

Effect of Prenatal Exposure to Bisphenols on Newborn Leucocyte Telomere Length: A Prospective Birth Cohort Study in China

Jun Liang

Guangxi Medical University

Yantao Shao

The First Affiliated Hospital of Guangxi Medical University

Dongping Huang

Guangxi Medical University

Chunxiu Yang

Guangxi Medical University

Tao Liu

Huaihua Centers for Disease Control and Prevention

Xiaoyun Zeng

Guangxi Medical University

Chunling Li

Guangxi Medical University

Zhenghua Tang

Guangxi Medical University

Jennifer Tan Hui Juan

Ngee Ann Polytechnic

Yanye Song

Guangxi Medical University

Shun Liu

Guangxi Medical University

Xiaoqiang Qiu (✉ xqqiu9999@163.com)

Guangxi Medical University <https://orcid.org/0000-0001-9881-3664>

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Abstract

Telomere length (TL) at birth is related to future diseases and long-term health. Bisphenols exhibit toxic effects and can cross the placenta barrier. However, the effect of prenatal exposure to bisphenols on newborn TL remains unknown. We aimed to explore the effects of prenatal exposure to bisphenols (i.e., bisphenol A (BPA), bisphenol B (BPB), bisphenol F (BPF), bisphenol S (BPS), and tetrabromobisphenol A (TBBPA)) on relative TL in newborns. A total of 801 mother–infant pairs were extracted from the Guangxi Zhuang Birth Cohort (GZBC). The relationships between bisphenol levels in maternal serum and relative TL in cord blood were examined by generalized linear models and restricted cubic spline (RCS) models. After adjusting for confounders, we observed a 3.19% (95% *CI*: -6.08%, -0.21%) reduction in relative cord blood TL among mothers ≥ 28 years with each 1-fold increase of BPS. However, each 1-fold increase of TBBPA, a 3.31% (95% *CI*: 0.67%, 6.01%) increased in relative cord blood TL among mothers < 28 years. The adjusted RCS models also revealed similar results (P overall < 0.05 , P non-linear > 0.05). This is the first study to show a positive association between serum TBBPA levels and newborn relative TL among younger mothers. However, BPS levels were inversely correlated with TL in fetus born to older mothers. The results suggest fetuses of older pregnant women are more sensitivity to BPS exposure and accelerated aging or BPS-related diseases in later life may stem from early-life exposure.

Background

Bisphenols are a group of substances share a common structure of two hydroxy-phenyl functionalities, such as bisphenol A (BPA), bisphenol B (BPB), bisphenol F (BPF), bisphenol S (BPS) and tetrabromobisphenol A (TBBPA). Among them, BPA is the largest production-volume bisphenols worldwide, with a global consumption exceeding 8 million tons in 2016 (Noszczyńska & Piotrowska-Seget 2018). It is a synthetic monomer used in polycarbonate plastics and epoxide resins such as plastic food containers, bottles, and electronics. BPA is the most common endocrine disruptor chemical, which has potential harm to fecundity and fetal growth (Wu et al. 2018). Due to concern about its potential health damage, government organizations in some countries have banned the use of BPA in certain products to limit its applications such as baby bottles (Wang et al. 2015). As a result, BPA analogues with similar structure to BPA have already been used as BPA substitutes in consumer products. The most widespread and commercially used substitutes include bisphenol B (BPB), bisphenol F (BPF), bisphenol S (BPS), and tetrabromobisphenol A (TBBPA) (Wang et al. 2015). Widespread use of these bisphenols has led to substantial exposure in the population, and the health effects caused by their exposure become one of the major public concerns.

Bisphenols have been detected in a wide range of environmental samples, including indoor dust, water, food, paper products, and personal care products (Gallo et al. 2017, Liao & Kannan 2014, Wang et al. 2015, Yamazaki et al. 2015). In addition, they were also widely detected in human samples such as blood, placenta, urine, breast milk, and adipose tissue (Cariou et al. 2008, Lee et al. 2018, Yang et al. 2014). Ideally, the toxicity and health risks of a substitute for a worrisome chemical would be much smaller than these of the original chemical. Unfortunately, recent studies found that many analogues exhibit toxic

effects similar to or even stronger than BPA (Siracusa et al. 2018). In particular, fetuses are more sensitive to the toxic effects of these compounds. Maternal exposure to BPA and its analogues has an adverse impact on the growth and development of fetus, such as low birth weight and premature birth (Aker et al. 2019, Aung et al. 2019, Hu et al. 2018, Huang et al. 2019). According to the theory of “developmental origins of adult diseases”, disease susceptibility and molecular longevity later in life originate through in utero and early life exposures (Leon 1998). Increasing evidences demonstrate that bisphenols may cause cell necrotic, apoptotic, and genotoxic changes by increasing reactive oxygen species (ROS) and/or damaging antioxidant defenses (Arita et al. 2018, Ullah et al. 2019). Prenatal exposure to bisphenols has suggested affecting the development, physiology, and metabolism of the fetus (Rochester & Bolden 2015, Siracusa et al. 2018), potentially becoming the origin of diseases in later life. However, fewer studies have focused on the long-term health effects of the bisphenols.

