

RESEARCH

Open Access



Associations between urinary concentrations of bisphenols and serum concentrations of sex hormones among US. Males

Chichen Zhang^{1†}, Yuehong Luo^{2†}, Shi Qiu^{1†}, Xinyi Huang³, Kun Jin¹, Jiakun Li¹, Mi Yang³, Dan Hu⁴, Xiaonan Zheng¹, Zhongyuan Jiang⁴, Mingda Wang⁴, Xiaoli Zou^{3*} and Qiang Wei^{1*}

Abstract

Background: Bisphenol A (BPA) exposure and its structural analogs (BPS and BPF) might cause endocrine alterations and adverse physiological effects. Few studies to date have directly explored the association between its structural analogs (BPS, BPF) and sex hormones in adult male participants. Therefore, we aimed to assess the associations between BPA, BPS, BPF, and sex hormones in American adult men.

Methods: We used data from the U.S. National Health and Nutrition Examination Survey 2011–2016. We excluded participants without data available on sex hormones and urinary bisphenols. Furthermore, participants consuming sex hormone medications were excluded. Multivariable regression models were performed to assess the association between bisphenols and sex hormones.

Results: In this study, 2367 participants were included. Of 2367, in 1575 participants, the data on BPS and BPF were available. We found that a per unit increase in BPF was associated with 0.575 ng/dL higher total testosterone (TT) (Model 2: 95% CI: 0.047, 1.103, $P=0.033$). However, there was no significant association between BPA or BPS and TT. Furthermore, increased BPA and BPS levels were associated with higher levels of sex hormone-binding globulin (SHBG) (Model 2: $\beta=0.364$, 95% CI: 0.158, 0.571; $\beta=0.25$, 95% CI: 0.071, 0.429, respectively). Additionally, participants in the highest BPA exposure quartile (quartile 4) had 4.072 nmol/L higher levels of SHBG than those in quartile 1 (Model 2: 95% CI: 0.746, 7.397, $P=0.017$; P for trend =0.005). Both BPA and BPS were negatively associated with free testosterone (FT, nmol/L) after full adjustment (Model 2, $\beta=-0.01\%$, $P=0.0211$, $P=0.0211$; Model 2, $\beta=-0.01\%$, $P=0.0258$, respectively). However, BPF was positively associated with FT (Model 2, $\beta=0.0029\%$, $P=0.0028$).

Conclusion: Our study indicated that exposure to both BPA and its substitutions could alter sex hormone levels. This finding supports the possibility that human exposure to bisphenols at environmental levels might affect the endogenous hormone balance.

Keywords: Sex hormones, Bisphenol A, Bisphenol F, Bisphenol S

[†]Chichen Zhang, Yuehong Luo and Shi Qiu contributed equally to this work.

*Correspondence: zouxl_1113@163.com; weiqiang933@126.com

¹ Department of Urology, Institute of Urology, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China

³ Department of Sanitary Technology, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, China
Full list of author information is available at the end of the article

Introduction

Sex hormones are critical in numerous biological processes, including reproduction, differentiation, development, inflammation, and metabolism [1]. The role of testosterone (T), the principal androgenic hormone, is important in human anabolism. It is essential for endocrine functions and spermatogenesis of the male testis.



The majority of the circulating testosterone in blood is commonly bound to sex hormone-binding globulin [SHBG] and albumin [2]. Estradiol (E_2) is essential for female reproductive functions. Estrogen hormone decline is associated with a higher risk of cognitive impairment and cardiovascular disease [3]. Moreover, the liver produces SHBG and secretes it into the blood. SHBG, the transport protein, binds to testosterone and estradiol and thus regulates their bioavailability [4]. Environmental insults impair human health around the world; sex hormones are reportedly vulnerable to environmental and chemical exposure, especially endocrine-disrupting chemicals (EDCs) [5].

Bisphenol A (BPA), characterized as an EDC [6], is widely produced primarily for manufacturing polycarbonate plastics (e.g., baby bottles, sports equipment, and dental sealants) [7] and epoxy resins [8] (e.g., food and beverage containers). In human, the major route of BPA exposure is food or beverage ingestion contaminated with leached BPA from the packaging material [9]; other routes include skin contact and indoor dust inhalation [10]. It has been established that BPA can bind to estrogen receptors and androgen receptors. Thus, it can affect steroidogenic enzyme expression and interfere with sex steroid levels [11]. Numerous animal studies have demonstrated that exposure to BPA has adverse reproductive and behavioral outcomes [12–14]. Similarly, emerging human researches suggest that BPA has unfavorable impacts on the reproductive system of both sexes, metabolic processes, like obesity and diabetes, and cardiovascular diseases [15, 16]. Therefore, safety concerns related to BPA exposure have resulted in the gradual substitution of BPA with its alternatives, including bisphenol S (BPS) and bisphenol F (BPF), in products advertised as “BPA free” [17–19].

