Endocrine Journal 2004, **51** (2), 165–169

Positive Relationship between Androgen and the Endocrine Disruptor, Bisphenol A, in Normal Women and Women with Ovarian Dysfunction

TORU TAKEUCHI*,**, OSAMU TSUTSUMI*,**, YUMIKO IKEZUKI*,**, YASUSHI TAKAI*,** AND YUJI TAKETANI*

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

Abstract. This study was performed to investigate the serum levels of bisphenol A (BPA), an endocrine disruptor, in women with ovarian dysfunction and obesity. Fasting serum samples were obtained from 19 non-obese and 7 obese women with normal menstrual cycles: 7 patients with hyperprolactinemia, 21 patients with hypothalamic amenorrhea, and 13 non-obese and 6 obese patients with polycystic ovary syndrome (PCOS). BPA was measured by an enzyme-linked immunosorbent assay. BPA was detected in all human sera. Serum BPA concentrations were significantly higher in both non-obese and obese women with polycystic ovary syndrome (1.05 ± 0.10 ng/ml, 1.17 ± 0.16 ng/ml; $p<0.05$, respectively) and obese normal women (1.04 ± 0.09 ng/ml, $p<0.05$) compared with those in non-obese normal women (0.71 ± 0.09 ng/ml). There was no difference among women with hyperprolactinemia, women with hypothalamic amenorrhea, and non-obese normal women. There were significant positive correlations between serum BPA and total testosterone ($r = 0.391$, $p<0.001$), free testosterone ($r = 0.504$, $p<0.001$), androstenedione ($r = 0.684$, $p<0.001$), and DHEAS ($r = 0.514$, $p<0.001$) concentrations in all subjects. These findings show that there is a strong relationship between serum BPA and androgen concentrations, speculatively due to the effect of androgen on the metabolism of BPA.

Key words: Bisphenol A, Polycystic ovary syndrome, Hyperprolactinemia, Hypothalamic amenorrhea, Obesity
(*Endocrine Journal* **51**: 165–169, 2004)

VARIOUS environmental chemicals are known to act as endocrine disruptors, which have been reported to exhibit estrogenic, anti-estrogenic and/or anti-androgenic actions in wildlife and human beings, causing a menace to living things. There is increasing concern about the implication of environmental chemicals in a number of human health disorders: testicular cancer, falling sperm count, endometriosis, precocious puberty, and breast cancer. Nevertheless, reliable evidence of the injurious influence of endocrine disruptors on human health has yet to be established.

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, phenolic 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], promotion of cell proliferation in a breast cancer cell line [7], and influence on preimplantation development [8], by animal experiments and *in vitro* studies. It was also reported that the activities of BPA depended on the mechanism via estrogen receptors (ERα and ERβ) [9, 10]. Recently, it was reported that there is human fetal exposure to BPA via amniotic fluid and placenta [11]. We previously reported that there was a gender difference in serum BPA concentrations in humans [12]. However, direct evidence of human effects is lacking. In this study, we measured serum BPA concentrations in humans and investigated the relationship between serum sex hor-

Received: October 17, 2003
Accepted: December 22, 2003
Correspondence to: Osamu TSUTSUMI, M.D., Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

mones and BPA concentrations in women with ovarian dysfunction and obesity.

Materials and Methods

After informed consent was obtained, fasting serum samples were collected from 19 non-obese and 7 obese healthy women in the midfollicular phase with normal menstrual cycles, 7 patients with hyperprolactinemia, 21 patients with hypothalamic amenorrhea and 13 non-obese and 6 obese patients with polycystic ovary syndrome (PCOS). Serum samples were obtained between 0900 and 1000 h from all subjects after overnight fasting. All sera were stored at –30°C until assayed.