Telomeres are nucleoprotein structures at the end of chromosomes (Zhang et al. 2019). They consist of a repeating DNA sequence (TTAGGG)_n and a specific binding protein. Telomere can avoid nucleic acid degradation, fusion, and repair, thereby maintaining the integrity of the genome (Collins 1996). The shortening of telomere occurs during each cell division in somatic tissues, from birth until the end of life. Telomere can induce cell senescence or apoptosis once its length reaches a minimum critical size (Youngren et al. 1998). Telomere maintenance has been proved to be vital to human health. A large body of evidences suggest that telomere length (TL) shortening is associated with many age-associated pathophysiological outcomes such as biological ageing, cardiovascular disease, type 2 diabetes, and cancer (De Vitis et al. 2018, Manolio et al. 2009, Samani & van der Harst 2008). The overall rate of reduction in TL in adulthood is relatively small and newborn TL largely determines interindividual TL variation among adults (Manolio et al. 2009). Therefore, the initial TL may be a major determinant of health status in later life, which can be used as a potential marker of the long-term health effects of bisphenols.

However, bisphenols exhibit toxic effects and can cross the placenta barrier (Usman & Ahmad 2016), potentially affecting fetal TL. In the present study, we hypothesized that prenatal exposure to bisphenols may be associated with TL at birth. Here, we aimed to explore the relationship between them by using the mother-child pairs from an ongoing prospective birth cohort study in Guangxi, China.

Methods

Study population and sample collection

The participants were selected from the Guangxi Zhuang Birth Cohort (GZBC) between June 2015 and May 2017. Details on the ongoing cohort have been described in our previous study (Liang et al. 2020). In the present study, the mothers met the following enrollment criteria: (1) singleton pregnancy and gestational age < 13 weeks at enrollment; (2) intend to deliver at the study hospitals; (3) agreed to provide blood samples and birth tissues at delivery. A total of 801 mothers with singleton pregnancy who met the

enrollment criteria were included in the final analysis. Ethics approval was approved by Guangxi Medical University (No.20140305-001). All participants were required to sign informed consent.

The demographic characteristics and lifestyles of pregnant women (e.g., age, pre-pregnancy weight, drinking, smoking, etc.) were collected via a face-to-face questionnaire. The maternal information (e.g., parity, pre-existing conditions, and pregnancy complications) and birth characteristics (e.g., gender, gestational age, and birth outcomes) were extracted from their medical records. Maternal pre-pregnancy body mass index (BMI, kg/m²) was calculated by their self-reported pre-pregnancy weight and height determined at their first examination. The last menstrual cycle of the mother was used to calculate gestational age (weeks). Ultrasound was used to estimate their gestational age if the menstrual date was uncertain.

Maternal blood samples were collected in a non-anticoagulated vacutainer during the first trimester. Cord blood was taken immediately from the umbilical vein into one ethylenediaminetetraacetic acid (EDTA) vacutainer after deliver. The blood samples were centrifuged at 3500 rpm for 10 min, and then separated into the plasma, serum, and hemocytes. After which, the samples were frozen at -80°C until assayed.

Maternal serum bisphenols measurement

Bisphenol levels in maternal serum were measured via ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS; Waters Xevo TQD) as previously described (Liang et al. 2020). Sample extraction and purification was used by liquid-liquid partition method. Briefly, 500 µl of serum and 2 µl of 1 µg/ml isotope internal standard containing BPA-d₁₆ (98.0%, Sigma, St. Louis), BPS-¹²C₁₃ (98.0%, Cambridge Osotope Lab, Inc.), and TBBPA-¹²C₁₃ (99.0%, Cambridge Osotope Lab, Inc.) were buffered with 500 µl of 1.0 M sodium dihydrogen phosphate dihydrate buffer (pH=5.4) and hydrolysed enzymatically with 50 µl of β-glucuronidase/sulfatase (2,00 units/ml) at 37°C overnight. After incubation, 2 ml of n-hexane/acetone (7:3, v/v) was added, and then the mixtures were vortexed for 2 min, sonicated for 15 min, and centrifuged at 4,000 rpm for 10 min. After centrifugation, the upper clear liquid was collected into a new and clean glass tube. Subsequently, 2 ml of methyl-tert-butyl ether was added to the underlying mixtures instead of n-hexane/acetone (7:3, v/v). The above extraction steps (n-hexane/acetone) were repeated, and the supernatants were collected. The extracts were evaporated to dryness at 40°C. The residue was dissolved with 100 µl of methanol: 0.1% ammonia solution (50:50, v/v), filtered using a microporous membrane filter, and 10 µl of these resultants were injected for instrumental analysis.

The mass spectrometer was achieved with the negative-ion electrospray ionization mass spectrometer and multiple reaction monitoring (MRM) mode. The capillary voltage and desolvation temperature were 2.9 kV and 550°C, respectively. The desolvation gas and cone gas were set at flow rates of 50 L/h and 1000 L/h, respectively. The chromatographic separation was operated with an Acquity UPLC BEH C18 (1.7µm 2.1x100 mm, Waters, USA) analytical column. The column temperature was set at 45 °C. The mobile phase was 0.1% ammonia solution (A) and acetonitrile (B), delivered at a flow velocity of 0.3

