Associations between Gut Microbiota and Metabolism of Bisphenol A in Sprague-Dawley Rat

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Research Article

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Abstract

Many studies have investigated the effect of bisphenol A (BPA) exposure on gut microbial community changes, while the influence of gut microbiota on metabolism of BPA in organisms remains not well known. To explore this, in this study, Sprague-Dawley rats were intermittently (a 7-day interval) or continuously dosed by oral gavage at 500 μg BPA/kg bw/day for 28 days. Under a 7-day interval BPA exposure, the metabolism of BPA in rats and gut microbiota structure did not change greatly with dosing time. In contrast, following continuous BPA exposure, the relative abundances of *Firmicutes* and *Proteobacteria* in rat gut were significantly increased, and the alpha diversity of rat gut bacteria was greatly reduced. Meanwhile, the mean proportion of BPA sulfate to total BPA in rat blood was gradually decreased from 30 (day 1) to 7.4% (day 28). After 28 days of continuous exposure, the mean proportion of BPA glucuronide to total BPA in rat urine elevated from 70 to 81%, and in rat feces the mean proportion of BPA gradually decreased from 83 to 65%. Under continuous BPA exposure, the abundances of 27, 25, and 24 gut microbial genera were significantly correlated with the proportion of BPA or its metabolites in rat blood, urine, and feces, respectively. Overall, this study first demonstrates that continuous BPA exposure disrupted the rat gut microbiota community, which in turn altered the metabolism of BPA in rats. This finding contributes to the better understanding of metabolism of BPA in humans.

Introduction

Bisphenol A (BPA) is an industrial chemical with a high global production volume, and is mainly polymerized to produce polycarbonate plastics and epoxy resins (Staples et al. 1998). Due to the incomplete polymerization, residual BPA has been detected in numerous consumer products, such as baby bottles, food containers, and beverage cans (Chen et al. 2016). The wide BPA presence, together with the leaching of BPA into foods, renders human BPA exposure ubiquitous (Chen et al. 2016, Vandenberg et al. 2007). For the general population, exposure to BPA predominantly occurs through dietary ingestion, with minor contributions from dermal absorption and dust inhalation (Vandenberg et al. 2007). BPA has been detected in human blood, urine, and other bodily fluids from around the world (Ginsberg and Rice 2009, Jin et al. 2018, Liao and Kannan 2012). For example, 94% of human urine samples (n = 294) from several Asian countries contained measurable BPA, with the concentrations of < 0.1 – 30 ng/mL (Zhang et al. 2011). However, BPA is biologically active, and shows a weak estrogenic activity *in vitro* and *in vivo* (Bonefeld-Jorgensen et al. 2007). Biochemical assays have demonstrated that BPA has a strong binding affinity with both estrogen receptors α and β, and also has a potential to disrupt normal hormonal functions in humans (Safe 2000, Volkel et al. 2002). Epidemiological data have linked BPA exposure to human diabetes, heart diseases, asthma, and onset of obesity (Nadal et al. 2009, Rochester 2013, vom Saal et al. 2005). Therefore, it has become a global concern on human exposure to BPA.

After ingestion, BPA is predominantly metabolized to BPA-glucuronide (BPA-G) and BPA-sulfate (BPA-S) in humans, and then rapidly excreted via urine and feces with the half-life of < 12 h (Volkel et al. 2002). It has long been considered that BPA-G and BPA-S are biologically inactive, and does not exhibit obvious *in*

vitro estrogenic activities (Dekant and Volkel 2008, Goodman et al. 2009). However, recent studies revealed the endocrine disrupting activities of BPA-G and BPA di-sulfate (BPA-DS) in rat prolactinoma cells, and cytotoxicity of BPA-G in human and mice preadipocytes (Boucher et al. 2015, René Viñas et al. 2013). Moreover, BPA-G and BPA-S can be deconjugated to BPA *in vivo*, which mainly happens in liver and intestine (Gauderat et al. 2016, Nishikawa et al. 2010), acting as a potential source of BPA. Following these toxicology studies, some biomonitoring results have examined the occurrence of BPA, BPA-G, and BPA-S in humans, mainly in serum and urine. Volkel et al. (2002) first developed a quantitation method for analyzing BPA-G and BPA in human urine and blood. Later, Liao and Kannan (2012) reported higher geometric mean BPA-G concentrations than BPA and BPA-DS in human urine and serum. Recently, human biomonitoring on maternal and cord serum reported higher BPA-G and BPA-S concentrations than BPA (Liu et al. 2017). Therefore, considering the demonstrated toxicities of BPA-G and BPA-S, to accurately and fully understand the toxic effects of BPA on humans, it is necessary to understand the mechanisms underlying the metabolism of BPA to its conjugated metabolites in humans.

Gut microbiota is composed of a large and complex composition of microbes in the gastrointestinal tract (Velagapudi et al. 2010). It plays an important role in metabolic processing, energy homeostasis, and nutrient absorption in humans (Chiu et al. 2020). Changes in the gut microbiome composition have been linked to the development of many human diseases (Rea et al. 2018). Gut microbiota composition is highly sensitive to exogenous stressors and environmental contaminants (Chen et al. 2018). Many studies have demonstrated that exposure to estrogenic compounds, including BPA, could remarkably change the composition of gut microbiota in organisms (Chiu et al. 2020). For example, Lai et al. (2016) reported that dietary exposure to BPA greatly altered the community of mouse gut microbiota, and enhanced the growth of *Proteobacteria*, a biomarker of intestinal microbial dysbiosis. Moreover, gut microbiota, besides the well-known liver, is also involved in the metabolism of a large variety of xenobiotics (e.g., drugs and heavy metals) in humans, which could significantly change the toxic effects of xenobiotics (Claus et al. 2016). Using the *in vitro* human intestinal microbial ecosystem, Wang et al. (2018) found that the bioavailability of BPA declined with the process of gastrointestinal digestion. However, the influence of gut microbiota change on metabolism of BPA to BPA-G and BPA-S in humans remains not well known.

In the present study, Sprague-Dawley (SD) rats were intermittently (a 7-day interval) or continuously dosed by oral gavage at 500 μg BPA/kg bw/day for 28 days. SD rat is a reliable model animal that has been widely used to understand the toxicokinetic behaviors of BPA in humans. This BPA dosing amount was chosen to render rat blood BPA levels similar to that in the general population. Then, we characterized the concentrations of BPA and its major conjugated metabolites in rat feces, urine, and blood with dosing time. The change of gut microbiome community structure with dosing time was also investigated using 16S rRNA amplicon sequencing. Finally, the influence of rat gut microbiota change on metabolism of BPA was explored by analyzing correlations between the gut microbiome abundance and proportion of BPA and its metabolites in rat feces, urine, and blood. This study contributes to the better understanding of metabolism of BPA in humans.

Materials And Methods

Standards and Reagents. BPA (2,2-(4,4'-dihydroxydiphenyl)propane; purity > 98%), bisphenol A mono-*β*-d-glucuronide (BPA-G; > 98%), bisphenol A mono-sulfate (BPA-S; > 98%), bisphenol A bis-*β*-d-glucuronide (BPA-BG; > 97%), bisphenol A di-sulfate (BPA-DS; > 97%), $^{13}C_{12}$-BPA, $^{13}C_{12}$-BPA *β*-d-glucuronide ($^{13}C_{12}$-BPA-G; > 98%), and D_6-BPA mono-sulfate (D_6-BPA-S; > 98%) were purchased from Toronto Research Chemicals (North York, ON, Canada). Dimethylsulfoxide (DMSO), methanol, physiological saline (0.9% NaCl solution), pure water, and ammonium acetate were obtained from Sigma-Aldrich (Oakville, ON, Canada).

Animal and Experimental Design. Sprague-Dawley rats (ten weeks old, body weight 230 – 280 g) were obtained from SLRC Animal Laboratory (Shanghai, China). All animals were housed (5 rats/cage) in a temperature (22–25°C), humidity (40–50%), and light (12/12 hr light/dark cycle) controlled house under specific pathogen-free conditions, and given *ad libitum* access to standard food (Medicience Ltd., Jiangsu, China) and water. Before conducting the experiment, all animals were allowed to acclimate for seven days. All SD rats were humanely treated throughout the experiment, following protocols approved by the Zhejiang University of Technology Animal Ethics Committee.

