eur Rev Med Pharmacol Sci 18:3477–3483

Geens T, Aerts D, Berthot C, Bourguignon JP, Goeyens L, Lecomte P, Maghuin-Rogister G, Pironnet AM, Pussemier L, Scippo ML, Van Loco J, Covaci A (2012) A review of dietary and non-dietary exposure to bisphenol-A. Food Chem Toxicol 50:3725–3740

Guisasola MC, Alonso B, Bravo B, Vaquero J, Chana F (2018) An overview of cytokines and heat shock response in polytraumatized patients. Cell Stress Chaperones 23:483–489

Han C, Hong YC (2016) Bisphenol A, hypertension, and cardiovascular diseases: epidemiological, laboratory, and clinical trial evidence. Curr Hypertens Rep 18:11

He Y, Miao M, Herrinton LJ, Wu C, Yuan W, Zhou Z, Li DK (2009) Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. Environ Res 109:629–633

Huygh J, Clotman K, Malarvannan G, Covaci A, Schepens T, Verbrugghe W, Dirinck E, Van Gaal L, Jorens PG (2015) Considerable exposure to the endocrine disrupting chemicals phthalates and bisphenol-A in intensive care unit (ICU) patients. Environ Int 81:64–72

Jin H, Zhu J, Chen Z, Hong Y, Cai Z (2018) Occurrence and partitioning of Bisphenol analogues in adults' blood from China. Environ Sci Technol 52:812–820

Kalaitzidis D, Gilmore TD (2005) Transcription factor cross-talk: the estrogen receptor and NF-kappaB. Trends Endocrinol Metab 16: 46–52

Kim MJ, Moon MK, Kang GH, Lee KJ, Choi SH, Lim S, Oh BC, Park DJ, Park KS, Jang HC, Park YJ (2014) Chronic exposure to bisphenol A can accelerate atherosclerosis in high-fat-fed apolipoprotein E knockout mice. Cardiovasc Toxicol 14:120–128

Konieczna A, Rutkowska A, Rachoń D (2015) Health risk of exposure to Bisphenol A (BPA). Rocz Panstw Zakl Hig 66:5–11

Kurosawa T, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fujiwara T, Inoue S, Muramatsu M, Momoeda M, Taketani Y (2002) The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. Endocr J 49:465–471

Lakind JS, Goodman M, Mattison DR (2014) Bisphenol A and indicators of obesity, glucose metabolism/type 2 diabetes and cardiovascular disease: a systematic review of epidemiologic research. Crit Rev Toxicol 44:121–150

Lee HR, Jeung EB, Cho MH, Kim TH, Leung PC, Choi KC (2013) Molecular mechanism(s) of endocrine-disrupting chemicals and their potent oestrogenicity in diverse cells and tissues that express oestrogen receptors. J Cell Mol Med 17:1–11

Liu Y, Mei C, Liu H, Wang H, Zeng G, Lin J, Xu M (2014) Modulation of cytokine expression in human macrophages by endocrine-disrupting chemical Bisphenol-A. Biochem Biophys Res Commun 451:592–598

Liu T, Zhang L, Joo D, Sun SC (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:17023

Magliano DJ, Lyons JG (2013) Bisphenol A and diabetes, insulin resistance, cardiovascular disease and obesity: controversy in a (plastic) cup? J Clin Endocrinol Metab 98:502–504

Maier HJ, Schips TG, Wietelmann A, Krüger M, Brunner C, Sauter M, Klingel K, Böttger T, Braun T, Wirth T (2012) Cardiomyocyte-specific IκB kinase (IKK)/NF-κB activation induces reversible inflammatory cardiomyopathy and heart failure. Proc Natl Acad Sci U S A 109:11794–11799

Malinen M, Niskanen EA, Kaikkonen MU, Palvimo JJ (2017) Crosstalk between androgen and pro-inflammatory signaling remodels androgen receptor and NF-κB cistrome to reprogram the prostate cancer cell transcriptome. Nucleic Acids Res 45:619–630

Meguro S, Ishibashi M, Takei I (2012) The significance of high sensitive C reactive protein as a risk factor for cardiovascular diseases. Rinsho Byori 60(4):356–361