ml/min; and linear gradient program, 30% B (0–1 min), 30%–100% B (1–4 min), 100% B (4–8 min), 100%–30% (8–8.5 min), and 100% B (8.5–10 min). BPA and its analogues levels were measured by comparing the chromatographic peak area of analytes with the area of the corresponding internal standards. BPA-d₁₆ was used as internal standard for BPA, BPB and BPF, and BPS-¹²C₁₃ and TBBPA-¹²C₁₃ for BPS and TBBPA, respectively. The standards substance (BPA (≥ 98.0%); BPB (≥ 98.0%); BPF (≥ 98.0%); BPS (≥ 98.0%), and TBBPA (≥ 97%)) were obtained from Sigma, St. Louis. The calibration curves with ten points displayed satisfactory linearity ($R^2 > 0.990$) over the range of bisphenol concentrations from 0.0 to 50.0 ng/ml. The limit of detection (LOD) was evaluated by the signal-to-noise ratio (S/N = 3). The LODs of BPA, BPB, BPF, BPS, and TBBPA were 0.193, 0.232, 0.507, 0.046, and 0.454 ng/ml, respectively. The average recoveries of the bisphenols ranged from 86.8% to 101.8%, and the relative standard deviations (RSD) were between 8.0% to 12.9%. Bisphenol was undetectable in the procedure blank and solvent blank. Glassware was used to avoid bisphenol contamination by plastic goods. The glassware was washed by methanol and ultra-pure water and then was baked.

Newborn relative leukocyte TL examination

The genomic DNA extraction of cord blood leukocytes was performed using a whole-genome DNA extraction kit and following the manufacturer's instructions (Aidlab Biotechnologies Co, Ltd, China). The concentration and purity of DNA were detected by the ultraviolet spectrophotometer (BioTek, USA). DNA purity was considered eligible if the OD₂₆₀/OD₂₈₀ rate was between 1.8 and 2.0.

Relative TL in cord blood was measured by StepOne Plus real-time polymerase chain reaction (RT-PCR; Applied Biosystems, USA). The relative TL was calculated as T/S ratio (telomere/single copy). The single-copy gene, 36B4, served as a reference gene in the telomere quantitative PCR signal. The primer sequence information of telomere and 36B4 gene is as follows: TeloF: 5'-CGGTTTGTGGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3'; TeloR: 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'; 36B4F: 5'-CAGCAAGTGGGAAGGTGTAATCC-3'; 36B4R: 5'-CCCATTCTATCATCAACGGGTACAA-3' (Cawthon 2002). Primers were purchased from Invitrogen, USA. The thermal cycling profile for the telomere PCR was: 50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 62°C for 1 min. For the 36B4 PCR, the thermal cycling profile was: 50°C for 2 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, 62°C for 20 s, and 72°C for 1 min. The reference standard curve was created by using seven serial dilutions of human DNA (200, 50, 25, 125.5, 6.25, 3.125 ng/μl) and validated in each reaction batch. The 35 ng/ml standard concentration was selected as the positive control, and the DNA of the remaining samples were diluted with Tris-EDTA buffer to 25-50 ng/μl. All samples were run in triplicates. Each 96-well plate contained negative and positive controls to create a standard curve. Good linearity was observed across this range ($R^2 > 0.980$). The Ct values generated were used to calculate the T/S ratio using the equation: Relative T/S = $2^{-(\Delta Ct_{\text{Sample}} - \Delta Ct_{\text{control sample}})}$, where $\Delta Ct_{\text{Sample}} = \text{Sample Ct}_{(\text{TL})} - \text{Sample Ct}_{(36B4)}$ and $\Delta Ct_{\text{control}} = \text{Control Ct}_{(\text{TL})} - \text{Control Ct}_{(36B4)}$ (Cawthon 2002).

Statistical analysis

Bisphenol levels below the LOD were replaced by LOD/2. The Kolmogorov-Smirnov normality test was used to examine the distribution of data. We found that bisphenol concentrations and relative TL showed a skewed distribution. Thus, the relative TL and bisphenol concentrations were converted to the natural log (Ln) –transformation to enhance normality. Demographic characteristics were expressed as mean and standard deviation (Mean \pm SD) values for normal distribution data, median (quartile range, IQR) values for skew distributional data, and number (percentages) for categorized data. The correlations of bisphenols were determined by using Spearman's correlation test.

Generalized linear models were used to estimate the regression coefficient (β) and standard error (SE) of the associations between bisphenol levels (Ln-transformed) and relative TL (Ln-transformed). The percentage change in relative TL for per doubling of bisphenol levels was calculated as $(e^{(\ln 2 \times \beta)} - 1) \times 100\%$, and the 95% confidence interval (95% CI) was calculated as $(e^{[\ln 2 \times (\beta \pm 1.96 \times SE)]} - 1) \times 100\%$ (Zhang et al. 2019). The covariates in the regression models included infant characteristics (gestational age, and infant gender) and maternal characteristics (maternal age, pre-pregnancy BMI, occupation, drinking before pregnancy, passive smoking during pregnancy, parity, hypertensive disorders in pregnancy (HDP), gestational diabetes mellitus (GDM)). We constructed different regression models, adjusted for infant characteristics only, maternal characteristics only and both of them. Considering the potential effect of maternal age and infant gender on the association between bisphenols and relative TL in offspring (Liang et al. 2020, Zhang et al. 2019), we conducted sub-group analysis stratified by maternal age (< 28 or ≥ 28 years, the median value) and infant gender. To ensure the robustness of the results, we also conducted sensitivity analyses by excluding women with GDM ($n = 37$) or HDP ($n = 37$).