A first experiment was conducted in 10 SD rats to investigate whether the metabolism of BPA was changed under stable gut microbiota community. On day 1, 8, 15, 22, and 29 (7-day interval), all SD rats were administered with BPA (reconstituted in 50% DMSO/water) once only by gavage at a dose of 500 μg/kg bw. After oral BPA intake, SD rats (n = 10) were individually placed into metabolic cages, and total rat feces and urine samples were collected after the natural micturition until 24 h after BPA administration. After that, rat whole blood samples (1 mL) were collected from vena caudalis (24 h after BPA administration).

A second experiment was performed in another 10 SD rats to assess whether the altered gut microbiota could change the metabolism of BPA. All SD rats were continuously administered with BPA (reconstituted in 50% DMSO/water) at 500 μg/kg bw by oral gavage once a day for 29 days. On day 0, 1, 3, 5, 7, 9, 15, and 29, BPA-exposed rats (n = 10) were individually placed in metabolic cages after dosing for 24 hours to collect urine and feces samples. After that, rat whole blood samples (1 mL) were collected from vena caudalis (24 h after BPA administration).

In both experiments, SD rats (n = 10), dosed with the same amount of 50% DMSO/water and given *ad libitum* access to clean water and food, were set as control groups. The total volume and weight of urine and feces collected from individual rats were accurately measured. Rat blood was collected in BD Vacutainer® tubes (embedded with sodium heparin; NJ, USA). Dry ice was placed surrounding the urine and feces collection glass vessels during the sample collection period. After collection, rat feces samples were transferred to RNase-free microfuge tubes (15 mL; Conical, Thermo Fisher; ON, Canada), and stored at -80 ^{o}C until extraction and analysis.

Sample Extraction. Prior to extraction, all samples were spiked with $^{13}C_{12}$-BPA, $^{13}C_{12}$-BPA-G, and D_6-BPA-S (5 ng each), working as internal standards. Rat urine samples were diluted with pure water, and then extracted by solid-phase extraction (SPE), following the method of Liao and Kannan (2012). Rat blood samples (500 µL) were directly extracted with methanol. Feces samples were freeze-dried, homogenized, and then extracted using 80% methanol/water, with additional purification using Supelco Envi-Carb cartridges (Sigma-Aldrich; ON, Canada). In additional, spike and recovery experiments were conducted to evaluate the extraction efficiency of analytes. Detailed extraction procedures and recovery experiments are provided in the Supporting Information (SI).

Instrumental Analysis. All sample extracts were analyzed with a ACQUITY liquid chromatography coupled with a triple quadrupole mass spectrometer (XEVO_TQS; Waters; Milford, MA, USA) (Jin et al. 2020, Zhao et al. 2021). The liquid chromatography was performed using an HSS T_3 column (1.8 µm, 2.1 × 50 mm; Waters, MA, USA), with the mobile phase composed of methanol and water (2.0 mM ammonium acetate, pH = 7). The column temperature and flow rate of mobile phase were maintained at 40 ^oC and 0.2 mL/min, respectively. The gradient elution was initially held at 20% methanol for 0.5 min, increased to 40% methanol by 1.0 min, and ramped to 95% methanol by 6.0 min, which was held for 2 min, and then returned to the starting condition. The mass spectrometer was operated in negative ionization mode, and spectral data was record by multiple reaction monitoring (MRM; 2 transitions per analyte). Detailed MRM transitions of target analytes are provided in the SI, Table S1.

QA/QC. Pure methanol (10 µL) was analyzed between every ten samples to monitor carryover contamination, and no obvious cross contamination between injections was found. Several steps were taken to achieve a very low or undetectable background BPA pollution. Despite glassware was used in the whole extraction procedures, procedural blanks analyzed along with every ten real samples still contained stable and low concentrations of BPA, which was possibly originated from SPE cartridges. BPA and BPA conjugates in collected samples were quantified by the internal calibration method. Background BPA concentrations were subtracted from quantified BPA concentrations in real rat samples. Limits of detection (LODs) were defined as the concentrations of analytes correspond to a signal-to-noise ratio of 3 in sample extracts from control rats, and were in the range of 0.047 – 0.088 ng/mL, 0.039 – 0.077 ng/g, and 0.039 – 0.15 ng/mL in rat blood, feces, and urine, respectively. Extraction recoveries of target analytes in rat blood, feces, and urine ranged from 72 to 114%. Detailed LODs and extraction recovery of analytes are shown in the SI, Table S2 and S3.

To avoid the in-source fragmentation of BPA conjugates, the capillary voltage of ion source was set at a low level (-1.0 kV) at a little sacrifice of detection sensitivity. Fragmentation of BPA conjugates in the second quadrupole could generate BPA, which greatly increased the quantified BPA concentrations. This interference was minimized by the baseline separation of BPA and its conjugated metabolites. A T3 column having strong retention capacity with hydrophilic compounds was used to reduce the serous tailing and shifting retention time of the peak of BPA-DG. Typical chromatograms and molecular structure of target analytes in the standard solution and rat blood are shown in Fig. 1 and SI, Figure S1. Given the instability of BPA conjugates (Waechter et al. 2007), special care was taken to avoid deconjugation

during rat sample collection and extraction. Rat blood, feces, and urine samples spiked with $^{13}C_{12}$-BPA-G and D_6-BPA-S (at 10 or 100 ng/mL) were analyzed along with real samples. No measurable $^{13}C_{12}$-BPA and D_6-BPA were detected in these fortified samples, demonstrating the negligible deconjugation of BPA metabolites in the sample analysis process. BPA metabolites were not detected in any control rat blood, urine, and feces samples. In control rats only BPA was detected in feces at levels around LOD (mean 0.07 ng/g), which was subtracted from BPA concentrations in feces of exposed rats.

Fecal Bacterial DNA Extraction and 16S rRNA Sequencing. Bacterial DNA was extracted from rat fecal samples using an E.Z.N.A.® Stool DNA Kit (50T, Omega Bio-Tek; Norcross, GA, USA), according to the manufacturer's protocol. The purity of extracted DNA was determined with NanoDrop 2000 (Thermo Scientific; USA). After that, the DNA was PCR-amplified with barcoded primers (27F: 5'-AGRGTTYGATYMTGGCTCAG-3' and 1492R: 5'-RGYTACCTTGTTACGACTT-3'), targeting the V1 – V9 regions of the bacterial 16S rRNA gene. The PCR reaction mixture (20 μL; performed in triplicate) included 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, and 20 ng of extracted DNA. The PCR (TransGen AP221-02: TransStart Fastpfu DNA Polymerase) program began with a denaturation step (94°C; 3 min), followed by denaturation (20 cycles of 1 min; 94°C), annealing (1 min; 65°C to 57°C with a 1°C reduction every two cycles, one cycle at 56°C, and one cycle at 55°C), elongation steps (72°C; 1 min), and a final 6 min extension at 72°C. The PCR products were pooled, purified with MiniBest DNA Fragment Purification Kit Ver 4.0 (TaKaRa; Tokyo, Japan), and then sequenced on an Illumina Miseq (Illumina; San Diego, CA, USA). Prior to sequencing, the DNA library was quantified using Library Quantification Kits (KAPA Biosystems; Merck, USA).

Sequencing Data Analysis. The obtained raw fastq files were demultiplexed (using 8-bp barcodes) and quality-filtered using the QIIME software (Caporaso et al. 2010). Reads with any unknown bases, > 2 mismatches to the primers, > 1 one mismatch to the barcode, or < 50 bp length were discarded. The DNA reads having 3 consecutive low-quality bases calls were truncated. After that, overlapped paired-end reads were merged to tags (USEARCH; http://www.drive5.com/usearch/), which were further stepwise clustered to Operational Taxonomic Unit (OTU) with ≥ 97% sequence similarity (UPARSE; http://drive5.com/uparse/). A representative sequence from each OTU was taxonomically classified, using the Ribosomal Database Project (RDP) classifier (http://rdp.cme.msu.edu/), against the SILVA database (http://www.arb-silva.de) with a confidence threshold of 80%. In this study, taxonomical classification was primarily focused on gut microbiota at phylum and genus levels. Unknown, unclassified, and unassigned classifications were omitted from the final dataset.

Results

The oral BPA dose is much lower than its reported lowest observed adverse effect level (200 mg/kg bw/day) for rodents (Mao et al. 2020). In the experiment process, no obvious diarrhea, dehydration, and vomiting were observed in any SD rats. In control rats, among target analytes, only BPA was detected in rat feces, with concentrations of < 0.l ng/g.

