Original Article

An Association of Elevated Serum Prolactin with Phthalate Exposure in Adult Men*

LI ShuGuang[1,Δ], DAI JiCan[2,Δ], ZHANG LiQian[1], ZHANG Jing[3], ZHANG ZiQiang[3], and CHEN Bo[1,#]

1.School of Public Health, Fudan University, Shanghai 200032, China; 2.Department of Urology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200001, China; 3.Tongji University Medical School, Shanghai 200092, China

Abstract

Objective To investigate the associations of hormone circulation with phthalate exposure in adult men.

Methods Semen and serum samples were collected from 118 men who were suspected of infertility. Phthalate diesters including dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) in both semen and serum samples were measured, along with serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estradiol (E_2) and prolactin (PRL).

Results Serum PRL was positively associated with serum DBP and DEHP and semen DEHP in all models of Spearman correlation, linear regression and binary logistic regression. In linear regression models adjusted for potential confounders and excluding subjects with undetectable phthalates, a 10-fold increase in semen DEHP was associated with a 23% increase in serum PRL, as well as a 26% increase in serum DBP and a 20% increase in serum DEHP. In logistic regression models all subjects demonstrated a dose-response relationship between above reference value PRL and semen DEHP (odds ratio per tertile adjusted for potential confounders = 1.0, 1.70, 3.50; *P* for trend = 0.01), and serum DBP (1.0, 1.10, 2.62; *P* for trend = 0.04), and serum DEHP (1.0, 1.46, 4.69; *P* for trend < 0.01). A positive correlation between serum estradiol and semen DEHP (linear regression), and an inverse correlation between semen DBP and serum testosterone and T:E_2 ratio (Spearman correlation) were also established.

Conclusions Serum PRL is suggested to be positively associated with both DBP and DEHP exposure in adult men.

Key words: Phthalate diesters; Prolactin; Testosterone; Estradiol; Male reproduction

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INTRODUCTION

Phthalates are a group of chemicals that are mainly used as plasticizers to soften polyvinyl chloride (PVC). As phthalate plasticizers are not chemically bound to PVC, they can leach, migrate or evaporate into air and atmosphere, foodstuffs and other materials[1]. As a result of their ubiquitous use, the general population was significantly exposed. The most widely used phthalate plasticizers are diethylhexyl phthalate (DEHP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and dibutyl phthalate (DBP). In recent years, Western Europe and North America

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ΔLI ShuGuang and DAI JiCan contributed equally to this work.

#Correspondence should be addressed to Dr CHEN Bo, Tel: 86-21-54237229; Fax: 86-21-64178160; E-mail: chenb@fudan.edu.cn

Biographical note of the first author: LI ShuGuang, male, born in 1955, professor and PhD, majoring in nutrition and food hygiene; DAI JiCan, male, born in 1968, associate chief physician and PhD, majoring in andrology.

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have started replacing highly toxic phthalates (DEHP, DBP, etc.) with less toxic phthalates (DINP, DIDP, etc.)[1-3]. However, the Chinese market is still dominated by DEHP and DBP due to their lower cost. In 2002, China produced and imported 772 783 tons of DEHP, 137 002 tons of DBP and DIBP (di-iso-butyl phthalate), but only 62 314 tons of DINP and DIDP[4]. Meanwhile, of over 800 000 tons of phthalates used in Western Europe in 2003, DEHP accounted for 24% and DINP and DIDP accounted for over 50%[1]. As China is the largest phthalates producer and consumer in the world, occupational and environmental exposure to phthalates and their adverse health effects in the Chinese population constitute a major concern.

Phthalates are chemicals with suspected endocrine-disrupting effects in animals and humans. Animal studies have shown that some phthalates can cause testicular toxicity in prenatal or perinatal exposure windows[5-6], as well as in pubertal or adult exposure windows[7-8]. *In vitro* studies have suggested that some phthalates are hormonally active[9-10]. Recent studies on humans have found that the metabolites of some phthalates, especially DBP and DEHP, are associated with altered semen quality[11-16] and reproductive hormones[17-21]. These studies have provided evidences that some endocrine-disrupting compounds (EDCs) may lead to declined reproductive capacity or possibly increased risk to testicular or prostate cancer in men and to ovarian or breast cancer in women[22-23]. Although the underlying biological mechanisms remain unclear, altered hormone levels may lead to infertility or cancer, which results possibly from environmental or occupational exposure to EDCs.