Currently, BPF and BPS have also been identified with estrogenic and anti-androgenic features because these analogs have similar chemical structures to BPA [20]. Additionally, as per *in vitro* and *in vivo* studies, they cause similar endocrine alterations and adverse physiological effects [14, 21, 22]. Recently, Wang et al. focused on associations of urinary bisphenol concentrations with adverse effects on sex hormones in children and adolescents, revealing that BPA analogs (BPS and BPF) could have comparable endocrine disrupting effects as those possessed by BPA [23]. However, literature exploring the impact of bisphenol A exposure on sex hormones homeostasis is scanty with contradictory data [24, 25]. Pollock et al. used data from the Canadian and U.S. populations and demonstrated that no association between BPA and total T (TT) or E_2 was found in male participants, but they did not assess the relationship between BPA and other sex hormone indicators (e.g., SHBG and calculated

free T). Lassen et al. showed that increased urinary BPA levels were related to higher TT and were not significantly associated with SHBG in young Danish men [26]. To date, few studies have directly explored the association of BPA's structural analogs with sex hormone levels in adult men [27].

Therefore, we aimed to explore the association of BPA and its analogs (BPS, BPF) with sex hormone levels in a nationally representative population of adult men.

Methods

Study population

The National Health and Nutrition Examination Survey (NHANES) is a continuous, national program that aims to evaluate the nutritional and health status of the U.S. population since 1999. The survey was conducted by the Center for Disease Control and Prevention (CDC) combining interviews and physical examinations. (Official website: <http://www.cdc.gov/nchs/nhanes.htm>). The Ethics Review Board (Continuation of Protocol #2011–17, National Center for Health Statistics) approved all procedures. Written informed consent was signed by all participants.

In this study, three-cycle data (2011–2012, 2013–2014, and 2015–2016) were extracted from the NHANES. Participants aged ≥ 20 years with data available on sex hormones, BPA, and its analogs (BPS, BPF) were included. Participants taking medication, including testosterone, estrogen, progesterone, or “other sex hormones (e.g., gonadotropin, sex hormone combination)” noted in the NHANES were excluded [28, 29]. Finally, 2367 participants were included. Of these, in 1575 participants, the data on BPS and BPF were available (NHANES 2013–2016). (Supplementary Fig. 1).

Exposure assessments

Details on the laboratory methods and procedures of BPA, BPS, and BPF have been published previously [30]. Urinary BPF, BPS, and BPA were measured using solid phase extraction coupled online to high-performance liquid chromatography and tandem mass spectrometry. The lower limit of detection (LOD) was 0.2 $\mu\text{g/L}$ for BPA and BPF and 0.1 $\mu\text{g/L}$ for BPS from the NHANES website. Urinary BPA, BPS, and BPF concentrations below the lower LOD were substituted by $\text{LOD}/\sqrt{2}$ based on the NHANES analysis guideline.

Sex steroid measures

The testosterone, estradiol, and SHBG assessment methodologies have been reported elsewhere [31]. The LODs of TT, E_2 , and SHBG were defined as 0.75 ng/mL, 2.994 pg/ml, and 0.800 nmol/L, respectively. Sex hormones level below LOD were replaced by the $\text{LOD}/\sqrt{2}$.

In addition, free testosterone (FT, nmol/L) was calculated utilizing testosterone, albumin, and SHBG values [32]; the TT to E_2 ratio (TT/ E_2) was employed to evaluate aromatase activity indirectly, which promoted the conversion of T to E_2 [33].

Covariates

As per previous studies of environmental bisphenols and sex hormones [23, 24, 34], several potential confounding variables, including age, race, poverty income ratio (PIR), body mass index, smoking status, urinary creatinine, time of sample collection, and the season of sample collection, were considered covariates. The classification of BMI and smoking status has been described in detail elsewhere [34]. Considering urinary dilution, unadjusted bisphenol concentrations were used as the predictor variable and adjusted for urinary creatinine in the model [35]. The LOD for urinary creatinine was 0.1 mg/dL. Since diurnal variations in sex hormones were observed, the time of sample collection was also adjusted (classification: morning, afternoon and evening) [36]. Moreover, the sample collection season was recorded as a six-month time period, and participants were randomly divided into two categories to measure sex hormones from November 1st through April 30th or May 1st through October 31st.