BPA-Induced Gut Microbiome Change. Under BPA exposure at 7-day interval, rat gut microbial community was stable for 29 days, and *Firmicutes* (mean 52 − 55%) and *Bacteroidetes* (31 − 35%) were consistently the predominant phyla in rat gut, followed by *Proteobacteria* (7.7 − 8.6%) and *Actinobacteria* (3.5 − 4.7%; Fig. 2a). This profile is consistent with the human gut, in which *Firmicutes* is always the major phylum (Zhernakova et al. 2016). At the genus level, rat gut microbial community was also consistent, with *Lactobacillus* (mean 36 − 39%), *Muribaculaceae spp.* (12 − 16%), and *Bacteroides* (11 − 14%) being the dominant genera (Fig. 2b). Moreover, alpha diversity analysis (SI, Figure S2), Chao1 estimator (mean 474 − 480), and Shannon index (5.5 − 5.7) also suggest that the diversity of the gut bacterial community was conserved under BPA exposure at 7-day interval. Overall, these results suggest that BPA exposure at 7-day interval did not significantly change the gut microbiome of rats for 29 days.

After 29 days of continuous oral BPA administration, rat gut microbial community structure had been significantly changed (Fig. 2c and 2d). At the phylum level, the relative abundances of *Firmicutes* and *Proteobacteria* were significantly ($p < 0.05$) increased, while that of *Bacteroidetes* and *Actinobacteria* were significantly ($p < 0.05$) declined. The continuous oral BPA exposure also greatly reduced the alpha diversity of rat gut bacteria, with the mean Chao1 and Shannon index decreased from 464 (day 1) to 342 (day 29) and from 5.6 to 4.1, respectively (SI, Figure S2). Principal coordinate analysis (PCoA) also suggests that the rat gut microbial community structures between day 1 and 28 were significantly different, as evaluated at the genus level (PERMANOVA; $p = 0.003$; SI, Figure S3). PCoA results showed that rats on day 1 and 29 were well separated, with 73% and 9.3% of the variation explained by principal component (PC) 1 and PC2, respectively.

BPA Metabolite Profile in Rat Blood. Under the 7-day interval BPA exposure, concentrations of BPA and its conjugated metabolites in rat blood did not change significantly with dosing time (ANOVA, $p = 0.15 - 0.33$; Fig. 3a). In rat blood, the majority (mean 94 − 95%) of BPA (total molar concentrations of BPA and its conjugated metabolites) was present in conjugated forms, with BPA-G (accounting for mean 73 − 76% of total BPA) being more abundant ($p < 0.01$) than BPA-S (mean 25 − 28% of total BPA) (Fig. 4a).

Following 29 days of exposure, remarkable changes in profiles of BPA-S and BPA-G were observed in rat blood (Fig. 4b). For example, the mean proportion of BPA-S to total BPA in rat blood was gradually decreased from 30 (day 1) to 7.4% (day 29; $p < 0.01$), while that of BPA-G was elevated from 71 (day 1) to 85% (day 29; $p = 0.021$). Proportion of BPA in rat blood was slightly increased with exposure time, from 6.4 (day 1) to 8.5% (day 29; $p < 0.01$). After 9 days of continuous BPA intake, rat blood concentrations of BPA and its metabolites became stable, as evidenced by no significant (ANOVA, $p = 0.35 - 0.40$; Fig. 3b) differences in their concentrations among day 9, 15, and 29. A previous study on female mice subjected to repeated oral BPA dosing for 28 days also did not find significant accumulation of BPA in mice serum (Pollock and deCatanzaro 2014).

BPA Metabolite Profile in Rat Excreta. The total amount of BPA excreted in rat urine and feces within 24 h after exposure was calculated, based on molar concentrations of BPA and its metabolites (SI, Figure S4). Under the 7-day interval BPA exposure, the relative amount of BPA excreted in rat urine and feces kept

stable, with mean 27 – 32% and 68 – 73% of BPA excreted in rat urine and feces, respectively. Specifically, in rat urine BPA was mainly present in conjugated forms, with BPA in glucuronide form accounted for mean 74 – 77% of total excreted BPA. While, in rat feces the majority (78 – 81%) of excreted BPA was present in the free form, and the remaining BPA was mainly in the sulfate form.

The profile of BPA and its metabolites in rat excreta greatly changed with continuous exposure. For example, in rat urine the mean proportion of BPA-G elevated from 70 (day 1) to 81% (day 29), while that of BPA-S declined from 24 (day 1) to 8.7% (day 29). In rat feces, the mean proportion of BPA gradually decreased from 83 (day 1) to 65% (day 29). Following continuous exposure, the total BPA recovered in rat urine and feces did not differ greatly after day 9. Based on the data on day 9, 15, and 29, the 24 h cumulative excretion of BPA via urine and feces accounted for 21 – 28% (mean 25%) and 72 – 79% (76%) of total excreted BPA, respectively (SI, Figure S4).

Association Between Gut Microbiome and Metabolism of BPA. To explore which kinds of gut microbiota were associated with the metabolism of BPA in rats, correlation analysis was performed between the abundance of gut microbiota at the genus level and molar proportion of BPA metabolites to total BPA (Fig. 5). The 7-day interval BPA exposure did not cause the great perturbation of rat gut microbiome and profiles of BPA metabolites in rat blood and excreta within 29 days. Despite that, significantly correlations were still occasionally found between abundances of gut microbiota and molar proportion of BPA in rat urine and blood. For example, greater *Negativibacillus* (Spearman's correlation coefficient, r_s = 0.41, p = 0.022) and *Parabacteroides* (r_s = 0.33, p = 0.035) abundances were significantly associated with a higher proportion of BPA in rat blood. The abundances of *Romboutsia* (r_s = 0.43, p = 0.024) and *Turicibacter* (r_s = 0.45, p = 0.016) were negatively correlated with the proportion of BPA in rat urine. In summary, under 7-day interval BPA exposure, 5 out of 32 identified gut microbial genera were associated with the proportion of BPA and its metabolites in rat urine or blood.

Under continuous BPA exposure, many significant correlations were exhibited between the perturbed gut microbial genera and altered profiles of BPA metabolites in rats. For example, abundances of 27, 25, and 24 gut microbial genera were significantly correlated with the proportion of BPA or its metabolites in rat blood, urine, and feces, respectively. Comparatively, less kinds of gut microbiota were correlated with the proportion of BPA-BG and BPA-DS in rats, relative to BPA-G and BPA-S. For instance, only four species of gut microbiota at the genus level (*Shuttleworthia*, *Lactococcus*, *Ruminococcaceae UCG-013*, and *Negativibacillus*) were correlated with the proportion of BPA-DS in rat blood.

Overall, these results showed that continuous BPA exposure induced a significant perturbation in rat gut microbiome, which in turn substantially altered the metabolism of BPA in rats, as evidenced by gut microflora-related changes of BPA metabolite profiles in rat blood and excreta.

Discussion

Diet intake is considered the major source of human exposure to BPA (Kubwabo et al. 2014). The oral administration adopted in this study is very relevant to the actual human BPA exposure scenario. Under continuous dosing at 500 μg BPA/kg bw/day, rat blood BPA concentrations did not accumulate significantly with time, which is mainly owing to the rapid elimination of BPA (Kim et al. 2004). Rat blood BPA concentrations under both intermittent and continuous exposure (mean < 2.0 ng/mL) are comparable to the reported human blood BPA levels (mean < 0.1 – 2.5 ng/mL) (Jin et al. 2018, Vandenberg et al. 2010).

Under the 7-day interval BPA administration, BPA in glucuronide form comprised the major fraction of total BPA in rat blood, besides minor BPA-S. This is consistent with the *in vivo* results in monkeys and humans (Kurebayashi et al. 2002, Teeguarden et al. 2015, Thayer et al. 2015). Draganov et al. (2015) et al. had reported that BPA was primarily present in the glucuronide form in mice blood following oral dosing, and BPA-S only contributed 0.46 – 4.8% of total BPA metabolites. The predominance of BPA-G in rat blood is mainly attributed to the strong first-pass metabolism occurred in the liver and intestines (Draganov et al. 2015). However, two biomonitoring studies had reported markedly higher BPA-S concentrations than BPA-G in human maternal and cord serum, and authors proposed that this was due to the back transfer of BPA-S in cord blood to maternal serum (Gerona et al. 2013, Liu et al. 2017).