It is only recently that phthalates were reported to be associated with altered steroid hormone levels[17-21]. A Danish/Finnish cooperative cohort study in infants reported positive correlations between monoethyl phthalate (MEP , a metabolite of diethyl phthalate) and monobutyl phthalate (MBP, a metabolite of DBP) in breast milk with serum sex hormone binding globulin (SHBG), and between monomethyl phthalate (MMP, a metabolite of dimethyl phthalate), MEP and MBP in milk with luteinizing hormone (LH):free testosterone (T) ratio, and between monoisononyl phthalate (MINP, a metabolite of DINP) and serum LH[19]. The study also reported an inverse association of MBP with free T. A cross-sectional study in Chinese adult men reported decreased serum free T levels in workers exposed to high levels of DBP and DEHP[21], while a Swedish study in young men who underwent medical examination before military service reported an inverse correlation between urinary MEP and serum LH[18]. An American study conducted in men seeking treatment from an infertility clinic found an inverse correlations between urinary monoethylhexyl phthalate (MEHP, a metabolite of DEHP) and serum testosterone, estradiol (E_2), and free androgen index (FAI)[20]. Meanwhile, this study also reported that the T:E_2 ratio was positively associated with MEHP and the proportion of DEHP metabolites in the urine measured as MEHP (MEHP%). The findings of all these studies suggested the declining trend in testosterone levels among adult males may be attributed to environmental or occupational exposure to phthalates.

In this study, we evaluated the effects of environmental exposure to DBP and DEHP on the circulating hormones including follicle stimulating hormone (FSH), LH, testosterone, estradiol, prolactin (PRL), T:LH ratio (a measure of Leydig cell function) and T:E_2 ratio (a measure of aromatase activity). The study, which was based on the hypothesis that phthalates might lead to hormone disturbance and consequently result in male infertility, reported a clear association of serum PRL with phthalates exposure.

MATERIALS AND METHODS

Subjects

The study was approved by the Ethics Committee of School of Public Health at Fudan University. All the participants gave written informed consents. They were seeking medical treatment from the Reproductive Medical Center at RenJi Hospital in Shanghai in 2007-2008 with a complaint of suspected infertility. They were required to provide a semen sample (2 mL) for phthalates measurement, and a serum sample (5 mL) for the analysis of serum phthalates and reproductive hormones, and to fill out a questionnaire which included age, educational background, and smoking and drinking habits. Height and weight were measured to calculate the body mass index (BMI). Of the 187 recruited men, 139 agreed to participate in the study, 11 lost their semen samples and 7 lost their serum samples in the process of phthalates determination, 3 currently took hormone medications, and consequently the final sample size was comprised of 118 subjects. Among the 48 subjects who disagreed to participate, only their age details were available and no difference was found. The other 21 excluded subjects had higher values in BMI and FSH, but were similar in other demographic characteristics and serum hormones.