Statistical analyses

The continuous variables were expressed as the median and interquartile range (IQR: 25th–75th percentile) or mean \pm standard deviation (SD). The categorical variables were expressed as frequencies (percentages). The Student's *t* test (normal distribution), Mann–Whitney *U* test (nonnormal distribution) and chi-square test were performed to compare the differences among different BPA groups. The geometric mean [GM, 95% confidence intervals (CI)] was also calculated because the distribution of bisphenol levels and sex hormones was not normal. Given the nonmonotonic association between BPA exposure and disease outcomes [37], urinary BPA was categorized into quartiles and modeled to allow for non-linear relationships. The same statistical management was performed for BPF and BPS. For BPF, quartile 1 and quartile 2 were merged into one category because of the low detection rate of 57.14% in total.

To evaluate the association between bisphenols (BPA, BPS, and BPF) and sex hormones, two multivariable regression model analyses, including a crude model (model 1) and model 2 (age, race, PIR, BMI, smoking status, urinary creatinine, time of sample collection, and six-month time period were adjusted) were performed. Linear trend tests were also performed by assigning the median value of each bisphenol category (BPA, BPS, and BPF) in the regression models. Finally, stratified and

interaction analyses to test the heterogeneity across subgroups were performed.

Software packages R (<http://www.R-project.org>, The R Foundation) and Empower (www.empowerstats.com) were used for conducting all data analyses. A two-tailed *p*-value < 0.05 was considered statistically significant.

Results

A total of 2367 subjects with an average age of 48.91 ± 17.64 years were included in the BPA group, while 1575 participants with an average age of 49.15 ± 17.59 years were included in the BPS/BPF group (Table 1). The mean BMI was 28.80 ± 6.17 kg/m² and 28.91 ± 6.12 kg/m² in the two groups, and over 80% of them were defined as overweight/obese. The median urinary creatinine level was 130.00 mg/dL in the BPA group and 130.50 mg/dL in the BPS/BPF group. Most participants were non-Hispanic white and who donated blood samples in the daytime in both groups. In the BPA group, approximately 17% of participants were reported to be current smokers. When stratified by BPA groups (quartile), the lowest BPA concentration group was slightly older, had a race other than White, Black, or Hispanic, with a normal BMI and the lowest urinary creatinine levels (Supplementary Table 1). The detection frequencies were 96.62, 92.32, and 57.14% for BPA, BPS, and BPF, respectively. The geometric means were 1.40 ng/mL for BPA, followed by 0.55 ng/mL for BPS and 0.44 ng/mL for BPF. Serum TT, E_2 , and SHBG were detected in 99.00, 99.74, and 100% of the overall samples, respectively. (Supplementary Table 2).

In order to evaluate the association between bisphenols and serum sex hormones concentrations (Table 2), the association of BPA and its analogs with TT in the crude model was first evaluated. Our results showed that significant associations were not observed between BPA or BPA analogs (BPS, BPF) and TT when bisphenols were treated as both continuous and categorical variables. (all $P > 0.05$, P for trend > 0.05). Next, a fully adjusted model was applied, which revealed no significant associations, except a positive association between BPF and TT. A per unit increase in BPF was associated with a 0.575 ng/dL higher TT (95% CI: 0.047, 1.103, $P = 0.033$). Then, the association of BPA or its analogs (BPS, BPF) with E_2 were evaluated which revealed that the associations between bisphenols (BPA, BPS, and BPF) and E_2 were also not statistically significant in non-adjusted and fully adjusted models. (all $P > 0.05$, P for trend > 0.05). Finally, for the association between BPA or BPA analogs (BPS, BPF) and SHBG, a positive association between BPA and SHBG was found (Model 2, $\beta = 0.364$, 95% CI: 0.158, 0.571; $P = 0.001$) after adjusting for all confounders. When BPA was treated as a categorical variable

Table 1 Population characteristics of study population in the 2011–2016 continuous NHANES

| Characteristic | BPA (NHANES 2011-2016) | BPS and BPF (NHANES2013-2016) |
|--|------------------------|-------------------------------|
| Number | 2367 | 1575 |
| Age | 48.91±17.64 | 49.15±17.59 |
| BMI (kg/m ²) | 28.80±6.17 | 28.91±6.12 |
| Urinary creatinine [mg/dL, median (IQR)] | 130.00 (80.00-194.00) | 130.50 (81.00-198.00) |
| % Race | | |
| Mexican American | 13.39% | 15.37% |
| Other Hispanic | 10.18% | 10.48% |
| Non-Hispanic White | 37.52% | 38.03% |
| Non-Hispanic Black | 22.48% | 20.89% |
| Other Race | 16.43% | 15.24% |
| % PIR | | |
| ≤1.3 | 31.48% | 31.18% |
| 1.3-3.5 | 36.15% | 36.23% |
| >3.5 | 32.37% | 32.59% |
| % Smoking status | | |
| never | 48.48% | 48.22% |
| former | 34.56% | 35.05% |
| current | 16.96% | 16.73% |
| % Time of venipuncture | | |
| morning | 49.39% | 49.71% |
| afternoon | 35.74% | 35.37% |
| evening | 14.87% | 14.92% |
| % BMI | | |
| Normal (<25kg/m ²) | 48.48% | 26.19% |
| Overweight (25–29.9 kg/m ²) | 34.56% | 38.96% |
| Obesity (>=30 kg/m ²) | 16.96% | 34.85% |
| Six-month time period | | |
| November 1 through April 30 | 48.92% | 49.02% |
| May 1 through October 31 | 51.08% | 50.98% |