Hyperprolactinemia was diagnosed by the presence of amenorrhea, elevated levels of prolactin (PRL >15 ng/ml). Prolactinoma, drug-induced hyperprolactinemia and hypothyroidism were excluded. Hypothalamic amenorrhea was diagnosed by the following criteria: a history suggestive of anovulation induced by psychogenic stressors such as emotional, familial or work problems, weight loss such as reduced food intake, absence of depression or psychiatric diseases, normal or slightly reduced luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, and an LH/FSH ratio <1. Polycystic ovary syndrome was diagnosed by the presence of oligomenorrhea or amenorrhea, elevated levels of LH with normal levels of FSH, an LH/FSH ratio >1, elevated testosterone (T) levels (>0.5 ng/ml), and a characteristic appearance of the ovaries on ultrasound images. All women in this study had normal thyroid hormone levels, and none were taking medication.

Serum BPA concentrations were assayed with a competitive ELISA [12, 13]. The intra- and inter-assay 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 the ELISA [13]. Serum total and free T, estradiol (E_2), androstenedione (A) and dehydroepiandrosterone sulfate (DHEAS) were assayed with commercial ^{125}I-RIA kits (DPC Co., Los Angeles, CA, USA). Serum LH, FSH and PRL were assayed with ^{125}I-immunoradiometric assay (IRMA) kits (Daiichi Radioisotope Co., Tokyo, Japan). Serum insulin was assayed with an ^{125}I-RIA kits (Pharmacia, Sweden). All assays were done in duplicate. All results obtained in this study were expressed as the mean ± the standard error of the mean (SEM). The intra- and inter- assay 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 $p<0.05$.

Results

The clinical data and fasting endocrine values are listed in Table 1. Obesity was defined by a body mass

Table 1. Clinical data and fasting endocrine values in normal women, patients with hyperprolactinemia, hypothalamic amenorrhea, and PCOS.

	Normal women		Hyperprolactinemia (n = 7)	HA (n = 21)	PCOS	
	Non-obese (n = 19)	Obese (n = 7)			Non-obese (n = 13)	Obese (n = 6)
Age (years)	27.5 ± 0.7	28.8 ± 2.0	27.7 ± 2.6	25.1 ± 1.0	26.5 ± 1.5	24.7 ± 1.9
BMI (kg/m^2)	19.7 ± 0.3	28.5 ± 1.7^b	20.8 ± 1.0	19.2 ± 0.6	19.1 ± 0.6	31.3 ± 3.0^b
LH (mIU/ml)	4.7 ± 0.3	4.1 ± 0.4	3.9 ± 1.5	3.4 ± 0.6^a	15.2 ± 1.7^b	11.8 ± 1.3^b
FSH (mIU/ml)	7.3 ± 0.5	6.4 ± 0.8	6.1 ± 0.8	5.5 ± 0.6^a	7.2 ± 0.7	5.7 ± 0.6
E_2 (pg/ml)	49.9 ± 6.1	41.3 ± 7.6	26.3 ± 4.8^b	28.7 ± 5.5^a	57.2 ± 8.6	73.1 ± 15.3
Total T (ng/ml)	0.20 ± 0.01	0.28 ± 0.05	0.27 ± 0.07	0.22 ± 0.02	0.63 ± 0.03^b	0.73 ± 0.07^b
Free T (pg/ml)	0.76 ± 0.09	1.53 ± 0.28^b	1.03 ± 0.24	0.68 ± 0.09	1.86 ± 0.24^b	2.77 ± 0.50^b
A (ng/ml)	1.75 ± 0.07	1.65 ± 0.05	2.47 ± 0.19^b	1.67 ± 0.14	2.82 ± 0.18^b	2.68 ± 0.29^a
DHEAS (μg/ml)	1.59 ± 0.13	1.76 ± 0.34	2.15 ± 0.24	1.64 ± 0.12	2.26 ± 0.30^a	2.80 ± 0.68^a
PRL (ng/ml)	6.9 ± 0.6	6.1 ± 1.0	20.8 ± 1.0^b	5.6 ± 0.5	6.5 ± 0.9	5.1 ± 0.9
Insulin (μU/ml)	6.0 ± 0.7	8.7 ± 2.1	6.1 ± 0.8	5.4 ± 0.5	7.6 ± 1.8	14.9 ± 3.7^b

Data are means ± SEM. ^{a}P<0.05, ^{b}P<0.01; compared with non-obese normal control group.