Phthalate Esters in Semen and Serum Samples

Semen and serum samples were stored at -80℃ until analysis. Determination of phthalate esters including DBP and DEHP was performed using the selective ion recording mode (SIR) of capillary column gas chromatography-mass spectrometry (GC/MS) coupled with organic solvent extraction according to Cai *et al*.[24] with minor modifications as described as follows. Defrosted semen samples were centrifuged at 2 000 g for 5 minutes and 500 μL of the upper aliquot layer was added into skellysolve B (0.8 mL). The mixture was then vortexed for 5 min and centrifuged at 4 ℃ at 2 000 g for 5 min. The organic phase was collected and the aqueous phase was extracted twice again with skellysolve B solvent. The combined organic phase was evaporated completely under nitrogen and the residue was redissolved in 2 mL of skellysolve B. The sample extract was filtered through a 0.45 μm glass-fiber filter into an autosampler vial for analysis. Pretreatment of serum samples was similar to the semen samples. 500 μL defrosted serum samples were extracted, evaporated, and redissolved following the same procedure mentioned above. Analysis of phthalate esters was accomplished using HP 5890 GC and HP 5972 MS (Hewlett-Packard, USA). The instrument was operated in EI +, SIR mode at 70 eV. The capillary column used was an HP-FFAP (30 m × 0.25 mm i. d., film thickness 0.25 μm; Hewlett-Packard, USA). Conditions were as follows: helium was used as carrier gas at a linear flow of 2.32 mL/min; column temperature was held at 150 ℃ for 2 min and then increased at 20 ℃/min to 300 ℃ and maintained at 300 ℃ for 1 min; the injector temperature was 280 ℃. DBP and DEHP were identified by comparison of retention times and spectra of reference standard compounds. Samples were quantified using the ions *m/z* 149 for both DBP and DEHP. The analyzing procedure had a detection limit of 1 ng for DBP and DEHP each. Because phthalates are ubiquitous contaminants, only glass wares were used during the sampling and analyzing process. All glass wares were washed carefully, rinsed with skellysolve B twice, and washed thoroughly with redistilled water again before being used. Semen and serum samples for phthalates measurement were transferred to glass containers immediately after drawing from subjects.

Serum Hormones

Blood samples were drawn before semen samples. Measurement of serum hormones and semen quality was done in the hospital within 2 hours after sample collection in the morning. Serum levels of FSH, LH, testosterone, estradiol, and PRL were measured by commercial radioimmunoassay kits (Bluegene Biotech CO. LTD. Shanghai, China). The reference values for the determinations provided by the hospital were 1.3-11.8 IU/L, 2.8-6.8 IU/L, 2.6-7.4 ng/mL, 0-56 pg/mL, and 4.1-18.5 ng/mL, respectively. The T:LH ratio, a measure of Leydig cell function, was calculated by dividing testosterone (ng/mL) by LH (IU/L). The T:E_2 ratio, a measure of aromatase activity, was calculated by dividing testosterone (ng/mL) by E_2 (pg/mL).

Statistic Analyses

Data analysis was performed using STATA/SE 8.0 for windows. All statistic tests were two sided (α = 0.05). The distribution of phthalate esters and reproductive hormones was given as medians, geometric means (GMs) (or arithmetic means, AMs), and percentiles (5, 25, 75, and 95th). Concentrations of LH and testosterone closely approximated normality and were used in statistical models untransformed, while the distribution of FSH, testosterone, estradiol, PRL, T:LH, and T:E_2 were skewed left and transformed by the natural logarithm for statistic analyses. In preliminary data analysis, hormone and phthalate ester concentrations were stratified by demographic categories to investigate the potential for confounding. Spearman correlation and multivariable linear regressions in stepwise models were then used to explore the associations between phthalate and hormone concentrations. In the linear regression models, age and BMI were included as a continuous variable, educational background was dichotomized by college or above versus high-school or below, current smoking status was trichotomized as non-smoker, moderate smoker (not higher than 10 cigarettes per day, ≤ 10 cig/day), and heavy smoker (> 10 cig/day), and current alcohol consumption habit was trichotomized as non-drinker, moderate drinker (not higher than 2 times of servings per week, ≤ 2 times/week), and heavy drinker (>2 times/week). Phthalate esters were undetectable in 9.3-36.4 percent of all samples. For these samples, an imputed value equal to one-half of the LOD was used. In the Spearman correlation analyses, linear regression models and binary logistic models, samples with undetectable phthalates were either excluded or included when exploring the associations between

hormones and phthalates. In the logistic models, hormones were dichotomized according to the reference values, and phthalates were trichotomized according to the tertile cut-points.

RESULTS

Subjects' Demographics

The $\bar{x}$ (±s) age and BMI of the 118 subjects was 30.40±4.23 years and 24.21±3.08 kg/m^2, respectively. Other demographic information of the subjects was given in Table 1. The larger proportion of them had high schooling or below (63%), and were current non-smokers (72%) and non-drinkers (83%).