Under 7-day interval exposure, BPA was predominantly excreted in rat feces after oral intake. Previous studies on rats had consistently pointed to fecal excretion as the major elimination route for BPA (Domoradzki et al. 2003, Kurebayashi et al. 2003, Pottenger et al. 2000). For example, Pottenger et al. (2000) reported that rats excreted approximately 80% of total BPA via feces at 24 hours after single oral dosing. In rat feces most BPA was primary present in the free form (accounting for mean 78 – 81% of total BPA), and the remaining BPA was present in the BPA-S form. BPA-G only accounted for < 1% of total BPA in rat feces. These results show some consistency with previous works, which consistently reported free-form BPA was predominant in rat feces after oral BPA dosing (Domoradzki et al. 2003, Kurebayashi et al. 2003, Snyder et al. 2000). This may be due to the enterohepatic circulation, which transfers BPA-G from the liver to intestine via bile secretion (Zalko et al. 2003), and then hydrolyzed to BPA by gut microflora (Kurebayashi et al. 2003, Snyder et al. 2000), resulting in elevated fecal excretion of free-form BPA. Due to the expected absence of enterohepatic circulation of BPA in humans (Volkel et al. 2002), the proportion of free-form BPA may be lower in human feces than in rat feces. However, we observed a distinct profile of BPA metabolites in rat urine, relative to rat feces, with BPA-G being much more abundant than BPA and BPA-S. This finding is consistent with previous outcomes (Domoradzki et al. 2003, Kurebayashi et al. 2003, Pottenger et al. 2000). For example, urinary BPA metabolites in rats following oral BPA administration also showed the predominance of BPA-G (84% of total BPA), compared with BPA (6.1%) and BPA-S (3.1%) (Pottenger et al. 2000). Rat urinary profile of BPA metabolites is similar to that reported in human urine from America (Gerona et al. 2016, Liao and Kannan 2012, Ye et al. 2005). The high hydrophilicity of BPA-G may contribute to its excretion predominantly via urine, and also to its less amount in rat feces.

After 29 days of continuous BPA exposure, the abundances of *Firmicutes* and *Proteobacteria* were greatly increased, while that of *Bacteroidetes* and *Actinobacteria* were significantly declined. Previous studies have demonstrated that BPA exposure greatly perturbed the gut microbial structure of organisms at the phylum level. Consistently, Feng et al. found that oral BPA exposure (equivalent to 50 μg BPA/kg bw/day) significantly increased ($p < 0.05$) the abundance of *Proteobacteria* in mice gut (Feng et al. 2020). Perinatal exposure of rabbits to BPA (200 μg/kg bw) declined the abundance of *Bacteroidetes* in the gut of their male offspring (Reddivari et al. 2017). Single BPA exposure had been reported could elevate the abundance of *Firmicutes* in zebrafish intestines (Chen et al. 2018). We observed that the alpha diversity of rat gut bacteria was also greatly decreased, consistent with previous studies (Chiu et al. 2020). For example, developmental BPA exposure reduced the diversity of mouse and rabbit gut microbiota composition (Javurek et al. 2016, Reddivari et al. 2017). The underlying mechanism through which BPA alters the gut microbiome remains unclear. Some studies proposed that BPA may exert effects on the gut microbiota community through an estrogenic mechanism (Javurek et al. 2016).

Comparison of intermittent and continuous BPA exposure suggests that changes in rat gut microbiome induced by continuous BPA exposure may in turn altered the metabolism of BPA in rats. At the phylum level, the abundances of *Firmicutes* and *Proteobacteria* were positively correlated with the proportion of BPA-G in rat blood. This partially explains the higher abundance of BPA-G in rat urine, since BPA-G was mainly excreted through urine. *Firmicutes* had shown a obviously positive correlation with the BPA degradation in Gonghu Bay sediment, and high concentrations of BPA can accelerate the BPA removal by *Firmicutes* (Yuxuan Liu et al. 2020). This suggests that *Firmicutes* may have the ability to biodegrade BPA. Consistently, Fernandez et al. (2018) had reported that many *β*-glucuronidase bacteria belong to the *Firmicutes* phylum in human feces. The abundance of *Bacteroidetes* was negatively correlated with the proportion of BPA in rat blood. Koestel et al. (2017) have reported that *Bacteroidetes*, capable of degrading bisphenols, was negatively associated with serum BPA concentrations in dogs, after oral diet BPA intake.

An obvious change of rat gut microbiota, at the genus level, was the increase of *Blautia* and decrease of *Lactobacillus*. The early life BDE-47 exposure also increased the abundance of the genus *Blautia* in adult male mice (Gomez et al. 2021). In Cd-treated (5 μg/L) zebrafish, the abundance of *Blautia* was more abundant than the control zebrafish (Xia et al. 2020). *Lactobacillus* was considered tolerant or resistant to BPA biodegradation (Ana López-Moreno et al. 2021), and this may lead to its decrease in abundance in rat gut after BPA intake. We also observed that the relative abundances of *Escherichia-Shigella*, *Ruminococcaceae UCG-005*, *Marvinbryantia*, and *Lachnoclostridium* were also increased in rat gut. Both *Escherichia-Shigella* and *Ruminococcaceae* bacterial groups possess *β*-glucuronidase enzymes (Fernandez et al. 2018, Rea et al. 2018). This partially explains the increased proportion of BPA-G in rat blood with more abundances of *Escherichia-Shigella* and *Ruminococcaceae UCG-005*. In contrast, we found that the abundance of *Muribaculaceae_norank* was decreased after continuous BPA exposure. The down-regulation of *Muribaculaceae* in gut of adult mice caused by low PCB dose had been reported (Lim et al. 2021). Notably, less kinds of gut microbiota were correlated with the proportion of BPA-BG and BPA-

DS in rats, relative to BPA-G and BPA-S. This may be because the conjugation of BPA to BPA-BG and BPA-DS is primarily driven by enzymes in rat liver.

Significance. Despite BPA is rapidly metabolized to BPA conjugates in humans and then rapidly excreted, but this does not necessary mean the negligible risk of BPA exposure, since BPA metabolites are still widely present in human blood (Ginsberg and Rice 2009). Moreover, BPA metabolites have been proved to have toxic effects on humans (Ginsberg and Rice 2009, Safe 2000, Volkel et al. 2002). Therefore, it is important to understand the underlying mechanisms responsible for the metabolism of BPA in humans. This study first demonstrates that continuous BPA exposure disrupted the rat gut microbiota community, which in turn altered the metabolism of BPA in rat. Rat gut microbiota significantly correlated with the profile of BPA metabolites in rat were also identified. This finding contributes to the better understanding of metabolism of BPA in humans. More studies are needed to further evaluate the relative contribution of gut microbiota to the occurrence of BPA metabolites in humans.

Declarations

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study procedures were approved by the Ethics Committee of Zhejiang University of Technology. All subjects have signed a written informed consent form.

CONSENT TO PUBLISH

The work in this manuscript has not been previously published and is not under consideration of other journals. All authors consent to publish this work.

AUTHOR CONTRIBUTIONS

Nan Zhao: Writing-Original draft preparation, Data curation, Methodology.

Lingling Mao: Data curation, Formal analysis, Methodology.

Weili Mao: Data curation, Methodology.

Meirong Zhao: Writing-Reviewing and Editing, Conceptualization.

Zefu Hu: Validation, Investigation, Data Curation.

Hangbiao Jin: Formal analysis, Methodology, Writing-Reviewing and Editing, Supervision.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AVAILABILITY OF DATA AND MATERIALS

Not applicable.

