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Bisphenol S exposure impairs glucose homeostasis in male zebrafish (*Danio rerio*)

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ABSTRACT

Bisphenol S (BPS) is a substitute of the plastic additive bisphenol A (BPA). Its concentrations detected in surface waters and urine samples are on the same order of magnitude as BPA. Human exposure to BPA has been implicated in the development of diabetes mellitus; however, whether BPS can disrupt glucose homeostasis and increase blood glucose concentration remains unclear. We extensively investigated the effects of environmentally relevant concentrations of BPS on glucose metabolism in male zebrafish (*Danio rerio*) and the underlying mechanisms of these effects. Male zebrafish were exposed to 1, 10, or 100 μg/L of BPS for 28 d. Fasting blood glucose (FBG) levels, glycogen levels in the liver and muscle, and mRNA levels of key glucose metabolic enzymes and the activities of the encoded proteins in tissues were evaluated to assess the effect of BPS on glucose metabolism. Plasma insulin levels and expression of preproinsulin and glucagon genes in the visceral tissue were also evaluated. Compared with the control group, exposure to 1 and 10 μg/L of BPS significantly increased FBG levels but decreased insulin levels. Gluconeogenesis and glycogenolysis in the liver were promoted, and glycogen synthesis in the liver and muscle and glycolysis in the muscle were inhibited. Exposure to 100 μg/L of BPS did not significantly alter plasma insulin and blood glucose levels, but nonetheless pronouncedly interfered with gluconeogenesis, glycogenolysis, glycolysis, and glycogen synthesis. Our data indicates that BPS at environmentally relevant concentrations impairs glucose homeostasis of male zebrafish possibly by hampering the physiological effect of insulin; higher BPS doses also pronouncedly interfered with glucose metabolism.

1. Introduction

Bisphenol S (BPS) is a substitute of the plastic additive bisphenol A (BPA) (Rochester and Bolden, 2015), and is being used in the production of epoxy glues, baby bottles, canned foodstuffs, paper currencies, food paperboard, and thermal receipt paper (Liao et al., 2012a; Liao and Kannan, 2013, 2014). In Europe, the annual production of BPS is between 1000 and 10,000 t, and steadily increases (Ivry Del Moral et al., 2016). Compared with BPA, BPS has a higher thermal stability and leaches to a lesser degree from plastic products, yet BPS at concentrations similar to or even greater than BPA was frequently detected in the abiotic environment and human urine from some regions (Chen et al., 2016). Mean BPS concentrations in sludge samples from wastewater treatment plants were detected to be 44.9 ng/g dry weight and 34.5 ng/g dry weight in Korea and the United States, respectively (Lee et al., 2015; Yu et al., 2015). For indoor dust, mean BPS concentrations were measured to be 220 ng/g in samples from 12 countries including China, Colombia, Greece, etc (Wang et al., 2015). In thermal receipt papers, BPS with concentrations as high as 22.0 mg/g was detected in samples from the United States, Japan, Korea, and Vietnam (Liao et al., 2012a). The highest measured concentration of BPS in surface waters was 0.006 μg/L and 7.2 μg/L in Taihu Lake in China (Jin and Zhu, 2016) and Adyar River in India (Yamazaki et al., 2015), respectively. BPS were also found in different categories of food items collected from nine cities in China, with mean concentrations reached 0.287 ng/g fresh weight (Liao and Kannan, 2014). These data indicate potential human exposure to BPS via various pathways. Indeed, high levels of BPS were reported in urine of humans, with values up to 12.2 ng/mL in

Abbreviations: BPS, bisphenol S; BPA, bisphenol A; FBG, fasting blood glucose; IRs, insulin receptors; IRS/PI3K/Akt, insulin receptor substrates/phosphotidylinositol 3-kinase/Akt; GP, glycogen phosphorylase; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; HK, hexokinase; GCK, glucokinase; PK, pyruvate kinase; GS, glycogen synthase; EDCs, endocrine-disturbing chemicals; BMI, body mass index; PCR, polymerase chain reaction; PEPCK-C, cytosolic PEPCK; PEPCK-M, mitochondrial PEPCK; G-6-P, glucose-6-phosphate; cAMP/PKA/CREB, cAMP/protein kinase A/cAMP response element binding protein; E_2, 17β-estradiol; T, testosterone