Melzer D, Harries L, Cipelli R, Henley W, Money C, McCormack P, Young A, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Galloway T (2011) Bisphenol A exposure is associated with in vivo estrogenic gene expression in adults. Environ Health Perspect 119:1788–1793

Melzer D, Gates P, Osborne NJ, Henley WE, Cipelli R, Young A, Money C, McCormack P, Schofield P, Mosedale D, Grainger D, Galloway TS (2012a) Urinary bisphenol a concentration and angiography-defined coronary artery stenosis. PLoS One 7:e43378

Melzer D, Osborne NJ, Henley WE, Cipelli R, Young A, Money C, McCormack P, Luben R, Khaw KT, Wareham NJ, Galloway TS (2012b) Urinary bisphenol A concentration and risk of future coronary artery disease in apparently healthy men and women. Circulation 125:1482–1490

Naji H (2017) The interaction of C-Reactive protein, bisphenol A, & cardiovrascular Disease: a demographical analysis. Global J Health Sci 9:63–72

Nanayakkara N, Cimmino I, Cerchione C, D'Esposito V, Oriente F, Troncone R, Formisano P, Barone MV, Valentino R (2020) Inflammation induced by very low-dose bisphenol-a can be prevented by probiotics. J Transl Sci 7:1–8

Nomiri S, Hoshyar R, Ambrosino C, Tyler CR, Mansouri B (2019) A mini review of bisphenol A (BPA) effects on cancer-related cellular signaling pathways. Environ Sci Pollut Res Int 26:8459–8467

Okazaki H, Takeda S, Kakizoe K, Taniguchi A, Tokuyasu M, Himeno T, Ishii H, Kohro-Ikeda E, Haraguchi K, Watanabe K, Aramaki H (2017) Bisphenol AF as an inducer of estrogen receptor β (ERβ): evidence for anti-estrogenic effects at higher concentrations in human breast cancer cells. Biol Pharm Bull 40:1909–1916

Olsén L, Lind L, Lind PM (2012) Associations between circulating levels of bisphenol A and phthalate metabolites and coronary risk in the elderly. Ecotoxicol Environ Saf 80:179–183

Park JH, Hwang MS, Ko A, Jeong DH, Lee JM, Moon G, Lee KS, Kho YH, Shin MK, Lee HS, Kang HS, Suh JH, Hwang IG (2016) Risk assessment based on urinary bisphenol A levels in the general Korean population. Environ Res 150:606–615

Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. J Clin Invest 111:1805–1812

Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ (2008) Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol 70:165–190

Rancière F, Lyons JG, Loh VH, Botton J, Galloway T, Wang T, Shaw JE, Magliano DJ (2015) Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environ Health 14:46

Rogers JA, Metz L, Yong VW (2013) Review: endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. Mol Immunol 53:421–430

Savastano S, Tarantino G, D'Esposito V, Passaretti F, Cabaro S, Liotti A, Liguoro D, Perruolo G, Ariemma F, Finelli C, Beguinot F, Formisano P, Valentino R (2015) Bisphenol-A plasma levels are related to inflammatory markers, visceral obesity and insulin-resistance: a cross-sectional study on adult male population. J Transl Med 13:169

Schönfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I (2002) Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect 110:A703–A707

Shankar A, Teppala S (2012) Urinary bisphenol A and hypertension in a multiethnic sample of US adults. J Environ Public Health 2012: 481641

Shekhar S, Sood S, Showkat S, Lite C, Chandrasekhar A, Vairamani M, Barathi S, Santosh W (2017) Detection of phenolic endocrine disrupting chemicals (EDCs) from maternal blood plasma and amniotic fluid in Indian population. Gen Comp Endocrinol 241:100–107

Sun W, Wu Y, Gao M, Tian Y, Qi P, Shen Y, Huang L, Shi L, Wang Y, Liu X (2019) C-reactive protein promotes inflammation through TLR4/NF-κB/TGF-β pathway in HL-1 cells. Biosci Rep 39