References

1. Ana López-Moreno Ángel, Ruiz-Moreno J, Pardo K, Cerk, Torres-Sánchez A, Ortíz P, Úbeda M, Aguilera M (2021) Human gut microbial taxa metabolizing dietary obesogens: A BPA directed-culturing and bioinformatics combined approach. 10.21203/rs.3.rs-754318/v1
2. Bonefeld-Jorgensen EC, Long M, Hofmeister MV, Vinggaard AM (2007) Endocrine-disrupting potential of bisphenol A, bisphenol A dimethacrylate, 4-n-nonylphenol, and 4-n-octylphenol in vitro: new data and a brief review. Environ Health Perspect 115(Suppl 1):69–76
3. Boucher JG, Boudreau A, Ahmed S, Atlas E (2015) In Vitro Effects of Bisphenol A beta-D-Glucuronide (BPA-G) on Adipogenesis in Human and Murine Preadipocytes. Environ Health Perspect 123(12):1287–1293
4. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7(5):335–336
5. Chen D, Kannan K, Tan H, Zheng Z, Feng YL, Wu Y, Widelka M (2016) Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity-A Review. Environ Sci Technol 50(11):5438–5453
6. Chen L, Guo Y, Hu C, Lam PKS, Lam JCW, Zhou B (2018) Dysbiosis of gut microbiota by chronic coexposure to titanium dioxide nanoparticles and bisphenol A: Implications for host health in zebrafish. Environ Pollut 234:307–317
7. Chiu K, Warner G, Nowak RA, Flaws JA, Mei W (2020) The Impact of Environmental Chemicals on the Gut Microbiome. Toxicol Sci 176(2):253–284

8. Claus SP, Guillou H, Ellero-Simatos S (2016) The gut microbiota: a major player in the toxicity of environmental pollutants? NPJ Biofilms Microbiomes 2:16003
9. Dekant W, Volkel W (2008) Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. Toxicol Appl Pharmacol 228(1):114–134
10. Domoradzki JY, Pottenger LH, Thornton CM, Hansen SC, Card TL, Markham DA, Dryzga MD, Shiotsuka RN, Waechter JM Jr (2003) Metabolism and pharmacokinetics of bisphenol A (BPA) and the embryo-fetal distribution of BPA and BPA-monoglucuronide in CD Sprague-Dawley rats at three gestational stages. Toxicol Sci 76(1):21–34
11. Draganov DI, Markham DA, Beyer D, Waechter JM Jr, Dimond SS, Budinsky RA, Shiotsuka RN, Snyder SA, Ehman KD, Hentges SG (2015) Extensive metabolism and route-dependent pharmacokinetics of bisphenol A (BPA) in neonatal mice following oral or subcutaneous administration. Toxicology 333:168–178
12. Feng D, Zhang H, Jiang X, Zou J, Li Q, Mai H, Su D, Ling W, Feng X (2020) Bisphenol A exposure induces gut microbiota dysbiosis and consequent activation of gut-liver axis leading to hepatic steatosis in CD-1 mice. Environ Pollut 265(Pt A),114880
13. Fernandez MF, Reina-Perez I, Astorga JM, Rodriguez-Carrillo A, Plaza-Diaz J, Fontana L (2018) Breast Cancer and Its Relationship with the Microbiota.Int J Environ Res Public Health15(8)
14. Gauderat G, Picard-Hagen N, Toutain PL, Corbel T, Viguie C, Puel S, Lacroix MZ, Mindeguia P, Bousquet-Melou A, Gayrard V (2016) Bisphenol A glucuronide deconjugation is a determining factor of fetal exposure to bisphenol A. Environ Int 86:52–59
15. Gerona RR, Pan J, Zota AR, Schwartz JM, Friesen M, Taylor JA, Hunt PA, Woodruff TJ (2016) Direct measurement of Bisphenol A (BPA), BPA glucuronide and BPA sulfate in a diverse and low-income population of pregnant women reveals high exposure, with potential implications for previous exposure estimates: a cross-sectional study.Environmental Health15
16. Gerona RR, Woodruff TJ, Dickenson CA, Pan J, Schwartz JM, Sen S, Friesen MW, Fujimoto VY, Hunt PA (2013) Bisphenol-A (BPA), BPA Glucuronide, and BPA Sulfate in Midgestation Umbilical Cord Serum in a Northern and Central California Population. Environ Sci Technol 47(21):12477–12485
17. Ginsberg G, Rice DC (2009) Does Rapid Metabolism Ensure Negligible Risk from Bisphenol A? Environ Health Perspect 117(11):1639–1643
18. Gomez MV, Dutta M, Suvorov A, Shi X, Gu H, Mani S, Cui Y, J (2021) Early Life Exposure to Environmental Contaminants (BDE-47, TBBPA, and BPS) Produced Persistent Alterations in Fecal Microbiome in Adult Male Mice. Toxicol Sci 179(1):14–30
19. Goodman JE, Witorsch RJ, McConnell EE, Sipes IG, Slayton TM, Yu CJ, Franz AM, Rhomberg LR (2009) Weight-of-evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. Crit Rev Toxicol 39(1):1–75
20. Javurek AB, Spollen WG, Johnson SA, Bivens NJ, Bromert KH, Givan SA, Rosenfeld CS (2016) Effects of exposure to bisphenol A and ethinyl estradiol on the gut microbiota of parents and their offspring in a rodent model. Gut Microbes 7(6):471–485

21. Jin HB, Xie JH, Mao LL, Zhao MR, Bai XX, Wen J, Shen T, Wu PF (2020) Bisphenol analogue concentrations in human breast milk and their associations with postnatal infant growth. Environ Pollut 259:113779
22. Jin HB, Zhu J, Chen ZJ, Hong YJ, Cai ZW (2018) Occurrence and Partitioning of Bisphenol Analogues in Adults' Blood from China. Environ Sci Technol 52(2):812–820
23. Kim CS, Sapienza PP, Ross IA, Johnson W, Luu HM, Hutter JC (2004) Distribution of bisphenol A in the neuroendocrine organs of female rats. Toxicol Ind Health 20(1–5):41–50
24. Koestel ZL, Backus RC, Tsuruta K, Spollen WG, Johnson SA, Javurek AB, Ellersieck MR, Wiedmeyer CE, Kannan K, Xue J, Bivens NJ, Givan SA, Rosenfeld CS (2017) Bisphenol A (BPA) in the serum of pet dogs following short-term consumption of canned dog food and potential health consequences of exposure to BPA. Sci Total Environ 579:1804–1814
25. Kubwabo C, Kosarac I, Lalonde K, Foster WG (2014) Quantitative determination of free and total bisphenol A in human urine using labeled BPA glucuronide and isotope dilution mass spectrometry. Anal Bioanal Chem 406(18):4381–4392
26. Kurebayashi H, Betsui H, Ohno Y (2003) Disposition of a low dose of 14C-bisphenol A in male rats and its main biliary excretion as BPA glucuronide. Toxicol Sci 73(1):17–25
27. Kurebayashi H, Harada R, Stewart RK, Numata H, Ohno Y (2002) Disposition of a low dose of bisphenol A in male and female cynomolgus monkeys. Toxicol Sci 68(1):32–42
28. Lai KP, Chung YT, Li R, Wan HT, Wong CK (2016) Bisphenol A alters gut microbiome: Comparative metagenomics analysis. Environ Pollut 218:923–930
29. Liao C, Kannan K (2012) Determination of Free and Conjugated Forms of Bisphenol A in Human Urine and Serum by Liquid Chromatography-Tandem Mass Spectrometry. Environ Sci Technol 46(9):5003–5009
30. Lim JJ, Dutta M, Dempsey JL, Lehmler HJ, MacDonald J, Bammler T, Walker C, Kavanagh TJ, Gu H, Mani S, Cui JY (2021) Neonatal exposure to BPA, BDE-99, and PCB produces persistent changes in hepatic transcriptome associated with gut dysbiosis in adult mouse livers. Toxicological Sciences
31. Liu JY, Li JG, Wu YN, Zhao YF, Luo FJ, Li SM, Yang L, Moez EK, Dinu I, Martin JW (2017) Bisphenol A Metabolites and Bisphenol S in Paired Maternal and Cord Serum. Environ Sci Technol 51(4):2456–2463
32. Mao J, Jain A, Denslow ND, Nouri MZ, Chen S, Wang T, Zhu N, Koh J, Sarma SJ, Sumner BW, Lei Z, Sumner LW, Bivens NJ, Roberts RM, Tuteja G, Rosenfeld CS (2020) Bisphenol A and bisphenol S disruptions of the mouse placenta and potential effects on the placenta-brain axis. Proc Natl Acad Sci U S A 117(9):4642–4652
33. Nadal A, Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB (2009) The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. Mol Cell Endocrinol 304(1–2):63–68
34. Nishikawa M, Iwano H, Yanagisawa R, Koike N, Inoue H, Yokota H (2010) Placental Transfer of Conjugated Bisphenol A and Subsequent Reactivation in the Rat Fetus. Environ Health Perspect