HA: hypothalamic amenorrhea, PCOS: polycystic ovary syndrome, BMI: body mass index, LH: luteinizing hormone, FSH: follicle-stimulating hormone, E_2: estradiol, T: testosterone, A: androstenedione, DHEAS: dehydroepiandrosterone sulfate, PRL: prolactin

index (BMI) ≥25 kg/m^2. There was no significant difference in the mean age among the study groups. The serum levels of LH, total T, free T, A, and DHEAS were significantly higher in the non-obese and obese PCOS groups than in the non-obese normal control group. The serum levels of LH, FSH and E_2 were significantly lower in the hypothalamic amenorrhea group than in the non-obese normal control group. The serum levels of PRL and A were significantly higher, but the serum levels of E_2 were significantly lower in the hyperprolactinemia group than in the non-obese normal control group. Free T levels were significantly higher in the obese normal control group than in the non-obese normal control group. Fasting insulin levels were significantly higher in the obese PCOS group than in the non-obese normal control group. Moreover, the insulin levels showed a tendency to increase in the obese normal control group and the non-obese PCOS group compared with the non-obese normal control group.

Serum BPA concentrations were significantly higher in both non-obese and obese women with PCOS (1.05 ± 0.10 ng/ml, 1.17 ± 0.16 ng/ml; p<0.05, respectively) and obese normal women (1.04 ± 0.09 ng/ml, p<0.05) compared with those in non-obese normal women (0.71 ± 0.09 ng/ml). There was no difference among women with hyperprolactinemia (0.83 ± 0.12 ng/ml), hypothalamic amenorrhea (0.84 ± 0.10 ng/ml), and non-obese normal women (Fig. 1).

Table 2 shows the correlation coefficients for BPA with age, BMI and hormone concentrations. There were significant positive correlations between BPA and BMI (r = 0.500, p<0.001), total testosterone (r = 0.391, p<0.001), free testosterone (r = 0.504, p<0.001), androstenedione (r = 0.684, p<0.001, and DHEAS (r = 0.514, p<0.001) in all the subjects, but not between the serum BPA and other hormone concentrations. Moreover, in the normal control group there were significant positive correlations between BPA and BMI (r = 0.564, p<0.01), free testosterone (r = 0.478, p<0.05), androstenedione (r = 0.627, p<0.01), and DHEAS (r = 0.455, p<0.05).

Table 2. Correlation coefficients between BPA and variables in all the women.

	BPA	p
Age	0.137	NS
BMI	0.500	P<0.001
LH	0.199	NS
FSH	0.174	NS
E_2	0.162	NS
Total T	0.391	P<0.001
Free T	0.504	P<0.001
A	0.684	P<0.001
DHEAS	0.514	P<0.001
PRL	0.090	NS
Insulin	0.213	NS