Mean \pm SD for continuous variables: *P* value was calculated by weighted t-test

% for Categorical variables: *P* value was calculated by weighted chi-square test

BMI Body Mass Index

(quartiles), participants in quartile 4 of the BPA group had 4.072 nmol/L higher levels of SHBG than those in quartile 1 (Model 2, $\beta=4.072$, 95% CI: 0.746, 7.397, $P=0.017$; *P* for trend =0.005). Additionally, a higher BPS level was associated with higher SHBG levels in non-adjusted and fully adjusted models. (Model 1, $\beta=0.276$, 95% CI: 0.068, 0.484, $P=0.009$; Model 2, $\beta=0.250$, 95% CI: 0.071, 0.429, $P=0.006$) However, no statistically significant associations between BPF and SHBG were observed.

In order to evaluate the association between bisphenols exposures and calculated FT (Supplementary Table 3) as well as TT/E₂ (Supplementary Table 4), it was demonstrated that there were no significant associations between bisphenols (BPA, BPS, and BPF) and calculated

FT in the crude model. (all $P>0.05$) Then, a fully adjusted model was applied which demonstrated that BPA and BPS were negatively associated with FT (nmol/L) (Model 2, $\beta=-0.01\%$, $P=0.0211$; Model 2, $\beta=-0.01\%$, $P=0.0258$, respectively), while BPF was positively associated with FT (Model 2, $\beta=0.0029\%$, $P=0.0028$). For TT/E₂, there were no significant associations between BPA, BPS, and TT/E₂ when treated with BPA and BPS in either the continuous or categorical variables. However, a per unit increase in BPS was significantly associated with a 0.0001 increase in TT/E₂ ($P=0.0492$).

To examine the potential modifying effect of BMI on the associations between bisphenols exposures and hormone concentrations, the association between bisphenols and TT among different BMI groups was evaluated.

Table 2 Association between Bisphenols and sex hormones (Total Testosterone, Estradiol, Sex hormone-binding globulin) among the US males in NHANES 2011-2016