Table 1. Subjects' Demographics (n=118)

Parameters	n(%)
Educational Background	
High-school or below	74(63)
College or above	44(37)
Smoking Status (cig/day)	
Non-smoker	85(72)
Moderate Smoker (⩽10)	13(11)
Heavy Smoker (> 10)	20(13)
Alcohol Consumption (times/week)	
Non-drinker	98(83)
Moderate Drinker (⩽2)	13(11)
Heavy Drinker (> 2)	7(6)

Phthalates' and Hormones' Distributions in Subjects and Their Respective Inner Correlation

Distributions of phthalate and hormone concentrations are presented in Table 2. Among the 118 subjects, DBP was detected in 64 percent of the semen samples (75 subjects) and 74 percent of the serum samples (87 subjects), and DEHP was detected in 71 percent of the semen samples (84 subjects) and 91 percent of the serum samples (107 subjects). Semen DBP was correlated to serum DBP with the spearman coefficient of 0.61, and semen DEHP correlated to serum DEHP with the coefficient of 0.71 (Data not shown). Hormones were detectable in all the samples. Significant spearman correlations between FSH and LH concentrations were 0.59, and between estradiol and PRL - 0.37 (Data not shown). T:LH ratio was correlated to testosterone or LH, respectively. T:E_2 ratio was correlated to testosterone or estradiol, respectively.

Hormones' Associations with Personal Demographics and Phthalates by Spearman Correlation Analyses

One-way ANOVA tests showed that phthalate and hormone concentrations were not associated with education level, smoking status or alcohol consumption (Data not shown). Age was significantly correlated to serum estradiol and PRL with the spearman coefficient of 0.22 and 0.27, respectively. BMI was correlated to T:LH ratio and T:E_2 ratio with the spearman coefficient of 0.20 and 0.26, respectively (Data not shown). When samples with undetectable phthalates were assigned a value of 1/2 LOD, phthalate esters demonstrating significant correlations with serum hormones were semen DBP with testosterone (r=-0.21, P=0.02) and with T:E_2 ratio

Table 2. Distribution of Phthalates and Hormones in Men's Semen or Serum

		Percentile							Geo. Mean
		Min.	5th	25th	50th	75th	95th	Max.	
Semen or Serum Phthalates (μg/mL)	Semen DBP	<LOD	<LOD	<LOD	0.02	0.08	0.20	1.40	0.02
	Semen DEHP	<LOD	<LOD	<LOD	0.03	0.09	0.49	1.36	0.03
	Serum DBP	<LOD	<LOD	<LOD	0.05	0.07	0.32	3.42	0.03
	Serum DEHP	<LOD	<LOD	0.02	0.05	0.21	1.81	2.79	0.07
Serum Hormones	FSH (IU/L)	1.76	2.88	5.34	7.32	8.58	17.66	31.00	6.87
	LH (IU/L)	1.49	2.74	4.01	5.25	6.35	7.83	8.70	5.22*
	T (ng/mL)	1.83	2.96	4.52	5.48	6.52	7.21	9.58	5.41*
	E_2 (pg/mL)	<12.00	12.00	15.60	19.50	26.00	37.47	61.40	20.27
	PRL (ng/mL)	6.06	8.71	11.93	15.67	21.36	34.96	50.63	16.54
	T:LH	0.24	0.45	0.84	1.05	1.37	2.08	3.15	1.05
	T:E_2	0.05	0.11	0.19	0.27	0.37	0.54	0.59	0.26

Note. * Arithmetic mean.

Table 3. Spearman Correlations Coefficients between Serum Hormones and Semen/Serum Phthalate Esters

		n	FSH	LH	T	E_2	PRL	T:LH	T:E_2
Samples with Undetectable Phthalates were Assigned a Value of 1/2 LOD	Semen DBP	118	-0.15	<0.01	**-0.21***	0.07	0.13	-0.10	**-0.18***
	Semen DEHP	118	-0.01	-0.10	0.07	0.15	**0.25****	0.10	-0.07
	Serum DBP	118	-0.05	0.04	-0.08	0.06	**0.23***	-0.07	-0.10
	Serum DEHP	118	0.08	-0.05	-0.03	0.15	**0.29****	<0.01	-0.12
Excluding Samples with Undetectable Phthalates	Semen DBP	75	-0.15	-0.12	-0.15	-0.06	0.15	<0.01	-0.02
	Semen DEHP	84	-0.03	-0.12	-0.07	**0.24***	**0.26***	0.06	**-0.19**$^{\#}$
	Serum DBP	87	-0.03	-0.05	-0.16	0.01	**0.26***	-0.02	-0.08
	Serum DEHP	107	0.02	-0.15	-0.07	0.09	**0.33****	0.07	-0.12

Note. *denotes $P < 0.05$; **denotes $P < 0.01$; $^{\#}$denotes $P < 0.1$.