Tehrani DM, Gardin JM, Yanez D, Hirsch CH, Lloyd-Jones DM, Stein PK, Wong ND (2013) Impact of inflammatory biomarkers on relation of high density lipoprotein-cholesterol with incident coronary heart disease: cardiovascular Health Study. Atherosclerosis 231: 246–251

Toole JF, Lefkowitz DS, Chambless LE, Wijnberg L, Paton CC, Heiss G (1996) Self-reported transient ischemic attack and stroke symptoms: methods and baseline prevalence. The ARIC Study, 1987-1989. Am J Epidemiol 144:849–856

Wan HT, Leung PY, Zhao YG, Wei X, Wong MH, Wong CK (2013) Blood plasma concentrations of endocrine disrupting chemicals in Hong Kong populations. J Hazard Mater 261:763–769

Wang T, Li M, Chen B, Xu M, Xu Y, Huang Y, Lu J, Chen Y, Wang W, Li X, Liu Y, Bi Y, Lai S, Ning G (2012) Urinary bisphenol A (BPA) concentration associates with obesity and insulin resistance. J Clin Endocrinol Metab 97:E223–E227

Wang L, Zhang Y, Zhang SY (2019) Immunotherapy for the rheumatoid arthritis-associated coronary artery disease: promise and future. Chin Med J 132:2972–2983

Wehbe Z, Nasser SA, El-Yazbi A, Nasreddine S, Eid AH (2020) Estrogen and Bisphenol A in hypertension. Curr Hypertens Rep 22:23

Wijnstok NJ, Twisk JW, Young IS, Woodside JV, McFarlane C, McEneny J, Hoekstra T, Murray L, Boreham CA (2010) Inflammation markers are associated with cardiovascular diseases risk in adolescents: the Young Hearts project 2000. J Adolesc Health 47:346–351

Windgassen EB, Funtowicz L, Lunsford TN, Harris LA, Mulvagh SL (2011) C-reactive protein and high-sensitivity C-reactive protein: an update for clinicians. Postgrad Med 123(1):114–119

Wiraagni IA, Mohd MA, Rashid RA, Haron D (2020) Trace level detection of Bisphenol A analogues and parabens by LC-MS/MS in human plasma from Malaysians. Biomed Res Int 2020:2581287

Włodarczyk M, Sobolewska-Włodarczyk A, Cygankiewicz AI, Jacenik D, Piechota-Polańczyk A, Stec-Michalska K, Krajewska WM, Fichna J, Wiśniewska-Jarosińska M (2017) G Protein-Coupled Receptor 30 (GPR30) expression pattern in inflammatory bowel disease patients suggests its key role in the inflammatory process. A preliminary study. J Gastrointestin Liver Dis 26:29–35

Wu W, Li M, Liu A, Wu C, Li D, Deng Q, Zhang B, Du J, Gao X, Hong Y (2020) Bisphenol A and the risk of obesity: a systematic review with meta-analysis of the epidemiological evidence. Dose-Response 18:1559325820916949

Yakimchuk K, Jondal M, Okret S (2013) Estrogen receptor α and β in the normal immune system and in lymphoid malignancies. Mol Cell Endocrinol 375:121–129

Yang YJ, Hong YC, Oh SY, Park MS, Kim H, Leem JH, Ha EH (2009) Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women. Environ Res 109:797–801

Yang M, Qiu W, Chen B, Chen J, Liu S, Wu M, Wang KJ (2015) The in vitro immune modulatory effect of bisphenol A on fish macrophages via estrogen receptor α and nuclear factor-κB signaling. Environ Sci Technol 49:1888–1895

Zhao J, Xu SZ, Liu J (2019) Fibrinopeptide A induces C-reactive protein expression through the ROS-ERK1/2/p38-NF-κB signal pathway in the human umbilical vascular endothelial cells. J Cell Physiol 234: 13481–13492

Zhu J, Jiang L, Liu Y, Qian W, Liu J, Zhou J, Gao R, Xiao H, Wang J (2015) MAPK and NF-κB pathways are involved in bisphenol A-induced TNF-α and IL-6 production in BV2 microglial cells. Inflammation 38:637–648