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samples from Indian children (Xue et al., 2015), and 21.0 ng/mL in those from American people (Liao et al., 2012b). As a structural analogue of BPA, BPS has been reported to exert similar toxic effects on animals as BPA. For example, BPS was reported to exhibit estrogenic, antiestrogenic, androgenic, and antiandrogenic potencies (Rochester and Bolden, 2015), alter plasma thyroxine and triiodothyronine levels of zebrafish (Naderi et al., 2014), cause cytotoxicity and genotoxicity in HepG2 cells (Fic et al., 2013), impact the reproductive neuroendocrine system during zebrafish embryonic and larval development (Qiu et al., 2016), and exhibit a significant effect on aryl hydrocarbon receptor (AhR)-mediated luciferase reporter gene activity in COS-7 cells at a high concentration (Ma et al., 2015). It was reported that BPA exposure can also interfere with glucose metabolism in humans, and epidemiological investigations showed a positive association between increased levels of urinary BPA and diabetes mellitus, especially in men (Beydoun et al., 2014; Shankar and Teppala, 2011). Nevertheless, to the best of our knowledge, only two researches investigated influence of BPS on energy metabolism with emphasis on its obesogen effects (Héliès-Toussaint et al., 2014; Ivry Del Moral et al., 2016), and neither of which has revealed that whether BPS can disrupt glucose homeostasis and elevate blood glucose levels in animals.

Glucose homeostasis is regulated by multiple hormones. Of these, insulin is the only hormone that can decrease glucose concentration; it thus plays a key role in glucose metabolism (Fritsche et al., 2008). Upon its release into the blood from the pancreatic islet β cells, insulin binds to insulin receptors (IRs) on the membranes of target tissues, subsequently activating signal pathways including the insulin receptor substrates/phosphotidylinositol 3-kinase/Akt (IRS/PI3K/Akt) (Caruso and Sheridan, 2011). Alterations in the structure and function of islet β cells, blood insulin levels, and protein phosphorylation of key molecules in the insulin signaling pathway therefore constitute hotspots for research into the mechanisms of glucose homeostasis impairment by BPA (Alonso-Magdalena et al., 2006; Batista et al., 2012; Jayashree et al., 2013). However, it should be noted that the final targets of insulin signaling pathway constitute various glucose metabolic enzymes; changes in their gene expression and activities may significantly affect the production and elimination of glucose, directly altering blood glucose concentration (Matsuoka et al., 2015; Röder et al., 2016). Normally, under fasting conditions, blood insulin levels decrease but the levels of hyperglycemic hormones (e.g., glucagon) increase, leading to a stimulation of glycogenolysis and gluconeogenesis. Accelerated transformation of glycogen and other non-carbohydrate carbon substrates into glucose, catalyzed by such key enzymes as glycogen phosphorylase (GP), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase), consequently increases blood glucose levels. In contrast, under high blood glucose conditions, insulin levels increase while glucagon levels decrease, resulting in an inhibition on glycogenolysis and gluconeogenesis but a promotion on glycolysis and glycogen synthesis. Activated hexokinase (HK) in the muscle or glucokinase (GCK) in the liver, as well as pyruvate kinase (PK) and glycogen synthase (GS) in both the liver and muscle, enhance glucose oxidation and storage in tissues, effectively reducing its concentration in the blood. Clarifying metabolic alterations caused by endocrine-disturbing chemicals (EDCs) may help to develop corresponding treatment strategies to maintain glucose homeostasis. Nevertheless, even though some glucose metabolic enzymes or pathways were investigated selectively in studies of BPA (Indumathi et al., 2013; Jayashree et al., 2013; Ma et al., 2013), the detailed influences of bisphenols, especially BPS, on animal glucose homeostasis were still unclear.