| Bisphenols | Total Testosterone(ng/dL) β(95%CI) | | Estradiol (pg/ml) β(95%CI) | | Sex hormone-binding globulin (SHBG) (nmol/L) β(95%CI) | |
|------------------|---------------------------------------|-----------------------------------|-------------------------------|-------------------------------|---|-----------------------------------|
| | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 | Model 2 |
| BPA (Continuous) | -0.156 (-1.248, 0.936) 0.779 | 0.611 (-0.805, 2.026) 0.398 | -0.009 (-0.078, 0.061) 0.807 | -0.039 (-0.143, 0.064) 0.456 | 0.135 (-0.024, 0.294) 0.096 | 0.364 (0.158, 0.571) 0.001 |
| BPA (Quartiles) | | | | | | |
| Q1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q2 | -6.109 (-26.68, 14.46) 0.561 | -6.314 (-25.840, 13.213) 0.526 | -1.430 (-2.835, -0.025) 0.046 | -1.534 (-2.948, -0.120) 0.034 | -3.933 (-7.329, -0.537) 0.024 | 0.746 (-2.209, 3.699) 0.621 |
| Q3 | -28.229 (-48.657, -7.801) 0.007 | -23.175 (-43.634, -2.715) 0.027 | -1.129 (-2.529, 0.272) 0.114 | -1.667 (-3.154, -0.180) 0.028 | -5.136 (-8.482, -1.791) 0.003 | -1.784 (-4.856, 1.289) 0.255 |
| Q4 | -15.770 (-36.341, 4.801) 0.1335 | -6.709 (-29.146, 15.729) 0.558 | 0.023 (-1.390, 1.436) 0.975 | -0.876 (-2.499, 0.747) 0.290 | 0.228 (-3.148, 3.604) 0.895 | 4.072 (0.746, 7.397) 0.017 |
| P for trend | 0.168 | 0.924 | 0.369 | 0.938 | 0.252 | 0.005 |
| BPS (Continuous) | -0.165 (-1.511, 1.181) 0.810 | 0.079 (-1.162, 1.321) 0.900 | -0.011 (-0.085, 0.064) 0.774 | -0.018 (-0.091, 0.056) 0.635 | 0.276 (0.068, 0.484) 0.009 | 0.250 (0.071, 0.429) 0.006 |
| BPS (Quartiles) | | | | | | |
| Q1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q2 | -27.161 (-54.670, 0.377) 0.053 | -17.705 (-43.628, 8.218) 0.181 | 0.706 (-0.829, 2.241) 0.366 | 0.306 (-1.236, 1.849) 0.697 | -4.067 (-7.821, -0.313) 0.034 | -2.195 (-5.465, 1.074) 0.188 |
| Q3 | 11.620 (-16.537, 39.776) 0.419 | 20.794 (-6.427, 48.016) 0.135 | 2.067 (0.496, 3.636) 0.011 | 1.738 (0.118, 3.357) 0.036 | -1.482 (-5.312, 2.349) 0.448 | 1.333 (-2.088, 4.754) 0.445 |
| Q4 | -11.482 (-39.870, 16.906) 0.428 | -8.295 (-36.676, 20.085) 0.567 | 0.503 (-1.079, 2.086) 0.533 | 0.140 (-1.548, 1.827) 0.871 | -4.017 (-7.868, -0.165) 0.041 | -1.678 (-5.254, 1.897) 0.358 |
| P for trend | 0.963 | 0.775 | 0.856 | 0.592 | 0.222 | 0.549 |
| BPF (Continuous) | 0.078 (-0.494, 0.649) 0.790 | 0.575 (0.047, 1.103) 0.033 | 0.017 (-0.015, 0.049) 0.295 | 0.010 (-0.022, 0.042) 0.542 | 0.008 (-0.066, 0.083) 0.825 | 0.025 (-0.039, 0.089) 0.444 |
| BPF (Quartiles) | | | | | | |
| Q1+2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Q3 | -8.203 (-30.453, 14.047) 0.470 | -13.633 (-34.227, 6.963) 0.195 | -0.278 (-1.518, 0.961) 0.660 | -0.372 (-1.599, 0.855) 0.553 | -0.941 (-3.914, 2.032) 0.535 | -2.453 (-4.997, 0.090) 0.059 |
| Q4 | 11.352 (-10.122, 32.825) 0.300 | 18.062 (-2.399, 38.522) 0.084 | 1.446 (0.255, 2.637) 0.017 | 1.172 (-0.043, 2.384) 0.059 | -1.266 (-4.153, 1.622) 0.390 | -1.659 (-4.188, 0.870) 0.199 |
| P for trend | 0.451 | 0.223 | 0.634 | 0.468 | 0.527 | 0.058 |

Model 1: crude model

Model 2: adjusted for age, race, BMI, poverty income ratio (PIR), smoking status, urinary creatinine, and time of sample collection, six-month time period.

95%CI 95% Confidence interval

In stratified analyses, it was found that associations were stronger between BPF and TT among obese men (Table 3, P for trend = 0.009; P for interaction = 0.027). Then, a fully adjusted model was applied which demonstrated that for men with obesity, compared with participants at quartile 2, those at quartile 3 had 39.027 ng/dL lower TT, and those at quartile 4 had 19.215 ng/dL higher TT (95% CI: -80.50, -27.86, P = 0.017; 95% CI: -22.28, 40.01, P = 0.225, respectively). Next, for the association between bisphenols exposures and E_2 , the interaction tests reported a significant effect of BMI on the association of BPS with E_2 . Furthermore, the interaction test and stratified analysis also indicated that the association was stronger between BPA

and SHBG in men with normal weight when stratified by BMI in a fully adjusted model (P for interaction = 0.023). Compared to those in quartile 1, those in quartile 4 had a 9.217 nmol/L higher SHBG (95% CI: 1.753, 16.68; P for trend = 0.018). (Table 3) Finally, supplementary Table 5 demonstrates that BMI might be a potential effector on the associations of BPA and FT, and the negative association was stronger in participants with normal BMI.