To further investigate the potential non-linear relationship between bisphenol concentrations (Ln-transformed) and relative TL (Ln-transformed), restricted cubic spline (RCS) function was used with three knots located at the 10th, 50th, and 90th percentiles of the Ln-transformed bisphenol levels. The median Ln-transformed bisphenol levels were selected as reference. Wald chi-square test was used to test for the overall and non-linear associations (Desquilbet & Mariotti 2010). RCS analysis was also adjusted for the above potential confounders. Data analysis was performed using the R 4.0. All P values were two-tailed, and $P < 0.05$ was defined as statistical significance.

Results

The demographic distribution data of mother-infant pairs ($n = 801$) are presented in Table 1. The mean maternal age was 28.3 ± 5.6 years and ranged between 18–48 years old. The average maternal pre-pregnancy BMI was 20.6 ± 3.0 kg/m², with 209 (26.1%) of them being underweight (BMI < 18.5) and 87 (10.9%) of them being overweight (BMI ≥ 24.0) before pregnancy. Most of women (83.8%) were employed and nearly half of them (48.6%) were nulliparous. Few women consumed alcohol (6.2%) before pregnancy. Almost two thirds of them (65.0%) were the victims of passive smoking. Among infants, the

average gestational age was 38.6 ± 1.5 weeks, and 439 (54.8%) of them were male. The median relative cord blood TL was 1.11 (0.85, 1.27).

The distributions of serum bisphenols are summarized in Table 2. The detection rate of BPA, BPB, and BPS were all nearly over 80%. Among these compounds, BPA had the highest geometric mean levels (1.447 ng/ml), followed by TBBPA (0.589 ng/ml), BPF (0.459 ng/ml) BPB (0.239 ng/ml), and BPS (0.102 ng/ml). The correlation analysis of serum bisphenol levels are showed in Fig. 1. The Spearman correlation coefficients among multiple bisphenols in serum ranged from 0 to 0.34. However, only the correlation between BPA and TBBPA was significant ($r_s = 0.34$).

The impacts of bisphenol levels in maternal serum on relative cord blood TL in the whole participants are listed in Table 3. We found prenatal serum levels of BPA, BPF and TBBPA were positively while BPB and BPS were negatively associated with relative cord blood TL in the whole participants. However, no significant association was found either in the unadjusted or adjusted models. **Table 4** shows the results of subgroup analysis. We found a doubling maternal serum BPS was significantly associated with 3.19% (95% *CI*: -6.08%, -0.21%) shorter relative TL of cord blood when adjusted by infant characteristics and maternal characteristics, among those mothers who were older than or equal to 28 years. However, when we adjusted for both maternal characteristics and infant characteristics, prenatal serum TBBPA levels were positively associated with relative TL among those younger mothers (< 28 years); a doubling of serum TBBPA was associated with 3.31% (95% *CI*: 0.67%, 6.01%) increased in the relative TL of cord blood. When stratifying the participant by infant sex, we found no significant association. In addition, the above results were essentially unchanged when we performed sensitivity analyses by excluding 37 subjects with HDP (**Table S1** and **Table S3**) or 37 subjects with GDM (**Table S2** and **Table S4**).

To identify the non-linear relationship between the prenatal serum bisphenols and relative cord blood TL, RCS analysis were performed by adjusted for the infant and maternal characteristics. Results revealed a negative linear relationship between Ln-transformed BPS levels and Ln-transformed relative TL (P overall = 0.041, P non-linear = 0.169) among mothers ≥ 28 years. However, a positive linear association was observed between Ln-transformed TBBPA levels and Ln-transformed relative TL (P overall = 0.037, P non-linear = 0.395) among mothers < 28 years (**Figure S1**) There was no evidence of a non-linear association between any other bisphenols and relative TL.

Discussion

In the present study, we investigated the impacts of prenatal exposure to bisphenols and relative cord blood TL by using a prospective cohort from Guangxi in China. We found that prenatal serum TBBPA levels were positively correlated with relative TL in fetus born to younger mothers. However, prenatal serum BPS levels were inversely associated with relative TL among fetus born to older mothers, which suggested BPS may have long-term health effects on the fetus.

In our study, the serum BPA levels of the participants was higher than studies from China (Median = 0.81 ng/ml) (Zhang et al. 2013) and Canada (Median = 0.548 ng/ml) (Kosarac et al. 2012), and lower than studies from Germany (Median = 3.1 ng/ml) (Schonfelder et al. 2002), Japan (Median = 2.24 mg/ml) (Minatoya et al. 2018), India (GM = 4.62 ng/ml) (Shekhar et al. 2017), and Korea (GM = 3.10 ng/ml) (Lee et al. 2008). In contrast to BPA, the data on its substitutes in human serum, plasma, and whole blood are very limited, especially for pregnant women. In a recent study including 181 serum samples from pregnant Chinese women, the detection frequency of BPS (72.4%), TBBPA (29.8%), BPB (41.4%), and BPF (20.4%), were all lower than our results, and the levels of TBBPA (Median < 0.009 ng/ml) and BPF (Median < 0.228 ng/ml) were lower than our results, but the levels of BPS (Median = 0.113 ng/ml) and BPB ($P_{75} = 0.849$ ng/ml) levels were higher than our results (Wazir & Mokbel 2019). Total BPS was detected in only 4 of 61 maternal serum samples in a Chinese study (< 0.03–0.07 ng/ml) (Liu et al. 2017), while the detection rate of BPS (90.9%) was much higher in the present study. The average concentrations of TBBPA in the serum of pregnant women in Korea (n = 12) (Kim & Oh 2014b), France (n = 91) (Cariou et al. 2008), and Japan (n = 10) (Fujii et al. 2014) were 10.7 ng/g lipid, 19.87 ng/g lipid, and 1.0 pg/g wet weight, respectively. Our results provided the first hand data about BPA analogues exposure in pregnant women in China.