118(9):1196–1203

35. Pollock T, deCatanzaro D (2014) Presence and bioavailability of bisphenol A in the uterus of rats and mice following single and repeated dietary administration at low doses. Reprod Toxicol 49:145–154
36. Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM Jr (2000) The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicol Sci 54(1):3–18
37. Rea D, Coppola G, Palma G, Barbieri A, Luciano A, Del Prete P, Rossetti S, Berretta M, Facchini G, Perdona S, Turco MC, Arra C (2018) Microbiota effects on cancer: from risks to therapies. Oncotarget 9(25):17915–17927
38. Reddivari L, Veeramachaneni DNR, Walters WA, Lozupone C, Palmer J, Hewage MKK, Bhatnagar R, Amir A, Kennett MJ, Knight R, Vanamala JKP (2017) Perinatal Bisphenol A Exposure Induces Chronic Inflammation in Rabbit Offspring via Modulation of Gut Bacteria and Their Metabolites. mSystems 2(5)
39. René Viñas RM, Goldblum, Watson CS (2013) Rapid estrogenic signaling activities of the modified (chlorinated, sulfonated, and glucuronidated) endocrine disruptor bisphenol A. Endocr Disruptors 1(1):24511
40. Rochester JR (2013) Bisphenol A and human health: a review of the literature. Reprod Toxicol 42:132–155
41. Safe SH (2000) Endocrine disruptors and human health–is there a problem? An update. Environ Health Perspect 108(6):487–493
42. Snyder RW, Maness SC, Gaido KW, Welsch F, Sumner SC, Fennell TR (2000) Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol 168(3):225–234
43. Staples CA, Dorn PB, Klecka GM, O'Block ST, Harris LR (1998) A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36(10):2149–2173
44. Teeguarden JG, Twaddle NC, Churchwell MI, Yang X, Fisher JW, Seryak LM, Doerge DR (2015) 24-hour human urine and serum profiles of bisphenol A: Evidence against sublingual absorption following ingestion in soup. Toxicol Appl Pharmacol 288(2):131–142
45. Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, Garantziotis S, Kissling GE, Easterling MR, Bucher JR, Birnbaum LS (2015) Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ Int 83:107–115
46. Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G (2010) Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect 118(8):1055–1070
47. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007) Human exposure to bisphenol A (BPA). Reprod Toxicol 24(2):139–177
48. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, Felin J, Perkins R, Boren J, Oresic M, Backhed F (2010) The gut microbiota modulates host energy and lipid metabolism in mice. J Lipid Res 51(5):1101–1112

49. Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W (2002) Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem Res Toxicol 15(10):1281–1287
50. vom Saal FS, Nagel SC, Timms BG, Welshons WV (2005) Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public.Toxicology212(2–3), 244–252, author reply 253 – 244.
51. Waechter J, Thornton C, Markham D, Domoradzki J (2007) Factors affecting the accuracy of bisphenol a and bisphenol a-monoglucuronide estimates in Mammalian tissues and urine samples. Toxicol Mech Methods 17(1):13–24
52. Wang Y, Rui M, Nie Y, Lu G (2018) Influence of gastrointestinal tract on metabolism of bisphenol A as determined by in vitro simulated system. J Hazard Mater 355:111–118
53. Xia Y, Zhu J, Xu Y, Zhang H, Zou F, Meng X (2020) Effects of ecologically relevant concentrations of cadmium on locomotor activity and microbiota in zebrafish. Chemosphere 257:127220
54. Ye X, Kuklenyik Z, Needham LL, Calafat AM (2005) Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 383(4):638–644
55. Yuxuan Liu Y, Wang Q, Wang B, Wang X, Liu, Wu B (2020) Adsorption and removal of bisphenol A in two types of sediments and its relationships with bacterial community. Int Biodeterior Biodegrad 153:105021
56. Zalko D, Soto AM, Dolo L, Dorio C, Rathahao E, Debrauwer L, Faure R, Cravedi JP (2003) Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environ Health Perspect 111(3):309–319
57. Zhang ZF, Alomirah H, Cho HS, Li YF, Liao CY, Minh TB, Mohd MA, Nakata H, Ren NQ, Kannan K (2011) Urinary Bisphenol A Concentrations and Their Implications for Human Exposure in Several Asian Countries. Environ Sci Technol 45(16):7044–7050
58. Zhao N, Hu H, Zhao M, Liu W, Jin HJES (2021) and TechnologyOccurrence of Free-Form and Conjugated Bisphenol Analogues in Marine Organisms
59. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, Mujagic Z, Vila AV, Falony G, Vieira-Silva S, Wang J, Imhann F, Brandsma E, Jankipersadsing SA, Joossens M, Cenit MC, Deelen P, Swertz MA, Weersma RK, Feskens EJ, Netea MG, Gevers D, Jonkers D, Franke L, Aulchenko YS, Huttenhower C, Raes J, Hofker MH, Xavier RJ, Wijmenga C, Fu J (2016) Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 352(6285), 565–569

Figures

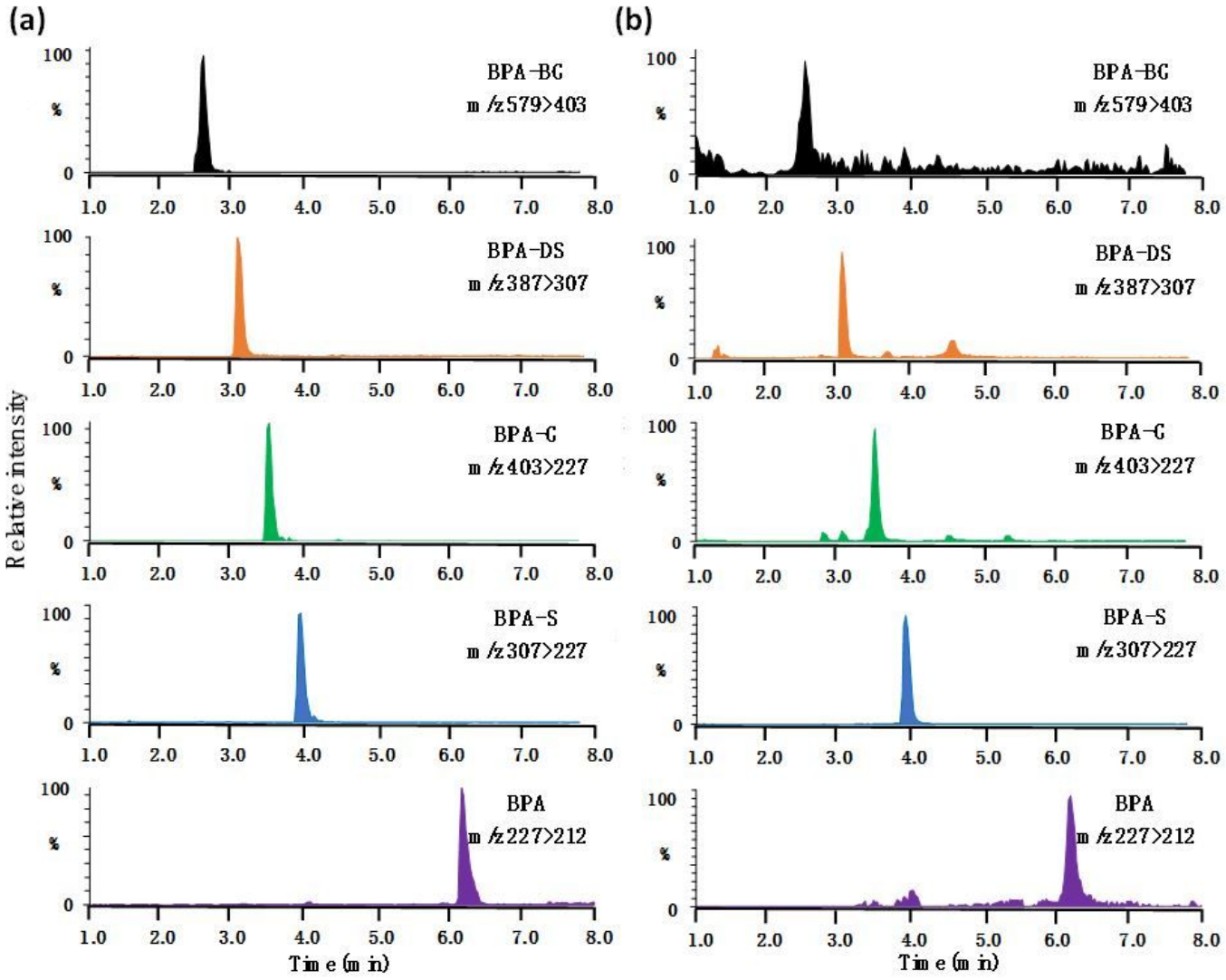

Figure 1

Representative chromatograms of BPA and its conjugated metabolites in (a) the 50% methanol/water solution and (b) rat blood.

