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Obesity aggravates toxic effect of BPA on spermatogenesis

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ABSTRACT

Both bisphenol A (BPA) and obesity affect male reproductive system. However, whether there is an interaction between them remains poorly understood. The aim of the present study was to evaluate the interaction between BPA exposure and obesity on semen quality and elucidate the mechanism in humans and animals. We firstly analyzed the interaction on semen volume, sperm count per ejaculate, sperm concentration and sperm motility in 357 men, and found that urinary BPA concentration was significantly correlated with sperm count per ejaculate in obese men ($\beta = -34.62$; 95% CI: -60.75, -8.48; $P = 0.01$). Then we validated the interaction using lean and obese mice with administration of BPA. Significant interactions between BPA exposure and obesity on sperm count and sperm concentration was observed in mice. Finally, we conducted metabolomics analyses to identify metabolites related to the interaction. Metabolites related to the interaction, including capric acid, dodecanoic acid, L-palmitoylcarnitine, niacinamide, etc., are known to play critical roles in fatty acid oxidation and tricarboxylic acid cycle indicating increased oxidative stress associated with male reproductive dysfunction. Thus, our study finds an interaction between BPA exposure and obesity on sperm count and reveals potential metabolic mechanisms. It emphasizes the importance to study interactions between endocrine disrupting chemicals and obesity, and opens avenues for the possible use of animal models in identifying the interactions.

1. Introduction

Bisphenol A (BPA) is a highly-produced monomer in polycarbonate plastics and epoxy resins, and it is present in various consumer products, including children's toys, food and water containers and dental sealants (Geens et al., 2011). With regard to the extensive chances for exposure, the US CDC reported that BPA was detectable in > 90% of Americans with the mean concentration of 2.6 ng/mL (Calafat et al., 2008). Similarly, the detectable rate of BPA is 84% in Chinese, with a geometric mean concentration of 1.01 ng/mL (Zhang et al., 2013). In the last decades, BPA has been well established to exhibit both estrogenic and antiandrogenic effects, and it is considered as a representative endocrine disrupting chemical (EDC) (Lee et al., 2003; Richter et al., 2007; Wetherill et al., 2007; Xu et al., 2005). There is strong evidence that BPA is a prostate and testicular toxicant (Li et al., 2009; Nakamura et al., 2010; Richter et al., 2007; Salian et al., 2009a; Salian et al., 2009b; Salian et al., 2009c), but due to the lack of epidemiological studies and the variety of administration doses, times, species and strains in experimental animal studies, it is hard to summarize whether BPA is associated with other male reproductive function (Peretz et al., 2014). And effects of BPA on reproductive hormones and semen quality in different epidemiologic studies are inconsistent (Mínguez-Alarcón et al., 2016), indicating that there may be other factors interacting with BPA exposure to modify its effect on male reproduction.

Additionally, overweight and obesity also have an influence on male reproductive system. There is a negative correlation between body mass index (BMI) and semen quality, and levels of seminal reactive oxygen species (ROS) and sperm DNA fragmentation are higher in obese man than normal-weight ones (Colaci et al., 2012; Hammoud et al., 2008; Hofny et al., 2010; Martini et al., 2010; Taha et al., 2016). Despite the reported impact of obesity on reproductive function, no clear evidence claims that obesity is an independent factor contributing to male reproductive dysfunction, and divergent results about associations

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between obesity and reproductive function have been reported to date (Hammoud et al., 2008; Macdonald et al., 2013; Sermondade et al., 2013). It is likely that there are other unknown factors interacting with obesity and contributing to male reproductive damage.

Notably, there seems to be a link between BPA and obesity. It has been shown that BPA induces oxidative stress which is associated with male infertility (Chen et al., 2012a; Rahman et al., 2016; Zhang et al., 2016). Meanwhile, as a low-grade systemic inflammatory status, obesity is reported to increase oxidative stress either (Yan et al., 2009). And a previous study has found that the regulation of BPA on the testicular endocrine function is affected by diet in male rats. (Nanjappa et al., 2014). Therefore, it is plausible that there is an interaction between BPA exposure and obesity on male reproduction. In addition, metabolomics is an emerging research area for studying toxic effects and underlying mechanism of EDCs. It is well known that both BPA and obesity alter metabolism (Cabaton et al., 2013; Elliott et al., 2015), suggesting that metabolic changes may also be the link connecting BPA and obesity to male reproductive damage. Although metabolic profiles have been explored in BPA and obesity independently, there is still a need to investigate the interaction between them on male reproduction from an aspect of metabolomics.

Thus, we conducted this study to identify whether there is an interaction between BPA and obesity on semen quality in humans, an indicator of male reproductive health, and verified the finding in a mice model. Potential mechanisms were further examined by metabolomics.