To our knowledge, there are few studies on the relationship between bisphenols and TL. One suggested an inverse relationship between urinary BPA concentrations and relative TL in peripheral blood of women (Awada et al. 2019), and the other reported the same relationship between serum BPA levels and relative TL in patients with type 2 diabetes (Soundararajan et al. 2019). Consistent with our results, (Michels et al. 2020) also found no association between prenatal BPA levels and relative TL in cord blood. The likely reason is that all the participants of those studies are adults, which have fewer buffers for harmful substances. As known to all, the placenta barrier can prevent the effects of harmful substances on the fetus to some extent. We found that TBBPA levels were positively correlated with TL in fetus born to younger mothers, while BPS could shorten relative cord blood TL among those mothers aged 28 years or older. So far, we have not found any studies on the effect of TBBPA and BPS on TL in offspring. However, BPS can lead to DNA damage and chromosomal abnormalities. For example, Ullah et al. found that BPS can cause generation of damage DNA in spermatozoa in both in vivo and in vitro rat models (Ullah et al. 2019). Another vitro data exhibited that BPS induced DNA damage in human leucocytes and bronchial epithelial cells (George & Rupasinghe 2018). Similarly, the study conducted by (Campen et al. 2018) showed that BPS induced spindle abnormalities and chromosome misalignment in bovine, and at concentrations that are orders of magnitude below those measured in humans. These results provide more substantial evidences for our findings, however, further needed larger sample studies to confirm.

The first trimester, a sensitive period for fetal growth, is highly susceptible to environmental exposure. Bisphenols can cross the placenta reaching the fetal compartment even at low environment-related concentrations. TBBPA, one of the most common persistent organic pollutants (POPs), has a relatively long half-life period (3.5–6.6 days in blood and 21–23.6 days in adipose tissue) (Geyer et al. 2004). In a previous study, serum TBBPA levels were 2–5 times higher in the infants than in the mothers (Kim & Oh

2014a). Similarly, a recent study from pregnancy sheep models found that BPS has the longest fetal half-life and higher persistence in the fetal compartment than other bisphenols (Gingrich et al. 2019). Therefore, the accumulation of BPS and TBBPA in the fetal compartment may lead to the long-term health effects on the fetus following gestational chronic exposure. Moreover, telomere shortens with age even in the germ cells, which can result in a carry-over effect of parental age to the next generation (Asghar et al. 2015). In addition, (Stefa et al. 2019) argued that genetic factors play an important role in TL, and that the TL of their mothers during pregnancy can affect TL of newborns. The study by (Iwama et al. 1998) showed that terminal restriction fragments in peripheral blood mononuclear cells decreased by about 84 bp per year in normal individuals (aged 4–39 years) and 41 bp per year in individuals aged ≥ 40 years. Evidence from human revealed that offspring TL was negatively correlated with their maternal age (Akkad et al. 2006). These evidences may partly explain why we found TBBPA levels were positively correlated with relative TL in fetus born to younger mothers and BPS levels was inversely associated with TL among fetus born to older mothers.

Some potential biological mechanisms may explain the relationship between prenatal BPS and TBBPA exposure and relative TL in newborns. Telomerase activity, known to be stimulated by estrogen, plays a role in maintaining telomere stability by antagonizing the effect of increased allostatic load on telomere attrition (Alnafakh et al. 2019, Sarkar et al. 2006). However, exogenous progesterone administration can inhibit telomerase and shorten TL (Hapangama et al. 2017, Heaphy et al. 2011, Valentijn et al. 2015). Bisphenols have endocrine disrupting effects and can change hormone levels in the body. TBBPA was an agonist for estrogen and an antagonist for progesterone (Li et al. 2010). Conversely, BPS has weaker estrogenic activity than other bisphenols, even no effect. However, it exhibits the strongest progesterone-producing effect compared to other bisphenols (Rosenmai et al. 2014). These findings are consistent with our results that TBBPA increased the relative cord blood TL and BPS shortened the relative cord blood TL. In addition, unlike BPA, BPB, BPF and TBBPA, BPS contains a sulfonic acid residue, shows a strong electron withdrawing effect on the nitrogen-containing organic compounds, and has a strong destruction effect on the structure of proteins (Chataigner et al. 2007). Moreover, telomere is also maintained by various proteins (Houben et al. 2008). For example, poly-ADP ribose polymerase (PARP) can catalyze the action of telomeric repeat binding factors to repair DNA damage and protect chromosome ends (Gomez et al. 2006). A recent study demonstrated that BPS can damage PARP in human peripheral blood mononuclear cells (Michałowicz et al. 2015). These evidences suggest that the relationship between BPS and TL in newborns may involve multiple regulatory biological mechanisms and further studies are needed. Since telomere loss is a crucial biomarker of aging, the results of this study provide additional evidence of the adverse effects of BPS exposure on aging. However, most previous studies have focused on BPA, but our result suggests that BPS may be more important with regard to health effects on the fetus.