(r=-0.18, P=0.05), and PRL with serum DBP (r=0.23, P=0.01), DEHP (r=0.29, P=0.001) and semen DEHP (r=0.25, P=0.006) (Table 3). Excluding samples lower than LOD, correlations between phthalate esters and serum hormones were similar for PRL, enhanced for estradiol with semen DEHP (r=0.24, P=0.03) and for T:E_2 ratio with semen DEHP (r=-0.19, P=0.08), but dismissed for testosterone and T:E_2 ratio with semen DBP.

Hormones' Associations with Phthalates by Linear Regression Analyses

All linear regression results presented in Table 4 were adjusted for age, BMI, educational background, smoking status, and alcohol consumption. Crude regression results (Data not shown) were similar to the adjusted results shown in Table 4. In the adjusted models excluding samples with undetectable phthalates, there were positive correlations between serum PRL and phthalates, where a 10-fold increase in semen DEHP was associated with a 23% increase in serum PRL [β=0.09, 23% = exp (β*ln10), P=0.01],and a 10-fold increase in serum DBP resulting in a 26% increase (β=0.10, P=0.04), and a 10-fold increase in serum DEHP resulting in a 20% increase (β=0.08, P<0.01). Linear regression models also demonstrated a slight inverse correlation between T:E_2 ratio and semen DEHP (β = -0.08, P = 0.05). When samples with undetectable phthalates were assigned a value of 1/2 LOD, the linear regression models showed diminished, but still significant, correlations between phthalates and serum PRL (β=0.06, 0.06 and 0.07 for semen DEHP, serum DBP and serum DEHP, respectively).

The overall R squares in all linear regression models with significant associations with phthalates were around 0.05-0.15, indicating that a 5%-15% variance could be explained by the models.

Hormones' Associations with Phthalates by Logistic Regression Analyses

Of the subjects who had abnormal serum hormones according to the reference values , 14 had abnormal FSH (all > 11.8 IU/L), 25 had abnormal LH (19 > 6.8 IU/L; 6 < 2.8 IU/L), 8 had abnormal testosterone (4 > 7.4 ng/mL; 4 < 2.6 ng/mL), 1 had estradiol > 56 pg/mL, and 49 had PRL > 18.5 ng/mL. Binary logistic regression analyses were conducted to explore the phthalates' associations with serum FSH, LH, and PRL. No associations were found in serum FSH or LH (Data not shown), but serum PRL was positively associated with phthalate ester concentrations, whether samples with undetectable phthalates were included or not (Table 5). When samples with undetectable phthalates were included, there were dose-response relationships between above reference value PRL and semen DEHP (odds ratio [OR] per tertile adjusted for age, BMI, educational background, smoking status, and alcohol consumption = 1.0, 1.70, 3.50; P for trend = 0.01), and serum DBP (1.0, 1.10, 2.62; P for trend = 0.04), and serum DEHP (1.0, 1.46, 4.69; P for trend < 0.01). The adjusted OR for each semen DBP tertile was also elevated (1.0, 1.07, and 2.11), although the P value for trend was not significant (0.10). Excluding the samples with undetectable phthalates, logistic models demonstrated an enhanced dose-res ponse relationship with semen DBP (1.0, 3.86, 4.07; P for trend = 0.05), serum DBP (1.0, 1.65, 3.64; P for trend = 0.04) and serum DEHP (1.0, 2.47, 4.94; P for trend < 0.01), and a weakened but still significant relationship with semen DEHP (1.0, 1.71, 3.43; P for trend = 0.03).