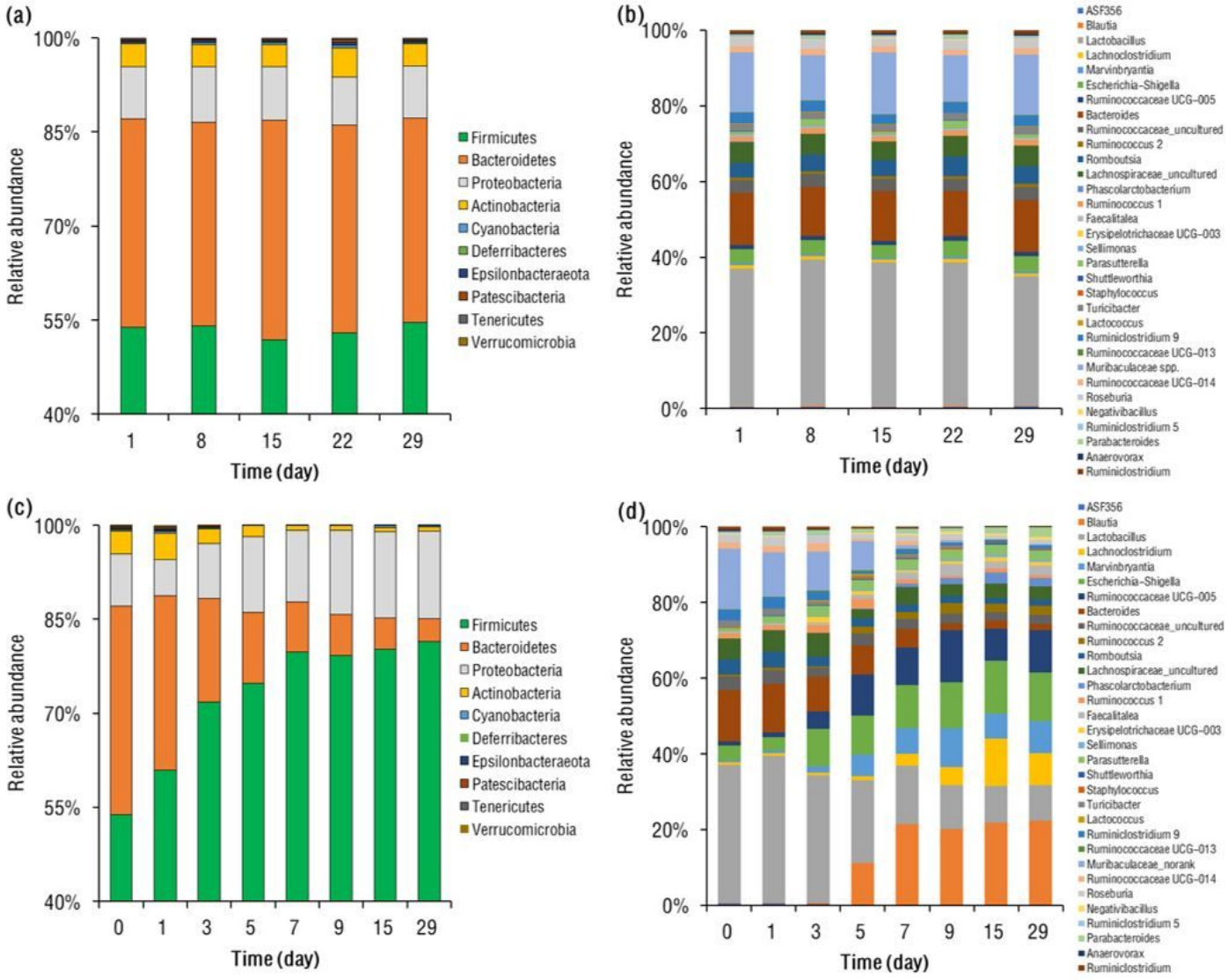

Figure 2

Change of relative abundance of gut microbiota at (a) phylum and (b) genus levels in SD rats with time, following intermittent oral administration of BPA. Change of relative abundance of gut microbiota at (c) phylum and (d) genus levels in SD rats with time, following continuous oral administration of BPA.

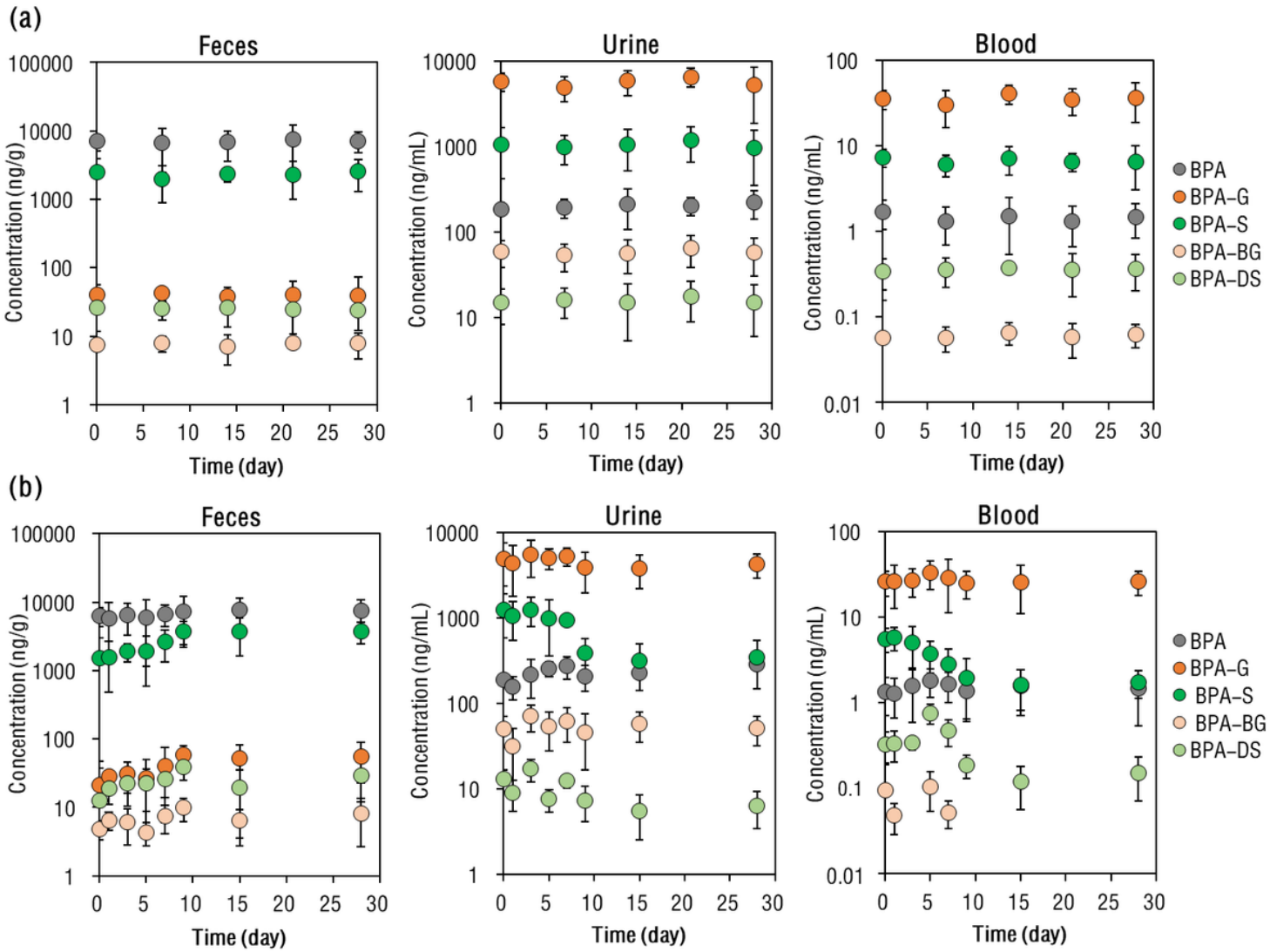

Figure 3

Mean concentrations of BPA and its conjugated metabolites in SD rat feces, urine, and blood, following (a) interval and (b) continuous oral BPA administration. The vertical bars represent standard deviation.