2. Material and methods

2.1. Study population and sample collection

Subfertile male volunteers of the present study were invited from affiliated hospitals of Nanjing Medical University between 2013 and 2014. A questionnaire was used to collect information including personal background, lifestyle factors, occupational and environmental exposures, genetic risk factors, sexual and reproductive status, medical history and physical activity. After the study was explained and all questions were answered, a total of 412 men (approximately 82% of the total invited males) participated. We excluded 48 participants who met any of the following criteria: (1) previous diagnosis with genitourinary inflammation, epididymitis, testicular injury, cryptorchidism; (2) abnormal sexual and ejaculatory functions; (3) other known risk factors related to male reproductive dysfunction, like vascular trauma, vasectomy, chromosome abnormalities and Y chromosome microdeletions of azoospermia factor (AZF) region, and 364 men were finally recruited. Taking into account the sample size in the relevant studies, the present sample size was a larger one with sufficient power to examine interactions on semen quality (Lassen et al., 2014; Louis et al., 2015; Meeker et al., 2011; Mendiola et al., 2010; Vitku et al., 2016). All subjects claimed that their life styles and exposures had not been changed for several months before sample collection. After completion of the questionnaire, the examiner measured the height and weight of each participant. Then, subjects were requested to donate a semen sample for semen analysis and a urine sample for the measurement of urinary BPA concentrations. The urine samples were stored at −20 °C until measurement.

This study was approved by the ethics review board of Nanjing Medical University, and all experimental protocol for human were in accordance with guidelines approved by the Institutional Review Board of Nanjing Medical University. All activities involved in this study were done under full compliance with government policies and the Helsinki Declaration.

2.2. Semen analysis in humans

The semen sample was obtained in a private room in the morning by masturbation into a sterile wide-mouth glass container after a recommended 2-day abstinence. As soon as the sample liquefaction at 37 °C, semen analysis was conducted in accordance with guidelines in the World Health Organization laboratory manual for the examination and processing of human semen (WHO, 2010). The semen volume was assessed by pipetting. We used a microcell slide and computer-aided semen analysis system (CASA, WLJY 9000, Weili New Century Science and Tech Dev.) to examine the sperm concentration, total sperm motility (progressive motility + non-progressive motility) and sperm motion parameters [curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity (LIN), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF)]. Sperm count per ejaculate was calculated as sperm concentration multiplied by semen volume. Each sample must be detected by two well-trained technicians without knowledge of subject's information in case of potential bias. If the difference between two replicates was smaller than 5% [(Larger − smaller) / smaller × 100%], the average number was reported. If the difference was larger than 5%, an additional measurement was repeated with two new aliquots.

2.3. Measurement of urinary BPA concentration

We measured urinary BPA concentration using ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Waters, USA) with a sensitive method as we previously described (Chen et al., 2012c). Briefly, urine samples were incubated in 1 M ammonium acetate buffer solution (pH = 5.0) for hydrolysis with β-glucuronidase/sulfatase (20,000 units/mL) overnight. After hydrolysis, BPA was extracted and preconcentrated using solid phase extraction (SPE) (500 mg/3 mL, Supelclean, USA) and then measured with UPLC-MS/MS in the negative ion mode by multiple reaction monitoring (MRM). The limit of detection (LOD) was 0.36 ng/mL. The intra- and inter-day precisions for BPA were between 3% and 6%, and the recoveries ranged from 98% to 105% at spiked concentration of 2 and 20 ng/mL. The detection rate of BPA in the present study was 58%. Urinary creatinine (CR) concentration was collected using an automated chemistry analyzer (7020 Hitachi, Tokyo, Japan), which was used to correct the variation of BPA concentration caused by fluctuated urine concentration and dilution.

2.4. Animals and treatment

Eight-week-old male CD-1 (ICR) mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and acclimated for 1 week before the study began. The animals were housed in a temperature, humidity-controlled (23 ± 1 °C, 53 ± 2%) room and maintained in a 12 h/12 h light/dark with food and water available ad libitum. We used glass water bottles in this study to avoid potential BPA related compounds leakage from plastic water bottles.

At the age of nine weeks, mice were randomly divided into two groups (n = 24 per group) for the generation of diet-induced obese (DIO) model (Lei et al., 2007): (a) DIO mice were placed on 45 kcal% High Fat Diet (HFD; Cooperative Medical Biological Engineering Co., Ltd., China); (b) lean mice were placed on 10 Kcal% Low Fat Diet (LFD; Cooperative Medical Biological Engineering Co., Ltd., China). All mice were maintained on the same diet till the end of this study.

At the age of 15 weeks, lean and DIO mice were then divided into two subgroups (n = 12 per group) respectively and started to receive either non-additive (solvent control) or BPA-added (239,658, Sigma-Aldrich) drinking water for 7 weeks. The BPA exposure model and dosage selection were established according to the previous study (Somm et al., 2009). BPA treated mice were exposed at concentration of 0.2 mg/L in water. We estimated the mean level of BPA exposure to be approximately 25 μg/kg bw per day, which was 200-fold less than the no observed adverse effect level (NOAEL) of 5 mg/kg bw per day (FDA, 2014) and relevant to the highest aggregated exposure of 1.01 μg/kg bw per day for adults (EFSA, 2015). Additionally, this dose