The present study has important strengths and limitations. An advantage is that we are the first to show that the possible effects of maternal exposure to BPA analogues during pregnancy on newborn relative blood TL in the Chinese population. Another advantage is that our study is a prospective cohort design that allows us to assess the relationship between prenatal exposure and offspring outcomes; thus the

extrapolation of the results may be more reliable. Moreover, most of the pregnant women we included are rural populations, and they did not migrate much during pregnancy, so the sources and pathways of exposure are relatively stable. Nonetheless, there were still some potential limitations that should be cautious. First, spot serum samples were used as a matrix for bisphenol exposure. Because BPA has a shorter half-life, ingested BPA may be rapidly converted to inactive metabolites. Bisphenol levels in serum are generally lower than in urine, and are considered to be at a high risk of contamination when compared to those in urine (Calafat et al. 2015). However, Evidences from a review conducted by (Vom Saal & Welshons 2014) have confirmed that bisphenols can be accurately estimated without contamination in human blood samples. Furthermore, given the relative stability of bisphenol exposure during pregnancy, spot samples can accurate measures of longer-term exposure (Rochester 2013). Thus, spot serum bisphenols concentrations might legitimately reflect bisphenols exposure during pregnancy. Second, we did not measure cord blood bisphenol concentrations, which might directly reflect the fetal exposure to bisphenols. However, previous studies have demonstrated a positive correlation between maternal blood bisphenol levels and cord blood bisphenol levels (Dalkan et al. 2019). Therefore, maternal serum bisphenol levels can to some extent reflect the levels of fetal exposure during pregnancy. Finally, the measuring method of TL we used only provided a mean relative TL of all the chromosomes in multiple cells and was unable to discriminate the TL of certain cells or chromosomes. More accurate measuring methods should be applied.

Conclusion

This is the first study to show that TBBPA levels were positively correlated with relative TL in fetus born to younger mothers and BPS levels were inversely associated with relative TL among fetus born to older mothers. The results suggest fetuses of older pregnant women are more sensitivity to BPS exposure and accelerated aging or BPS-related diseases in later life may stem from early-life exposure.

Abbreviations

BPA

Bisphenol A; BPB: Bisphenol B; BPF: Bisphenol F; BPS: Bisphenol S; *CI*: Confidence interval; EDTA: Ethylenediaminetetraacetic acid; GDM: Gestational diabetes mellitus; GM: Geometric mean; GSD: Geometric standard deviation; GZBC: Guangxi Zhuang Birth Cohort; HDP: Hypertensive disorders in pregnancy; IQR: Interquartile range; LOD: Limit of detection; PAR: Poly-ADP ribose polymerase; RCS: Restricted cubic spline; RSD: relative standard deviations; RT-PCR: Real-time polymerase chain reaction; SD: Standard deviation; TBBPA: Tetrabromobisphenol A; TL: Telomere length; UPLC-MS: Ultra-high performance liquid chromatography-tandem mass spectrometry.

Declarations

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Author Contributions:

Jun Liang: Conceptualization/design, Methodology, Formal analysis, Investigation, Writing-Original Draft, Visualization, Writing-Review; Yantao Shao: Methodology, Data Curation, Investigation; Formal analysis, Writing - Review & Editing; Dongping Huang: Conceptualization/design, Funding acquisition, Resources, Funding acquisition, Writing-Review & Editing, Supervision; Chunxiu Yang: Formal analysis, Investigation, Writing - Original Draft, Visualization; Xiaoyun Zeng: Investigation, Supervision, Data Curation, Writing-Review & Editing; Chunling Li: Investigation, Data Curation, Writing-Review & Editing; Zhenghua Tang: Investigation, Data Curation, Writing-Review & Editing, Shun Liu: Conceptualization/design, Methodology, Data Curation, Formal analysis, Writing - Review & Editing; Xiaoqiang Qiu: Project administration, Resources, Data Curation, Writing-Review & Editing, Supervision, Funding acquisition. All authors have given approval to the final version of the manuscript. †These authors contributed equally.

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Ethics approval and consent to participate

Ethics approval was approved by Guangxi Medical University (No.20140305-001). All participants were required to sign informed consent.

Competing interests

The authors declare no conflicting interests.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Consent for publication

Not applicable.

Consent to publish

All of co-authors agreed that the article will be published in Environmental Science and Pollution Research.

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Tables

| Table 1. Characteristics of the participants in this study (n=801). | |
|--|---|
| Characteristics | Number (%) / Mean \pm SD / median (IQR) |
| Mothers | |
| Age (years) | 28.3 \pm 5.6 |
| Pre-pregnancy BMI (kg/m ²) | |
| < 18.5 | 209 (26.1) |
| 18.5–23.9 | 505 (63.0) |
| \geq 24.0 | 87 (10.9) |
| Occupation | |
| Unemployment | 130 (16.2) |
| Employment | 671 (83.8) |
| Parity | |
| Nulliparous | 389 (48.6) |
| Multiparous | 412 (51.4) |
| Drinking | |
| Yes | 50 (6.2) |
| No | 751 (93.8) |
| Passive smoking during pregnancy | |
| Yes | 521 (65.0) |
| No | 280 (35.0) |
| Hypertensive disorders in pregnancy | |
| Yes | 37 (4.6) |
| No | 764 (95.4) |
| Gestational diabetes mellitus | |
| Yes | 37 (4.6) |
| No | 764 (95.4) |
| Infants | |
| Gestational age (weeks) | 38.6 \pm 1.5 |
| Gender | |