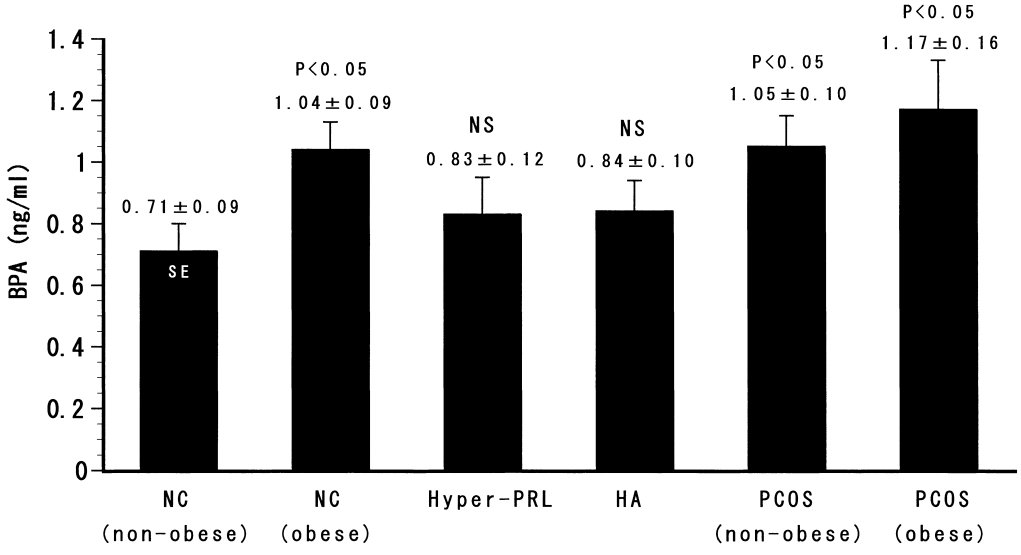

Fig. 1. Comparison of serum bisphenol A (BPA) concentrations among the study groups. Serum BPA concentrations were significantly higher in both non-obese and obese women with PCOS and obese normal women compared with those in non-obese normal women. There was no difference among women with hyperprolactinemia, women with hypothalamic amenorrhea, and non-obese normal women.

Discussion

To our knowledge, this is the first report of serum BPA concentrations in women with or without ovarian dysfunction and obesity. The present study showed that BPA could be detected in all human serum samples, and that the serum BPA concentrations were significantly higher in both non-obese and obese PCOS groups than in the non-obese normal control group. The serum BPA concentrations, moreover, were significantly higher in the obese normal control group than in the non-obese normal control group, with the result that there was a significant positive correlation between BPA levels and BMI. The serum BPA concentrations were also significantly correlated with serum androgen concentrations in all the subjects. It is generally known that obesity is associated with peripheral insulin resistance and hyperinsulinemia [14]. Since hyperinsulinemia has a direct inhibitory effect on hepatic sex hormone-binding globulin (SHBG) production [14], free T, a biologically active fraction, is higher in obese than in non-obese women (Table 1). The present findings suggest that BPA concentrations in blood are influenced by a hyperandrogenic environment. Our previous study showed that serum BPA concentrations were significantly higher in normal men compared with those in normal women [12]. We speculated that the gender difference in serum BPA concentrations was due to differences in the androgen-related metabolism of BPA. Actually, the present study shows that not only in men but also in hyperandrogenic women serum BPA concentrations are higher than in non-obese normal women.

There are possible explanations for the findings: stimulation of androgen production by BPA, or the differences in intake, metabolism, or excretion of BPA. Metabolic variation is also implicated in the difference in serum BPA concentrations. BPA is known to be glucuronidated by liver microsomes and catalysed by an isoform of uridine diphosphate-glucuronosyl transferase (UGT), and then rapidly excreted in the feces and urine [15]. There are several lines of evidence to indicate that the metabolism of BPA is influenced by androgen. It was reported that the level of UGT activity and transcripts was down-regulated by androgen [16]. The difference in metabolism of BPA thus seems to be the greatest possible cause with the metabolism of BPA being suppressed by androgen. Free T, which is the best parameter of androgenicity, is significantly higher in the obese normal control group and the non-obese and obese PCOS groups than in the non-obese normal control group. Accordingly, the glucuronidation of BPA might be suppressed in these groups under the hyperandrogenic environment.

On the other hand, it was reported that BPA significantly decreased the activity of testosterone 2α-hydroxylase (T2AH) and testosterone 6β-hydroxylase (T6BH), which are cytochrome P450 isoforms, and also decreased CYP2C11/6 and CYP3A2/1 protein levels in rat livers [17]. Thus, BPA might also affect the metabolism of testosterone hydroxylation, creating a vicious cycle between BPA and androgen. Recently, it was reported that BPA treatment decreased UGT activities toward sex hormones and BPA in the livers of male rats, but not of female rats [18]. Moreover, since BPA was identified as a potent sex hormone-binding globulin (SHBG)-ligand [19], it might also displace endogenous sex steroid hormones from SHBG binding sites and disrupt the androgen-to-estrogen balance.

We have shown that there is a strong relationship between serum BPA and androgen concentrations, speculatively due to the influence of androgen on the metabolism of BPA. The findings in the present study may provide some insights into the metabolism of endocrine disruptors in humans and the pathophysiology of endocrine disorders such as PCOS.

