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Urinary concentrations of benzophenone-type ultra violet light filters and reproductive parameters in young men

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ABSTRACT

Background: Benzophenone (BP)-type ultraviolet (UV) light filters are chemicals frequently added to personal care products, insect repellents, sunscreens, and beverage and food packaging to diminish the harmful effects of UV sunlight on human skin or foodstuffs. BP-type UV filters have shown negative effects on male reproduction function in *in vitro* and animal models, but human epidemiologic studies are limited. The goal of this study was to examine associations between urinary concentrations of BP-type UV filters and semen quality and reproductive hormone levels.

Methods: This is a cross-sectional study with 215 young university students (18–23 years old) recruited between 2010 and 2011 in Southern Spain (Murcia Region). All men provided a urine, blood and semen sample on a single day. Urinary concentrations of 2,4-dihydroxybenzophenone (BP-1); 2,2′,4,4′-tetrahydroxybenzophenone (BP-2); 2-hydroxy-4-methoxybenzophenone (BP-3); 2,2′-dihydroxy-4-methoxybenzophenone (BP-8) and 4-hydroxybenzophenone (4OH-BP) were measured by dispersive liquid–liquid microextraction and ultra-high performance liquid chromatography with tandem mass spectrometry detection. Semen quality was evaluated by measuring volume, sperm counts, motility and morphology. Serum samples were analyzed for reproductive hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), inhibin B and estradiol (E2). Associations between urinary concentrations of BP-type UV filters and semen quality parameters and reproductive hormone levels were examined using linear regression, adjusting for potential confounders.

Results: Ninety-seven percent of the men had detectable urinary concentrations of at least one of the five BP-type UV filters quantified. After adjustment for important covariates (body mass index, smoking status and time of blood sample collection), there was a significant positive association between urinary BP-1 and BP-3 concentrations and serum FSH levels ($\beta = 0.08$, 95%CI: 0.009; 0.15 and $\beta = 0.04$, 95%CI: 0.0002; 0.08, respectively). Urinary BP-1 concentration was also significantly positively associated with T/E2 ($\beta = 0.04$, 95%CI: 0.002; 0.07) and negatively with inhibin b/FSH ($\beta = -0.11$, 95%CI: -0.21; -0.006) ratio. No significant associations were found between other urinary BP-type UV filters and other reproductive hormone levels or between any semen parameters and any of the urinary BP-type UV filters quantified.

Conclusions: Our results suggest that, in young men, urinary BP-type UV filters may be associated with a modest alteration of some reproductive hormones, but the effects we report on reproductive function are likely to be

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small, and of unclear clinical significance. Further research is needed to replicate these findings in other male populations.

1. Introduction

Benzophenone (BP)-type ultraviolet (UV) light filters are chemicals coming from different sources generally added to personal care products, insect repellents and sunscreens to diminish the harmful effects of UV sunlight on human skin (Pillai et al., 2005). These compounds are also used for coating surfaces exposed to sunlight due to their UV absorption properties, including protecting colors from bleaching, photoinitiators in ink and adhesives, or food packaging and water containers, where they can leach to foodstuffs (Kawamura et al., 2003; Muncke, 2011; Suzuki et al., 2005). Widespread human exposure to BP-type UV filters is mainly through dermal contact (Jiang et al., 1999; Schlumpf et al., 2004) and dietary ingestion (Muncke, 2011), and are excreted via urine (Janjua et al., 2004, 2008). International biomonitoring studies have reported that BP-type UV filters exposure is common among different populations (USA, Germany, Switzerland), with detectable concentrations in the urine of most of the study participants (Calafat et al., 2008; CDC, 2015; Moos et al., 2014; Philippat et al., 2015; Schlumpf et al., 2010).

BP-type UV filters represent around twenty-nine different chemicals, but only a few have been examined with regard to endocrine-disrupting properties. Some BP-type UV filters have been reported to show estrogenic, anti-estrogenic and anti-androgenic effects in *in vitro* and *in vivo* studies (Ma et al., 2003; Nakagawa and Tayama, 2011; Kawamura et al., 2005; Schlumpf et al., 2001; Schreurs et al., 2005; Suzuki et al., 2005).