| Table 1. Characteristics of the participants in this study (n=801). | |
|--|---|
| Characteristics | Number (%) / Mean \pm SD / median (IQR) |
| Male | 439 (54.8) |
| Female | 362 (45.2) |
| Relative cord blood TL | 1.11 (0.85, 1.27) |
| SD, standard deviation; IQR, interquartile range; BMI, body mass index. | |

| Table 2. Distribution of bisphenol levels (ng/ml) in serum samples | | | | | | |
|---|--------------------|-------|-----------------|-----------------|-----------------|-----------------|
| | Detection rate (%) | GM | P ₂₅ | P ₅₀ | P ₇₅ | P ₉₅ |
| BPA | 99.1 | 1.447 | 0.520 | 1.378 | 3.916 | 11.499 |
| BPB | 90.1 | 0.239 | 0.233 | 0.235 | 0.246 | 0.362 |
| BPF | 65.4 | 0.459 | < LOD | 0.606 | 0.609 | 0.628 |
| BPS | 90.9 | 0.102 | 0.096 | 0.098 | 0.110 | 0.239 |
| TBBPA | 76.4 | 0.589 | 0.463 | 0.482 | 0.675 | 3.507 |
| GM: geometric mean; GSD: geometric standard deviation; LOD: limit of detection; P: percentiles. | | | | | | |

| Table 3. Associations between prenatal exposure to bisphenols and relative cord blood TL (n = 801). | | | | | |
|---|--|--|--|--|--|
| | BPA | BPB | BPF | BPS | TBBPA |
| | Percentage change (%) ^a (95% CI) |
| Unadjusted | 0.58 (-0.78, 1.96) | -1.01 (-5.73, 3.96) | 0.53 (-2.86, 4.03) | -1.21 (-3.26, 0.89) | 0.68 (-1.15, 2.55) |
| Model one ^b | 0.54 (-0.52, 1.61) | -1.09 (-5.82, 3.87) | 0.53 (-2.86, 4.03) | -1.21 (-3.27, 0.88) | 0.69 (-1.14, 2.56) |
| Model two ^c | 0.75 (-0.60, 2.13) | -0.98 (0.95, -2.88) | 0.09 (-3.29, 3.59) | -1.32 (-3.37, 0.77) | 0.92 (-0.92, 2.79) |
| Model three ^d | 0.78 (-0.57, 2.15) | -0.84 (-5.58, 4.14) | 0.11 (-3.27, 3.62) | -1.32 (-3.37, 0.76) | 0.92 (-0.92, 2.79) |
| ^a The percentage change in relative cord blood TL was calculated for each doubling of bisphenol concentrations. | | | | | |
| ^b Adjusted for gestation age and infant gender. | | | | | |
| ^c Adjusted for maternal age, pre-pregnancy BMI, occupation, drinking before pregnancy, passive smoking during pregnancy, parity, hypertensive disorders in pregnancy, and gestational diabetes mellitus. | | | | | |
| ^d Adjusted for gestation age, infant gender, maternal age, pre-pregnancy BMI, occupation, drinking before pregnancy, passive smoking during pregnancy, parity, hypertensive disorders in pregnancy, and gestational diabetes mellitus. | | | | | |

Table 4. Associations between prenatal exposure to bisphenols and relative cord blood TL, stratified by maternal age and infant gender.

| Subgroup | BPA | BPB | BPF | BPS | TBBPA |
|--------------------------|--|--|--|--|--|
| | Percentage change (%) ^a (95% CI) |
| Maternal age (years) | | | | | |
| < 28 (n = 371) | | | | | |
| Model one ^b | -0.18 (-1.97, 1.64) | -4.97 (-10.81, 1.26) | 1.90 (-2.5, 6.51) | 0.82 (-2.05, 3.78) | 2.92 (0.32, 5.60) |
| Model two ^c | 0.09 (-1.75, 1.96) | -4.56 (-10.48, 1.76) | 1.54 (-2.91, 6.18) | 0.76 (-2.14, 3.74) | 3.26 (0.63, 5.97) |
| Model three ^d | 0.04 (-1.80, 1.90) | -4.41 (-10.33, 1.90) | 1.36 (-3.06, 5.99) | 0.64 (-2.25, 3.61) | 3.31 (0.67, 6.01) |
| ≥ 28 (n = 430) | | | | | |
| Model one ^b | 1.29 (-0.68, 3.29) | 3.57 (-3.98, 11.71) | -1.02 (-6.22, 4.46) | -3.00 (-5.90, 0.00) | -1.19 (-3.74, 1.42) |
| Model two ^c | 1.46 (-0.55, 3.52) | 3.30 (-4.26, 11.46) | -1.64 (-6.82, 3.82) | -3.25 (-6.15, -0.26) | -0.79 (-3.38, 1.87) |
| Model three ^d | 1.53 (-0.47, 3.57) | 3.33 (-4.21, 11.46) | -1.65 (-6.79, 3.78) | -3.19 (-6.08, -0.21) | -0.84 (-3.42, 1.81) |
| Infant gender | | | | | |
| Male (n = 439) | | | | | |
| Model one ^e | 0.22 (-1.57, 2.04) | -1.59 (-7.54, 4.75) | 1.49 (-3.64, 6.89) | -1.74 (-4.58, 1.19) | 0.95 (-1.49, 3.46) |
| Model two ^c | 0.74 (-1.07, 2.58) | -1.60 (-7.52, 4.71) | 0.85 (-4.22, 6.19) | -1.60 (-4.43, 1.32) | 1.07 (-1.36, 3.56) |
| Model three ^f | 0.77 (-1.05, 2.61) | -1.46 (-7.41, 4.87) | 0.62 (-4.44, 5.96) | -1.47 (-4.32, 1.45) | 1.01 (-1.42, 3.5) |
| Female (n = 362) | | | | | |