Table 4. Linear Regression Coefficients (95% Confidence Intervals) between Serum Hormones and Semen/Serum Phthalate Esters[a]

		Semen DBP[b]	Semen DEHP[b]	Serum DBP[b]	Serum DEHP[b]
All Subjects (Samples with Undetectable Phthalates were Assigned a Value of 1/2 LOD)	FSH[c]	-0.06 (-0.13, 0.01)	0.00 (-0.05, 0.06)	-0.02 (-0.08, 0.05)	0.02 (-0.03, 0.08)
	LH[c]	-0.01 (-0.05, 0.04)	-0.01 (-0.05, 0.03)	0.01 (-0.04, 0.05)	-0.01 (-0.04, 0.03)
	T[c]	-0.17 (-0.36, 0.02)	0.02 (-0.14, 0.18)	-0.07 (-0.26, 0.12)	-0.07 (-0.22, 0.09)
	E_2[c]	0.54 (-0.65, 1.74)	0.37 (-0.63, 1.36)	0.45 (-0.72, 1.62)	0.37 (-0.57, 1.31)
	PRL[c]	0.03 (-0.03, 0.09)	**0.06 (0.02, 0.11)****	**0.06 (0.01, 0.12)****	**0.07 (0.02, 0.11)****
	T:LH[c]	-0.04 (-0.10, 0.02)	0.01 (-0.04, 0.06)	-0.03 (-0.09, 0.03)	0.00 (-0.05, 0.05)
	T:E_2[c]	-0.06 (-0.12, 0.00)	-0.02 (-0.08, 0.03)	-0.04 (-0.10, 0.02)	-0.03 (-0.08, 0.02)
Excluding Samples with Undetectable Phthalates	*N*	75	84	87	107
	FSH[c]	-0.05 (-0.18, 0.08)	0.02 (-0.06, 0.10)	-0.01 (-0.12, 0.11)	0.01 (-0.05, 0.07)
	LH[c]	-0.02 (-0.12, 0.07)	-0.01 (-0.06, 0.05)	-0.01 (-0.08, 0.07)	-0.03 (-0.07, 0.01)
	T[c]	-0.14 (-0.55, 0.27)	-0.12 (-0.36, 0.11)	-0.23 (-0.58, 0.13)	-0.11 (-0.27, 0.06)
	E_2[c]	-0.73 (-3.22, 1.76)	0.86 (-0.59, 2.31)	-0.13 (-2.28, 2.01)	0.14 (-0.95, 1.24)
	PRL[c]	0.04 (-0.08, 0.15)	**0.09 (0.03, 0.16)****	**0.10 (0.01, 0.20)***	**0.08 (0.03, 0.13)****
	T:LH[c]	-0.01 (-0.13, 0.12)	-0.02 (-0.09, 0.05)	-0.05 (-0.15, 0.06)	0.01 (-0.05, 0.06)
	T:E_2[c]	0.00 (-0.13, 0.13)	**-0.08 (-0.15, 0.00)***	-0.05 (-0.17, 0.06)	-0.04 (-0.10, 0.02)

Note. [a]Adjusted for age, BMI, educational background, smoking status, and alcohol consumption using stepwise models. [b]In all models ln-transformations of phthalate esters were used. [c]Ln-transformations of FSH, LH, PRL, T:LH, and T:E_2 were used. T and E_2 were modeled untransformed. *denotes $P<0.05$; **denotes $P<0.01$.

DISCUSSION

In this study, both DBP and DEHP concentrations in semen or serum samples were positively associated with circulating PRL levels in adult men who were seeking treatment from a reproductive medical center with a complaint of suspected infertility. These findings were not seen in 425 American infertile men, in whom Meeker et al.[20] found that serum PRL was not associated with urinary concentrations of the metabolites of several phthalates including DEP, DBP, DBzP (dibenzyl phthalate), and DEHP.