The glucose metabolism of fish is similar to that of mammals. Further, the function of insulin is conserved among vertebrates, i.e., it plays a key role in the regulation of glucose metabolism also in fish (Caruso and Sheridan, 2011). The fish model, e.g., zebrafish (*Danio rerio*), with the advantages of high fecundity, short generation period, low breeding costs, and the ease of genetic manipulation, has therefore been proposed as a good model for the research of human diseases, including diabetes mellitus (Craig and Moon, 2011; Kimmel and Meyer, 2016; Olsen et al., 2012; Zang et al., 2017). Additionally, by conducting comparative transcriptome analysis, results of Zang et al. (2017) revealed that diabetic zebrafish and human patients share common pathological pathways. However, researches using zebrafish to investigate the influences and underlying mechanisms of EDCs on glucose homeostasis are relatively rare. Since the association between urinary bisphenol compounds and the risk of diabetes mellitus was shown to be stronger in men than in women (Beydoun et al., 2014), male zebrafish were utilized as model animals in the current study. To investigate the effects of BPS on glucose metabolism in detail, zebrafish were exposed to 1, 10, or 100 μg/L BPS for 28 d, and fasting blood glucose (FBG) levels, glycogen levels in the liver and muscle, and tissue mRNA levels and activities of the encoded key glucose metabolic enzymes were evaluated. The underlying mechanisms were further explored by analyzing plasma insulin levels and mRNA levels of preproinsulin and glucagon genes in the visceral tissue. The results of this study may inform the evaluation of potential health risks of BPS, and provide a reference for the use of zebrafish model in mechanistic studies of human metabolic disease caused by EDCs exposure.

2. Materials and methods

2.1. Animals and exposure

T strain zebrafish have been bred at the Biotoxicity Laboratory in Ocean University of China for over 2 years. Male zebrafish aged 9 months (n = 128) were used in this study (average length of 2.61 ± 0.15 cm; average weight of 0.29 ± 0.05 g). Fish were exposed to 1, 10, or 100 μg/L of BPS (purity 99.9%; Sigma), or solvent control (dimethyl sulfoxide, 0.002% V/V) for 28 d. A semi-static approach (with 100% daily water renewal) was adopted to maintain stable concentrations of the test compound. For each treatment and control group, 32 fish were randomly and evenly distributed between four beakers, each containing 4 L of well-aerated tap water. The experimental conditions were as follows: water temperature of 28 ± 1 °C; dissolved oxygen level of 7.0 ± 0.1 mg/L; pH of 7.6 ± 0.2; with 14 h light/10 h darkness photoperiod. Newly-hatched *Artemia saline* were used for fish feeding, twice daily, and equal and appropriate amounts of food were provided to each beaker to guarantee that all food was taken up by fish within 5 min. No fish deaths were observed during the experiment. All animal use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China.

To measure actual concentrations of BPS in test solutions, water samples from each beaker in each group were collected before (0 h) and after 24 h of exposure (24 h) on day 9, day 18, and day 27, respectively. BPS concentrations in water samples were analyzed according to the methods described by Yang et al. (2014). Briefly, an aliquot of 100 μL water samples was spiked with 20 μL of internal standards (50 μg/L of BPS-$^{13}C_{12}$) and diluted to 1 mL. A Calibration standard (ranged from 0.1 to 5 μg/L) was prepared with ultrapure water samples previously spiked with standard mixture and internal standards. These samples with concentrations of BPS above the calibration range were diluted to bring the concentration to within the calibration range. BPS quantification was performed with an Acquity ultra performance liquid chromatography system (UPLC) coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, USA). UPLC separation was conducted on an Acquity BEH C18 column (2.1 mm × 100 mm; 1.7 μm; Waters). MS/MS acquisition was operated in negative-ion mode with multiple reaction monitoring (MRM). Quantification was based on the peak areas relative to the corresponding internal standards. The quantification limit was 0.1 μg/L. Table A.2 shows that BPS concentrations in the control beakers were below the quantification limit, while the mean concentrations (mean ± standard deviation, n = 12) of BPS in the test solutions were 1.46 ± 0.34, 12.37 ± 1.46, and 121.95 ± 10.19 μg/L at 0 h, and were 1.22 ± 0.06, 11.29 ± 0.68, and