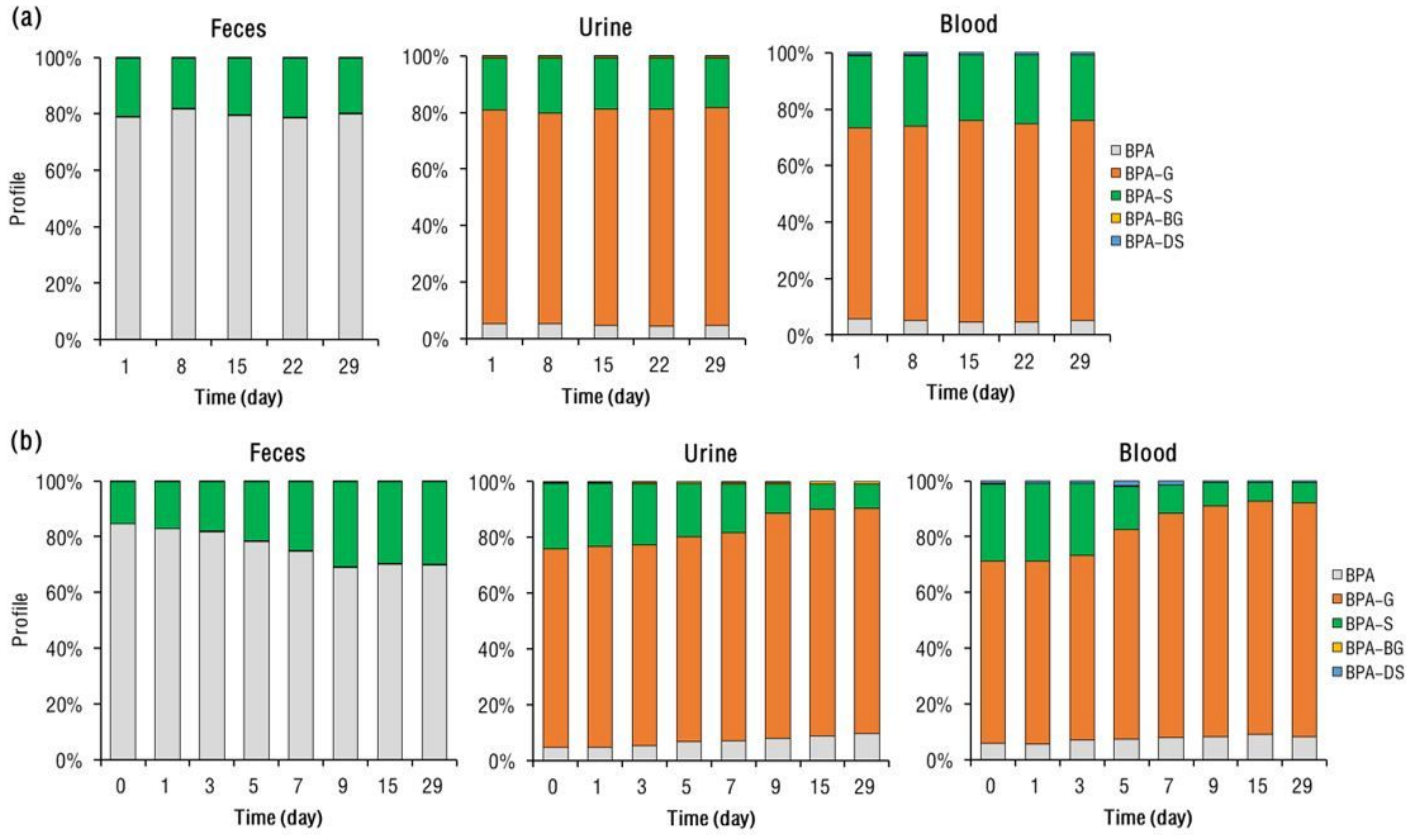

Figure 4

Change of molar concentration profile of BPA and its conjugated metabolites in feces, urine, and blood of SD rats with time, following (a) interval and (b) continuous oral BPA exposure.

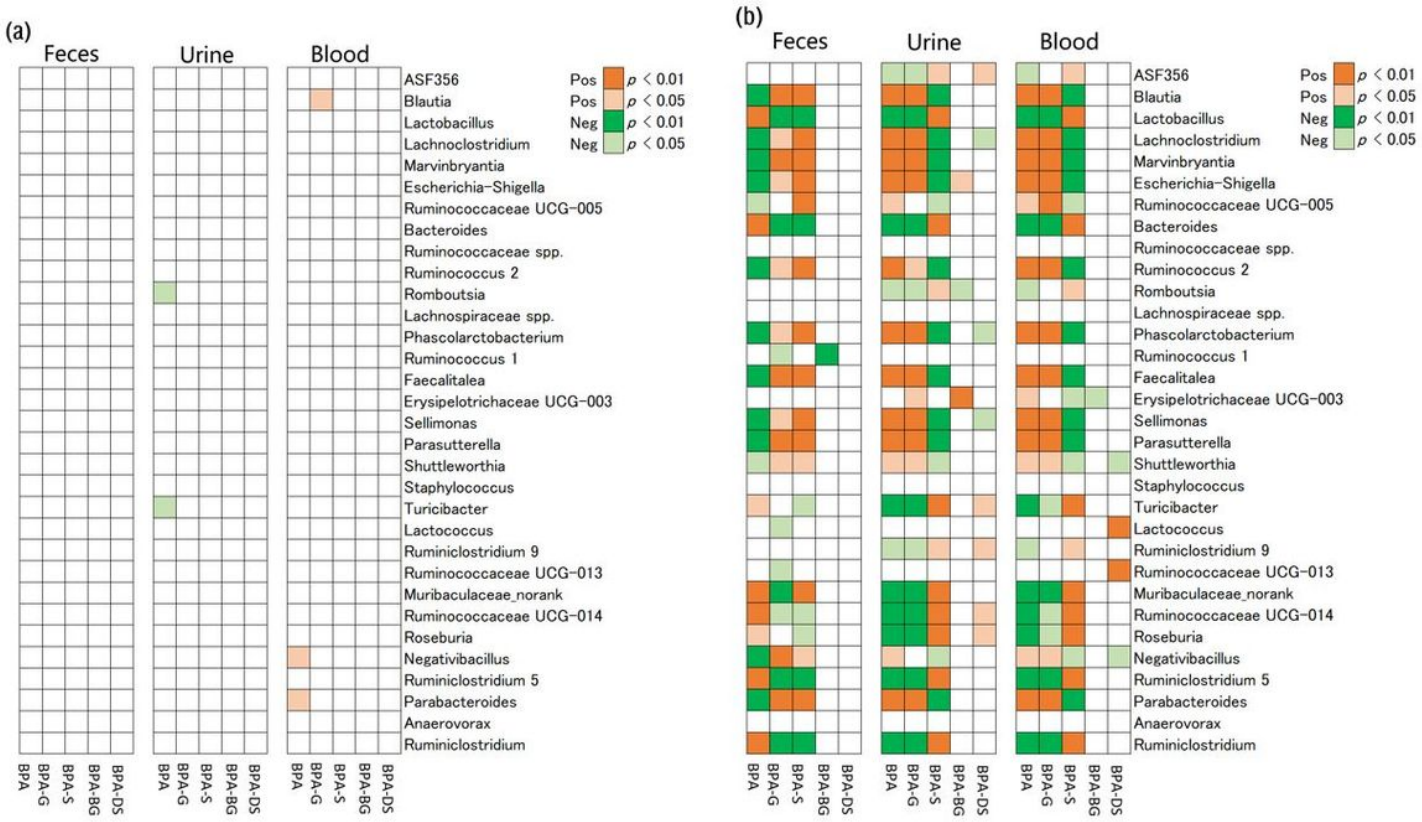

Figure 5

Correlations between abundance of rat gut bacteria at the genus level and proportion of BPA and its metabolites in feces, urine, and blood of rat following (a) interval and (b) continuous BPA exposure. The red and green squares indicate the significantly positive and negative correlations, respectively.

Supplementary Files

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

- TOC.jpg
- SIMetabolismofBPAinSDRats011.docx