In animal models, mainly fish, some studies have demonstrated (Blüthgen et al., 2012; Kim et al., 2014; Weisbrod et al., 2007) a negative impact of BP-type UV filters on male reproductive function, although not all (Daston et al., 1993). However, studies examining the relationship between BP-type UV filters exposure and human reproductive function are limited (Ghazipura et al., 2017). Janjua et al. (2004) observed minor differences in testosterone levels, but not in gonadotrophins (FSH or LH), between levels measured before and two weeks after whole-body topical application of selected sunscreens in healthy volunteers. In a case-control study on male infertility, Chen et al. (2013) found no association between urinary concentrations of 2-hydroxy-4-methoxybenzophenone (BP-3) and idiopathic male infertility. Buck Louis et al. (2015), in a cohort study following US couples attempting pregnancy, reported significant inverse associations between urinary concentrations of 2,2′,4,4′-tetrahydroxybenzophenone (BP-2) and sperm concentration, motility and morphology. Recently, urinary BP-3 has been associated with significantly lower total testosterone in male adolescents (12–19 years) participating in the National Health and Nutrition Examination Survey (NHANES) 2011–2012 (Scinicariello and Buser, 2016). However, this is the first study to explore urinary concentrations of BP-type UV filters in relation to semen quality and serum reproductive hormone levels in unselected young men.

2. Material and methods

2.1. Study population

The Murcia Young Men's Study (MYMS) is a cross-sectional study of university students 18–23 years old in the Murcia Region (Southern Spain), aimed at studying the influence of environmental and lifestyle factors on reproductive parameters. The study rationale and design have been previously described in detail (Cutillas-Tolín et al., 2015; Mendiola et al., 2013; Mínguez-Alarcón et al., 2017). Briefly, a total of 215 students (90% of the total contacted) agreed to participate and completed the study visit between October 2010 and November 2011. At the study visit men underwent an andrological examination (assessment of breast, lower abdomen, testicles and penis), provided semen, urine and blood samples and completed questionnaires on general health and lifestyles. The Research Ethics Committee of the University of Murcia approved this study and written informed consent was obtained from all subjects.

2.2. Physical examination and semen analysis

Body weight and height were measured using a digital scale (Tanita SC 330-S, London, UK). Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. The presence of varicocele or other scrotal abnormalities was evaluated and recorded. Semen analyses were carried out as described in detail elsewhere (Mendiola et al., 2013). Briefly, men were asked to abstain from ejaculation for at least 48 h before sample collection by masturbation without lubrication. Abstinence time was recorded as the time between current and previous ejaculation as reported by the study subject. Ejaculate volumes were estimated by specimen weight, assuming a semen density of 1.0 g/ml. Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hauser Scientific, Inc., Horsham, PA, USA). The spermatozoa were classified as either motile or immotile (WHO, 2010) to report the percentage of motile spermatozoa [progressive (PR) and non-progressive (NP)]. Total sperm count (TSC) (volume × sperm concentration) was also calculated. Smears for morphology were made, air-dried, fixed, Papanicolaou stained and assessed using strict criteria (Menkveld et al., 1990). The same specialized biologist, that was unaware of the men's exposure status, carried out all the semen analyses. An external quality control on semen samples throughout the study period was carried out in collaboration with the University of Copenhagen's Department of Growth and Reproduction. No systematic difference was shown and the mean inter-examiner coefficient of variation was 4.0% with a range for the sets between 1.7 and 7.1%.

2.3. Hormonal analyses

Hormone analysis methods have been described previously (Asklund et al., 2007; Cutillas-Tolín et al., 2015). Briefly, blood samples were drawn from participants' cubital veins on the same time of the day of semen sample collection and were stored and frozen. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone–binding globulin (SHBG) were determined using time-resolved immunofluorometric assays (DELFIA; PerkinElmer, Skovlund, Denmark). Intra- and inter-assay variations were < 5% in each of the three assays. Serum testosterone levels were determined using a time-resolved fluoroimmunoassay (DELFIA; PerkinElmer) with intra- and inter-assay variation of < 8%. Estradiol was measured by radioimmunoassay (Pantex, Santa Monica, CA) with an intra-assay variation of < 8% and an inter-assay variation of < 13%. Inhibin b levels were determined by a specific two-sided enzyme immunometric assay (Oxford Bio-Innovation Ltd, Bicester, UK) with intra- and inter-assay variation of 13% and 18%, respectively. Free testosterone (FT) was calculated using the equation of Vermeulen et al. (1999) assuming a fixed albumin of 43.8 g/L. Hormone ratios were also calculated in order to assess potential hormonal dysregulation.