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Serum Bisphenol A Concentrations Showed Gender Differences, Possibly Linked to Androgen Levels

Toru Takeuchi* and Osamu Tsutsumi*,†,[1]

**Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan; and †CREST, Japan Science and Technology, Honcho, Kawaguchi, Saitama 332-0012, Japan*

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To investigate human exposure to bisphenol A (BPA), a widely used endocrine disruptor, we measured serum BPA concentrations and analyzed the interrelation of BPA with sex-related hormones. BPA was detected in all human sera by a novel enzyme-linked immunosorbent assay. Serum BPA concentrations were significantly higher in normal men (1.49 ± 0.11 ng/ml; $P < 0.01$) and in women with polycystic ovary syndrome (1.04 ± 0.10 ng/ml; $P < 0.05$) compared with normal women (0.64 ± 0.10 ng/ml). There were significant positive correlations between serum BPA and total testosterone ($r = 0.595$, $P < 0.001$) and free testosterone ($r = 0.609$, $P < 0.001$) concentrations in all subjects and likewise between serum BPA and total testosterone ($r = 0.559$, $P < 0.01$) and free testosterone ($r = 0.598$, $P < 0.001$) concentrations in all female subjects, but not between serum BPA and other sex-related hormone concentrations in any group. These findings showed that there are gender differences in serum BPA concentrations, possibly due to differences in the androgen-related metabolism of BPA.

Key Words: **bisphenol A; endocrine disruptor; gender difference; human; polycystic ovary syndrome; testosterone.**

Bisphenol A (2,2-bis (4-hydroxyphenyl) propane; BPA), a chemical with weak estrogenic activity, is widely used in polycarbonate plastic products, epoxy resins, polyester-styrene resins, phenolics resins, polyacrylates, dental resin composites and sealants, and the lining of food cans (1, 2). BPA has been reported to have estrogenic actions such as uterotrophic effects (3), decreased sperm production (4, 5), stimulation of prolactin release (6), and promotion of cell proliferation in a breast cancer cell line (7) by animal experiments and *in vitro* studies. However, direct evidence of human effects is lacking. In this study, we measured serum BPA concentrations in humans using a novel enzyme-linked immunosorbent assay (ELISA). Furthermore, we investigated the gender differences in serum BPA concentrations, and the relationship between BPA and sex-related hormones.

MATERIALS AND METHODS

After informed consent was obtained, fasting serum samples were collected from 14 healthy women (normal women group) in the mid-follicular phase with normal menstrual cycles, 16 women with polycystic ovary syndrome (PCOS group), and 11 healthy men (normal men group). Serum BPA concentrations were assayed with a competitive ELISA (8), which was recently developed by Otsuka Assay Laboratories and Yanaihara Institute Inc. Briefly, after a 1-ml serum sample was washed with 10% methanol, the eluate was obtained from a solid-phase column (Oasis HLB column) with the sample in 1 ml of methanol: acetonitrile (3:1 v/v), and the solvent was evaporated under a stream of nitrogen gas. An aliquot (0.2 ml) of phosphate buffer was added to the dry residue in the test tube. Fifty microliters of the sample extract and 50 μl of peroxidase-labeled BPA were placed on microtiter plates coated with a solid-phase containing rabbit anti-BPA polyclonal antibody, and incubated at room temperature for 2 h. After washing, an aliquot (0.1 ml) of *ortho*-phenylenediamine was added as a luminescence substrate and the plate was allowed to stand for 30 min. The reaction was stopped by addition of 0.1 ml 1 N H_2SO_4. The optical absorbance was measured at a wavelength of 490 nm by a microplate reader in a vertical-beam photometer. A standard curve was prepared for the analysis. The cross-reaction substances were bis (4-hydroxyphenyl) methane (0.8%), 1,1-bis (4-hydroxyphenyl) ethane (10.4%), 2,2-bis (hydroxyphenyl) butane (40.9%), bis (hydroxymethylphenyl) propane (1.6%), and other related substance (<0.1%). The intra- and interassay coefficients of variance were 7.7 and 9.7%. A significant correlation ($r = 0.971$) was confirmed between the BPA values obtained from the HPLC analysis and ELISA (8). Serum total and free testosterone, estradiol, androstenedione and dehydroepiandrosterone sulfate were assayed with commercial ^{125}I-RIA kits (DPC Co., Los Angeles, CA). Serum LH, FSH, and prolactin were assayed with ^{125}I-immunoradiometric assay (IRMA) kits (Daiichi Radioisotope Co., Tokyo, Japan). All assays were done in duplicate. The intra- and interassay coefficients of variation were less than 10% in all assays. Statistical analyses among the groups were performed by analysis of variance (ANOVA) and the least significant difference test. Correlation coefficients were calculated by linear regression analysis. Significance was determined as $P < 0.05$.

[1] To whom correspondence and reprint requests should be addressed at Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Fax: +81-3-3816-2017. E-mail: osamut-tky@umin.ac.jp.