Discussion

This study revealed that higher urinary BPF concentrations were significantly associated with increased TT after adjustment for all confounders. Furthermore, the

Table 3 Stratified analyses for the association between Bisphenols and sex hormones among different BMI groups in NHANES 2011–2016^a

| Bisphenols | Total Testosterone(ng/dL) β(95%CI) | | | Estradiol pg/mL β(95%CI) | | | sex hormone-binding globulin (SHBG) (nmol/L) β(95%CI) | | |
|--|---------------------------------------|----------------------------------|---------------------------------------|-------------------------------|------------------------------|------------------------------|---|-------------------------------|----------------------------|
| | BPA | BPS | BPF ^b | BPA | BPS | BPF ^b | BPA | BPS | BPF ^b |
| Normal (BMI<25 kg/m²) | | | | | | | | | |
| Q1 | 0 | 0 | - | 0 | 0 | - | 0 | 0 | |
| Q2 | -15.301 (-56.008, 25.406) | -42.339 (-97.7169, 13.039) | 0 | -0.300 (-2.852, 2.252) | -0.018 (-2.800, 2.764) | 0 | 5.700 (-0.363, 11.763) | -4.155 (-10.778, 2.467) | 0 |
| Q3 | -44.446 (-90.232, 1.340) | -1.444 (-59.518, 56.629) | 19.930 (-26.249, 66.109) | -1.387 (-4.369, 1.595) | -0.406 (-3.322, 2.509) | -0.748 (-3.081, 1.584) | -1.159 (-8.042, 5.724) | -1.604 (-8.544, 5.335) | 1.097 (-4.300, 6.495) |
| Q4 | -20.640 (-72.017, 30.736) | -20.426 (-79.364, 38.512) | 2.424 (-45.380, 50.229) | -2.128 (-5.400, 1.144) | -0.429 (-3.399, 2.540) | -0.187 (-2.581, 2.207) | 9.217 (1.753, 16.681) | -6.470 (-13.603, 0.663) | -0.245 (-5.895, 5.405) |
| P for trend | 0.705 | 0.980 | 0.446 | 0.208 | 0.758 | 0.330 | 0.018 | 0.125 | 0.765 |
| Overweight (BMI 25–29.9 kg/m²) | | | | | | | | | |
| Q1 | 0 | 0 | - | 0 | 0 | - | 0 | 0 | |
| Q2 | -21.057 (-52.519, 10.405) | -20.682 (-61.043, 19.680) | 0 | -1.212 (-3.364, 0.941) | -2.157 (-4.487, 0.173) | 0 | 2.254 (-2.296, 6.805) | -0.121 (-5.177, 4.934) | 0 |
| Q3 | -44.487 (-77.418, -11.555) | -3.530 (-47.245, 40.185) | -14.431 (-46.821, 17.960) 0.383 | -2.627 (-4.878, -0.376) | -1.040 (-3.561, 1.480) | 0.071 (-1.792, 1.934) | -0.136 (-4.859, 4.587) | 2.647 (-2.736, 8.031) | -2.164 (-6.091, 1.763) |
| Q4 | -18.700 (-55.833, 18.432) | -26.164 (-71.089, 18.762) | 21.759 (-10.731, 54.249) 0.190 | -0.572 (-3.146, 2.002) | -2.160 (-4.739, 0.419) | 1.937 (0.073, 3.801) | 3.933 (-1.373, 9.239) | 2.236 (-3.249, 7.720) | 0.664 (-3.305, 4.634) |
| P for trend | 0.754 | 0.377 | 0.651 | 0.875 | 0.378 | 0.684 | 0.203 | 0.359 | 0.419 |
| Obesity (BMI≥30 kg/m²) | | | | | | | | | |
| Q1 | 0 | 0 | - | 0 | 0 | - | | 0 | |
| Q2 | 11.538 (-19.286, 42.361) | 6.812 (-35.172, 48.796) | 0 | -2.901 (-5.562, -0.239) | 4.064 (1.187, 6.940) | 0 | 0 | -2.416 (-8.132, 3.299) | 0 |
| Q3 | 6.8150 (-23.995, 37.625) | 59.052 (16.075, 102.029) | -39.027 (-70.978, -7.077) | -1.056 (-3.748, 1.637) | 6.394 (3.449, 9.340) | -0.674 (-2.903, 1.554) | -4.360 (-9.545, 0.825) | 2.348 (-3.558, 8.254) | -5.074 (-9.395, -0.753) |
| Q4 | 13.431 (-19.277, 46.138) | 14.184 (-31.819, 60.188) | 19.215 (-11.780, 50.210) | -1.020 (-3.848, 1.807) | 3.289 (0.136, 6.442) | 1.068 (-1.089, 3.225) | -2.813 (-8.011, 2.386) | -1.824 (-8.211, 4.564) | -5.202 (-9.372, -1.031) |
| P for trend | 0.558 | 0.897 | 0.009 | 0.787 | 0.855 | 0.413 | 0.610 | 0.655 | 0.019 |
| P for interaction | 0.249 | 0.288 | 0.027 | 0.227 | 0.007 | 0.796 | 0.023 | 0.732 | 0.101 |

95%CI 95% Confidence interval

^a adjusted for adjusted for age, race, poverty income ratio (PIR), smoking status, urinary creatinine, time of sample collection, and six-month time period^b for BPF, Q1 and Q2 were merged into Q2, Q3 and Q4 were Q3 and Q4, respectively

association was stronger among men with obesity. Additionally, both urinary BPA and BPS concentrations were found to be positively associated with SHBG levels.