Acknowledgements

This work was supported in part by grants-in-aid from the Ministry of Education, Science, Sports and Culture, from the Ministry of Health, Labour and Welfare, from the National Institute for Environmental Studies, and from the Science and Technology Agency, Japan.

References

1. Feldman D (1997) Editorial: Estrogens from plastic —Are we being exposed? *Endocrinol* 138: 1777–1779.
2. Noda M, Komatsu H, Sano, H (1999) HPLC analysis of dental resin composites components. *J Biomed Mater*

Res 47: 374–378.
3. Ashby J, Tinwell H (1998) Uterotrophic activity of bisphenol A in the immature rat. *Environ Health Perspect* 106: 719–720.
4. Nikula H, Talonpoika T, Kaleva M, Toppari J (1999) Inhibition of hCG-stimulated steroidogenesis in cultured mouse leydig tumor cell by bisphenol A and octylphenols. *Toxicol Appl Pharmacol* 157: 166–173.
5. Von Saal F, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nage SC, Stetano P, Welshons WV (1998) A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol Indust Health* 14: 239–260.
6. Steinmetz R, Brown NG, Allen DL, Bigsby RM, Ben-Jonathan N (1997) The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo. *Endocrinol* 138: 1780–1786.
7. Krishman AV, Stathis P, Permuth S, Tokes L, Feldman D (1993) Bisphenol A : An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinol* 132: 2279–2286.
8. Takai Y, Tsutsumi O, Ikezuki Y, Kamei Y, Osuga Y, Yano T, Taketani Y (2001) Preimplantation exposure to bisphenol A advances postnatal developement. *Reprod Toxicol* 15: 71–74.
9. Hiroi H, Tsutsumi O, Momoeda M, Takai Y, Osuga Y, Taketani Y (1999) Differential interactions of bisphenol A and 17β-estradiol with estrogen receptorα (ERα) and ERβ. *Endocr J* 46: 773–778.
10. Kurosawa T, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fujiwara T, Inoue S, Muramatsu M, Momoeda M, Taketani Y (2002) The activity of bisphenol A depends on both the estrogen receptor subtype and cell type. *Endocr J* 49: 465–471.
11. Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y (2002) Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum Reprod* 17: 2839–2841.
12. Takeuchi T, Tsutsumi O (2002) Serum bisphenol A concentrations showed gender diffeerences, possibly linked to androgen levels. *Biochem Biophys Res Commun* 291: 76–78.
13. Kodaira T, Kato I, Li J, Mochizuki T, Hoshino M, Usuki Y, Oguri H, Yanaihara N (2000) Novel ELISA for the measurement of immunoreactive bisphenol A. *Biomed Res* 21: 117–121.
14. Peris AN, Sothmann MS, Aiman EJ, Kissebah AH (1989) The relationship of insulin to sex hormone-binding globulin: role of adiposity. *Fertil Steril* 52: 69–72.
15. Yokota H, Iwano H, Endo M, Kobayashi T, Inoue H, Ikushiro S (1999) Glucuronidation of the environmental oestrogen bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver. *Biochem J* 340: 405–409.
16. Guillemette C, Levesque E, Beaulieu M, Turgeon D, Hum DW, Belanger A (1997) Differential regulation of two uridine diphospho-glucuronosyltransferases, UGT2B15 and UGT2B17, in human prostate LNCaP cells. *Endocrinol* 138: 2998–3005.
17. Hanioka N, Jinno H, Nishimura T, Ando M (1998) Suppression of male-specific cytochrome P450 isoforms by bisphenol A in rat liver. *Arch Toxicol* 72: 387–394.
18. Shibata N, Matsumoto J, Nakada K, Yuasa A, Yokota H (2002) Male-specific suppression of hepatic microsomal UDP-glucuronosyltransferase activity toward sex hormones in the adult male rat administered bisphenol A. *Biochem J* 368: 783–788.
19. Déchaud H, Ravard C, Claustrat F, de la Perrière AB, Pugeat M (1999) Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). *Steroids* 64: 328–334.