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RESULTS

Serum BPA concentrations were significantly higher in the normal men group (1.49 ± 0.11 ng/ml; i.e., 6.5 ± 0.5 nM) than in the normal women group (0.64 ± 0.10 ng/ml; i.e., 2.8 ± 0.4 nM). Moreover, the serum BPA concentrations in the PCOS group, which was characterized by hyperandrogenism, were also significantly higher than in the normal women group (Table 1).

Table 2 shows the correlation coefficients between the serum BPA, hormone concentrations, age, and BMI. There were significant positive correlations between the serum BPA and total testosterone ($r = 0.595$, $P < 0.001$) and free testosterone ($r = 0.609$, $P < 0.001$) concentrations in all the subjects and likewise between serum BPA and total testosterone ($r = 0.559$, $P < 0.01$) and free testosterone ($r = 0.598$, $P < 0.001$) concentrations in all female subjects, but not between serum BPA and other sex-related hormone concentrations in any group.

DISCUSSION

It was of interest that BPA could be detected in all human serum samples, and that the serum BPA concentrations were significantly higher in the normal men group than in the normal women group. Of note, these BPA levels were higher than the level (1 nM) reported to affect preimplantation development (9). To our knowledge, this is the first direct examination of serum BPA concentrations in humans by ELISA, and the first detection of gender differences. We considered that there are only two explanations for the findings: stimulation of testosterone production by BPA, or suppression of the metabolism of BPA by testosterone. Metabolic variation is also implicated in the difference in serum BPA concentrations. BPA is known to be glucuronidated by liver microsomes and catalyzed by an isoform of uridine diphosphate–glucuronosyl transferase (UGT), then rapidly excreted in the feces and urine (10). It was reported that the level of UGT activity and transcripts was down-regulated by androgens (11). On the other hand, it was reported that BPA significantly decreased testosterone 2α-hydroxylase (T2AH) and testosterone 6β-hydroxylase (T6BH) activities, which are cytochrome P450 isoforms, and also decreased CYP2C11/6 and CYP3A2/1 protein levels in rat liver (12). Thus, BPA might also affect the metabolism of testosterone hydroxylation, and a vicious cycle may be created between BPA and testosterone. Moreover, since BPA was identified as a potent sex hormone-binding globulin (SHBG)–ligand (13), it might also displace endogenous sex steroid hormones from SHBG binding sites and disrupt the androgen-to-estrogen balance.

In conclusion, we have shown that there are gender differences in serum BPA concentrations, possibly due to the difference in androgen-related enzyme activity levels, and the findings in this study may provide some insight into the metabolism of endocrine disruptors in humans.

TABLE 1

Serum Bisphenol A (BPA) and Hormone Concentrations in Normal Women and Men and Women with Polycystic Ovary Syndrome (PCOS)

	Normal women (n = 14)	Normal men (n = 11)	PCOS (n = 16)
Age (years)	28.7 ± 0.7	29.4 ± 1.1	25.7 ± 1.4
BMI (kg/m^2)	19.4 ± 0.3	22.4 ± 0.9	21.2 ± 1.1
BPA (ng/ml)	0.64 ± 0.10	1.49 ± 0.11**	1.04 ± 0.10*,***
LH (mIU/ml)	4.8 ± 0.3	4.1 ± 0.5	13.8 ± 1.1**,***
FSH (mIU/ml)	7.4 ± 0.5	4.2 ± 0.3**	6.6 ± 0.6
E_2 (pg/ml)	45.6 ± 4.1	26.1 ± 6.1*	65.0 ± 8.8***
Total T (ng/ml)	0.20 ± 0.02	4.58 ± 0.36**	0.68 ± 0.04**,***
Free T (pg/ml)	0.72 ± 0.10	21.35 ± 2.09**	2.01 ± 0.22**,***
A (ng/ml)	1.85 ± 0.06	1.88 ± 0.21	2.82 ± 0.16**,***
DHEAS (μg/ml)	1.67 ± 0.15	2.43 ± 0.33*	2.33 ± 0.29
PRL (ng/ml)	6.9 ± 0.7	5.6 ± 1.1	5.6 ± 0.6

Note. Data are means ± SEM. BMI, body mass index; T, testosterone; A, androstenedione; DHEAS, dehydroepiandrosterone sulfate.
* $P < 0.05$, ** $P < 0.01$, compared with normal women.
*** $P < 0.01$, compared with normal men.

TABLE 2

Correlation Coefficients between BPA and Variables in All Women and in All Subjects

	All women		All subjects	
BPA vs	r	P	r	P
Age	−0.302	NS	−0.135	NS
BMI	0.302	NS	0.319	NS
LH	0.333	NS	0.028	NS
FSH	−0.091	NS	−0.343	NS
E_2	−0.019	NS	−0.228	NS
Total T	0.559	<0.01	0.595	<0.001
Free T	0.598	<0.01	0.609	<0.001
A	0.396	NS	0.141	NS
DHEAS	0.203	NS	0.112	NS
PRL	−0.129	NS	−0.108	NS

Note. NS, not significant.

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