Recently, several studies have investigated the associations between BPA exposure and serum sex hormones, but the findings are contradictory. Our finding showed that there was no significant association between BPA and TT or E₂ in U.S. adult men, which was consistent

with previous studies [24, 38]; and Mendiola et al. included 360 U.S. fertile men with a median PSA concentration of 1.7 ng/mL³⁸ (1.40 ng/mL in our study). Furthermore, consistent results have also been reported elsewhere across different adult populations (i.e. China, Spanish) [27, 39]. In contrast, both Galloway et al. [25] and Lassen et al. [26] demonstrated that there was a significantly positive association between BPA and TT

concentrations from a prospective cohort of young Danish men and Italian adult men, respectively. The median BPA concentration in both studies was more than two-fold (3.25 ng/mL and 3.5 ng/mL) than that in our study. In conclusion, these divergent results might be associated with geographic disparities and dissimilarities in exposure levels. Interestingly, inconsistent with previous findings [23, 40], our study reported a positive association between BPF and TT in adult men, which might be explained by the developmental stage of the exposed individual. Our results also indicated that a higher BPA concentration was associated with increased SHBG levels and decreased FT levels, and the positive association between BPA and SHBG has been reported in another study [38]. Currently, SHBG has been identified as a disease risk biomarker [4]; its level is inversely associated with metabolic syndrome and type 2 diabetes, whereas it is positively associated with HDL-cholesterol concentration [41–44]. Regarding FT, it has also been found to be associated with bone health, frailty, and other clinical endpoints [45, 46]. And a previous study reported that determining the FT level avoids under- and over-diagnosis of male hypogonadism and facilitates adequate prescription of hormonal replacement therapy [47]. Therefore, the variations in SHBG and FT concentrations in serum can not only be associated with adverse health outcomes but also impact the sex hormones' utility as sensitive biomarkers for several clinical outcomes.

The androgenic effect of BPA has been reported elsewhere in some experimental and clinical studies. The possible mechanism for BPA action could be a reduction in aromatase activity due to BPA, which decreased testosterone conversion to E_2 and increased the TT level [48, 49]. Furthermore, the metabolism of BPA is catalyzed through uridine diphosphate-glucuronosyl transferase (UGT) in the liver and intestine, which produces most of the urinary metabolite BPA-glucuronide [50]. Androgens have been found to reduce the level of UGT activity and transcription [51], which could lead to an increased serum BPA concentration under high androgenic conditions. However, whether BPF acting as a BPA analog might possess similar effects is still unknown. The plausible explanation for the androgenic activity of BPF could be that BPF increased the testicular weight [52] and the cumulative BPF effect increased the Cowper's gland's weight [53], showing possible androgenic activity. However, existing evidence could not fully explain the androgenic activity of BPF. To date, the regulation of SHBG has not been fully investigated. In humans, androgen action suppresses the serum SHBG concentration, while estrogen action increases it. Mendiola et al. proposed that the positive association between BPA and SHBG levels could be a direct

effect of the estrogenic action of BPA. In addition, BPA might reduce androgenic effects through estrogen receptor-mediated reduction in steroid production [38]. However, associations between BPA analogs (BPS, BPF) and SHBG levels have not been examined. Hence, future studies should be conducted to provide enough evidence to illustrate the role of BPA and its analogs in altering sex hormone levels.

Based on our knowledge, this is the first study to explore the association between urinary concentrations of bisphenol analogs (and not only BPA) and sex hormones in noninstitutionalized adult men using the NHANES database. Our results suggested that BPA substitutes (BPS and BPF) might also have endocrine-disrupting features, and the epidemiologic evidence in our study combined with previous experimental studies suggested that they might not be potentially safe substitutes for BPA. Although our study detected no significant association between BPA and TT, further study should concentrate on the mechanism by which different BPA concentrations may have different effects on TT.

Our study still has some limitations. First, because of the cross-sectional study design, it could not establish causality and could not indicate the impact of long-term exposure. Second, bisphenol exposure was only assessed in a single-spot urine sample. This could result in exposure misclassification and would not establish longitudinal exposure to bisphenol compounds. Previous studies have reported poor reproducibility of BPA and its analogs in spot urine samples through intraclass correlation coefficient estimates ($ICC < 0.4$) [54–56]. Therefore, repeated urinary specimens are required to characterize bisphenol exposure levels and reduce exposure misclassification. Furthermore, unidentified or unmeasured confounders, like co-exposure to other environmental chemicals, such as phthalate, and dietary patterns related to sex hormones cannot be excluded because the main source of BPA is diet. Finally, although we excluded participants taking sex hormone medications, individuals using other products that could alter sex hormones, like Rogaine and aromatase inhibitors were not excluded.