PRL is mainly synthesized and secreted by the lactogenic cells of the adenohypophysis.PRL secretion is modulated not only by dopamine, but also by several other neurotransmitters including serotonin, γ-aminobutyric acid (GABA) glycine, and glutamate[25]. This modulation by either dopamine or by other neurotransmitters may be dose-dependent and will result in different serum PRL levels. For example, cadmium have shown a biphasic dose-dependent effect on serum PRL[26], where cadmium chloride at a lower dose (5 or 10 ppm in drinking water) increases plasma PRL levels while at a higher doses (25 or 50 ppm) it decreases them instead. Phthalates may also possibly increase the PRL secretion at a relatively lower level of exposure, but decrease it at a higher level of exposure, and the underlying mechanism may be related to the involvement of different neurotransmitters. This speculation may explain the discrepancies in findings between our study and Meeker's study[20]. Without the data of urinary metabolite concentrations of phthalates, we cannot compare the exposure levels in subjects between our study and Meeker's[20]. Studies by Pant[14] and Zhang[15] provided, mean concentrations of semen phthalates, which were much higher than the mean levels of phthalates in our study (semen DBP: 0.06 μg/mL; semen DEHP: 0.12 μg/mL). It was likely that the subjects in our study were exposed to lower levels of phthalates. Yet the data of altered hormones should remind us to attach great importance to the assessment of the health effects of phthalate exposure at a low level.

We also found in this study some correlations between phthalates and serum testosterone, estradiol and T:E_2 ratio, where the serum testosterone and T:E_2 ratio were decreased following the increase of phthalate exposure, but serum estradiol was increased. Meeker et al.[20] reported that urinary MEHP was inversely associated with

serum testosterone and estradiol, and positively associated with T:E_2 ratio. The inverse association between serum testosterone and phthalate exposure was also reported by Pan et al.[21].

The mechanisms underlying hormones secretion and their relationships are complex[27]. Testosterone is produced by Leydig cells (LCs) in males. LC steroidogenesis is controlled primarily by LH with negative feedback of testosterone on the hypothalamic-pituitary-testis (HPT) axis. Estradiol is produced in the Sertoli cells (SCs) of the testes in males. Some testosterone is converted to estradiol by SC-derived aromatase enzyme. The release of FSH and LH at the pituitary gland is controlled by pulses of gonadotropin-releasing hormone (GnRH). Those pulses, in turn, are subject to the estrogen feedback from the gonads. PRL exerts effects on the HPT axis and can inhibit pulsatile GnRH secretion from the hypothalamus and alter the activity of certain steroidogenic enzymes. In men, excessive PRL secretion causes decreased testosterone and sperm production.

Phthalates are generally suspected to possess both anti-androgenic and estrogenic activity. There is evidence that DEHP may inhibit expression of genes or proteins related to steroidogenesis in LCs[28].

Table 5. Associations of Tertiles of Phthalate Ester Concentrations in Semen/Serum Samples with Serum PRL (Logistic Regression Analyses)[a]

Phthalate Tertile[c]		All Subjects (n = 118)		Subjects with Detectable Phthalates[b]	
		N[d]	Adjusted OR (95% CI)	N[d]	Adjusted OR (95% CI)
Semen DBP	1	15	1.0	7	1.0
	2	15	1.07 (0.43-2.67)	16	**3.86 (1.17-12.76)**
	3	19	2.11 (0.85-5.24)	11	**4.07 (1.11-14.90)**
R Square[e]			0.05		**0.14**
P for trend[f]			0.10		**0.05**
Semen DEHP	1	9	1.0	10	1.0
	2	16	**1.70 (0.63-4.62)**	13	**1.71 (0.58-5.08)**
	3	24	**3.50 (1.32-9.33)**	17	**3.43 (1.13-10.37)**
R Square[e]			**0.10**		**0.10**
P for trend[f]			**0.01**		**0.03**
Serum DBP	1	11	1.0	8	1.0
	2	13	**1.10 (0.41-2.96)**	14	**1.65 (0.54-5.11)**
	3	25	**2.62 (1.04-6.64)**	17	**3.64 (1.18-11.22)**
R Square[e]			**0.08**		**0.13**
P for trend[f]			**0.04**		**0.04**
Serum DEHP	1	9	1.0	8	
	2	16	**1.46 (0.54-3.96)**	17	**2.47 (0.89-6.90)**
	3	24	**4.69 (1.71-12.85)**	20	**4.94 (1.71-14.29)**
R Square[e]			**0.14**		**0.13**
P for trend[f]			**<0.01**		**<0.01**