| | | | | | |
|---|--------------------|---------------------|---------------------|---------------------|--------------------|
| Model one ^e | 0.95 (-1.05, 2.98) | -0.28 (-7.85, 7.92) | -0.08 (-4.57, 4.61) | -0.74 (-3.69, 2.3) | 0.01 (-2.38, 3.21) |
| Model two ^c | 1.11 (-0.95, 3.22) | 1.07 (-6.81, 9.61) | 0.08 (-4.50, 4.88) | -0.74 (-3.73, 2.34) | 0.48 (-2.33, 3.37) |
| Model three ^f | 1.07 (-0.98, 3.17) | 1.01 (-6.85, 9.52) | 0.04 (-4.53, 4.82) | -0.74 (-3.72, 2.34) | 0.48 (-2.33, 3.37) |
| ^a The percentage change in relative cord blood TL was calculated for each doubling of bisphenol concentrations. | | | | | |
| ^b Adjusted for gestational age and infant gender. | | | | | |
| ^c Adjusted for maternal age, pre-pregnancy BMI, occupation, drinking before pregnancy, passive smoking during pregnancy, parity, hypertensive disorders in pregnancy, and gestational diabetes mellitus. | | | | | |
| ^d Adjusted for gestational age, infant gender, maternal age, pre-pregnancy BMI, occupation, drinking before pregnancy, passive smoking during pregnancy, parity, hypertensive disorders in pregnancy, and gestational diabetes mellitus. | | | | | |
| ^e Adjusted for gestation age. | | | | | |
| ^f Adjusted for gestation age, maternal age, pre-pregnancy BMI, occupation, drinking before pregnancy, passive smoking during pregnancy, parity, hypertensive disorders in pregnancy, and gestational diabetes mellitus. | | | | | |

Figures

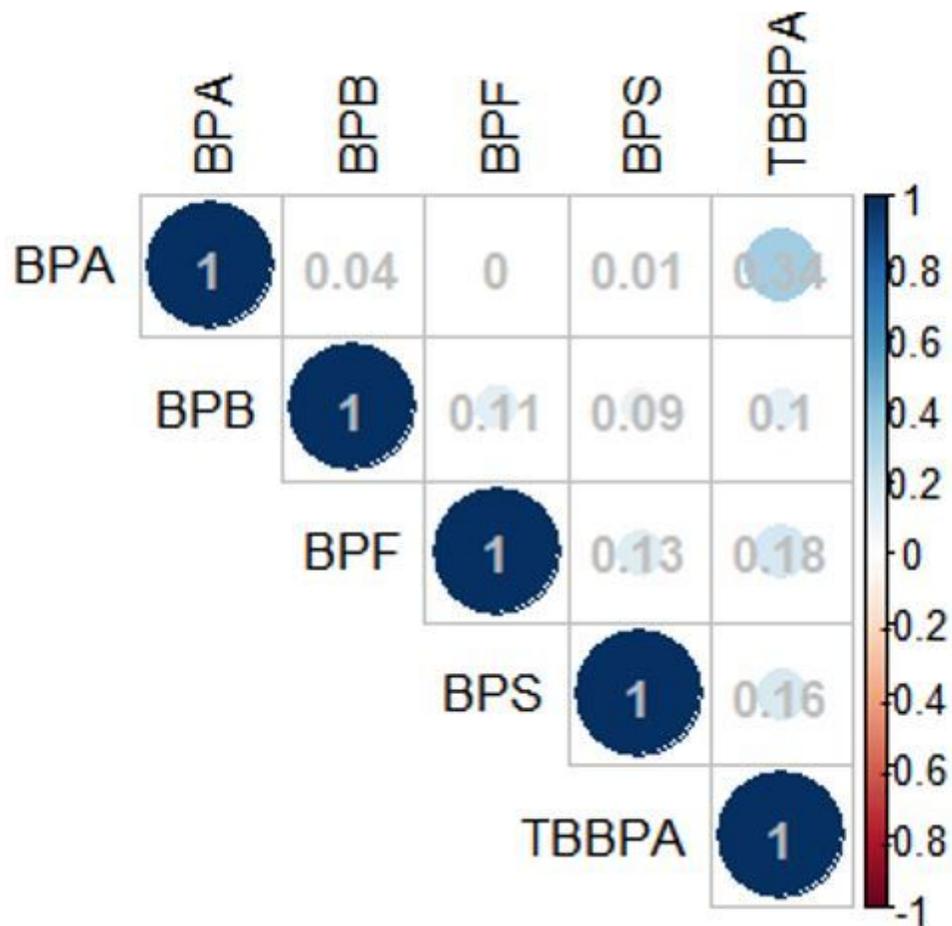


Figure 1

The correlation analysis of serum bisphenol levels in pregnant women. The value indicates the magnitude of the correlation. Blue indicates a positive association, and red indicates a negative correlation.

Supplementary Files

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