Note. [a]Adjusted for age, BMI, educational background, smoking status, and alcohol consumption using stepwise models. [b]Subjects with undetectable phthalates (lower than limit of detection [LOD]) were excluded in the models. The sample size for semen DBP was 75 subjects, for semen DEHP 84, for serum DBP 87, and for serum DEHP 107. [c]For the analyses in all subjects, tertile cut points (μg/mL) were as follows: semen DBP: <LOD, 0.01-0.05, 0.06-1.40; semen DEHP: <LOD, 0.01-0.06, 0.07-1.36; serum DBP: <LOD-0.01, 0.02-0.05, 0.06-3.42; serum DEHP: <LOD-0.02, 0.03-0.08, 0.09-2.79. For the analyses in subjects with detectable phthalates, tertile cut points were as follows: semen DBP: 0.01-0.03, 0.04-0.08, 0.09-1.40; semen DEHP: 0.01-0.04; 0.05-0.10, 0.11-1.36; serum DBP: 0.01-0.04, 0.05-0.07, 0.08-3.42; serum DEHP: 0.01-0.03, 0.04-0.14, 0.15-2.79. [d]Indicates the number of men with above reference PRL value. [e]Cox & Snell R Square of the regression model. [f]P for trend reflects Mantel-Haenszel Chi-square distribution of categorized serum PRL among phthalate tertiles.

While DBP and DEHP exhibit both estrogen receptor alpha mediated estrogenic activity and androgen receptor mediated anti-androgenic activity, DEHP surprisingly shows anti-estrogenic activity in an in vitro assay using highly sensitive reporter genes[29]. This mechanism may explain why the decreased testosterone levels are associated with phthalate exposure in both our study and Meeker's study[20], but the findings in estradiol in these two studies are inconsistent. $T:E_2$ ratio is another inconsistent finding between our study and Meeker 's study[20]. It is doubted whether the decreased $T:E_2$ ratio is associated with aromatase activity or is due to the altered steroid hormone levels. Like two other studies (Meeker et al.[20] and Pan et al.[21]), we have not found any associations of phthalate exposure with FSH or LH secretion, which should have been increased if the negative feedback mechanism takes action. As mentioned by Pan et al.[21], the most likely explanation for the simultaneous occurrence of Significantly decreased testosterone and insignificant increase of LH and FSH levels is that the phthalate exposure may have caused dysfunction of both testosterone biosynthesis in the testis and the normal feedback regulation of the HPT axis.

Our study has some limitations which need to be addressed. First, since urine samples were not included as part of our study, no urinary metabolites of phthalates were measured, which have been suggested to be a better biomarker of phthalate exposure[30]. Alternatively, we have determined phthalate diesters in serum and semen samples for exposure assessment, which are also accepted as a biomarker in some recent epidemiological surveys [14,15]. Phthalates are rapidly metabolized and excreted, and ester concentrations in semen or serum samples only reflect exposure within 1 or 2 days. Hauser *et al.*[31] have reported that a single urine sample (also reflect exposure in the preceding 1 or 2 days) is moderately predictive of each subject's exposure over a 3-month period. A single semen or serum sample in the present study is presumed to predict each subject's exposure over months.

Second, the limitations in the determination of serum hormones should also be addressed. Apart from the diurnal variation for many of the hormones used as an end point, it is well known that PRL is a "stress hormone". The best sampling time for hormone determination is in the afternoon after a brief rest. In the present study, all blood samples were sampled in the morning when consultations with specialists were accessible at the medical center. Considering that fact that the medical laboratory in hospital is always busy in the morning, subjects were usually waiting for 10 min before getting sampled. However, the information of the sampling time and resting time had not been recorded as adjusted variables in the statistical analyses. Krüger et al.[32] have reported that plasma PRL levels in men increase immediately after orgasm. To avoid the influence of orgasm, blood samples were taken before semen samples in this study.

In conclusion, the present study has found that serum PRL is positively associated with both DBP and DEHP in semen or serum samples. However, conclusions should be drawn carefully due to some limitations in the study. Future studies are needed to confirm the effects of phthalate exposure on circulating PRL secretion and other steroid hormone levels. The potential clinical impact of these changes in terms of phthalate toxicity will also have to be addressed.

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