Conclusion

In conclusion, this study revealed that BPF was positively associated with TT. It also found that higher BPA and BPS were associated with increased levels of SHBG. Additionally, this study might have potential reproductive health-related impacts, where we demonstrated that BPS and BPF exposure could also have endocrine-disrupting abilities and alter sex hormone levels. This suggests that BPF and BPS might not be safe substitutes for BPA. Further investigation should focus on the endocrine

disruption potencies and the mechanisms of BPS and BPF.

Abbreviations

TT: Total testosterone; E₂: Estradiol; SHBG: Sex hormone-binding globulin; EDCs: Endocrine disrupting chemicals; BPA: Bisphenol A; BPS: Bisphenol S; BPF: Bisphenol F; LOD: Lower limit of detection.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12940-022-00949-6>.

Additional file 1: Supplementary Figure 1. Participant selection flowchart.

Additional file 2: Supplementary Table 1. Population Characteristics of participants stratified by BPA in the 2011–2016 continuous NHANES.

Additional file 3: Supplementary Table 2. Distribution of selected Bisphenols and sex hormones in study population, NHANES, USA.

Additional file 4: Supplementary Table 3. Association between Bisphenols and free testosterone among the US males in NHANES 2011–2016.

Additional file 5: Supplementary Table 4. Association between Bisphenols and Testosterone/estradiol ratio among the US males in NHANES 2011–2016.

Additional file 6: Supplementary Table 5. Stratified Analyses for the association between Bisphenols and free testosterone and Testosterone/estradiol ratio among the US males in NHANES 2011–2016*.

Acknowledgments

The authors thank Dr. Chi Chen, Changzhong Chen, and Xing-Lin Chen for providing statistical methodology consultation.

Authors' contributions

CCZ, YHL, and SQ contributed equally as first authors of this manuscript. QW and XLZ were responsible for the conception and design of the study. SQ, YHL, MY, XYH, and XNZ interpreted the analysis. CCZ, JKL, DH, ZYJ, and MDW were responsible for the acquisition of data. CCZ and SQ wrote the first draft of the manuscript, interpreted the data and wrote the final version. All authors critically revised the Article for important intellectual content and approved the final version.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81902578, 81974098, 81974099), the National Key Research and Development Program of China (Grant No. SQ2017YF5F090096), the China Postdoctoral Science Foundation (2017 M61 2971), the Postdoctoral Science Research Foundation of Sichuan University (2020SCU12041), the PostDoctor Research Project, West China Hospital, Sichuan University (2018HXBH085), and the National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University (Z2018C01).

Availability of data and materials

The data sets generated and/or analyzed during the current study are available from the NHANES repository, <https://www.cdc.gov/nchs/nhanes/>.

Declarations

Ethics approval and consent to participate

The survey was performed by the National Center for Health Statistics (NCHS) and approved by the NCHS Institutional Review Board (IRB). All informed consents had been obtained from the eligible subjects before initiating data collection and NHANES health examinations. (<https://www.cdc.gov/nchs/nhanes/irba98.htm>).

All authors confirmed that all methods were carried out in accordance with relevant NHANES Analytic Guidelines. (<https://www.cdc.gov/nchs/nhanes/analyticguidelines.aspx#analytic-guidelines>).

Consent for publication

Not applicable.

Competing interests

All authors in the study declare no conflicts of interest.

Author details

¹Department of Urology, Institute of Urology, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China. ²Department of Anesthesia, First Affiliated Hospital, School of Medicine, Zhejiang University, Zhejiang 310009, Hangzhou, China. ³Department of Sanitary Technology, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, China. ⁴Department of Clinical Research Management, West China Hospital of Sichuan University, Chengdu 610041, China.

Received: 20 June 2022 Accepted: 16 December 2022

Published online: 22 December 2022

References

- Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol*. 2005;67:335–76.
- Walker WH. Non-classical actions of testosterone and spermatogenesis. *Philos Trans R Soc Lond Ser B Biol Sci*. 2010;365:1557–69.
- MohanKumar SMJ, Balasubramanian P, Subramanian M, MohanKumar PS. Chronic estradiol exposure - harmful effects on behavior, cardiovascular and reproductive functions. *Reproduction*. 2018;156:R169–r86.
- Simó R, Sáez-López C, Barbosa-Desongles A, Hernández C, Selva DM. Novel insights in SHBG regulation and clinical implications. *Trends Endocrinol Metab*. 2015;26:376–83.
- Peters A, Nawrot TS, Baccarelli AA. Hallmarks of environmental insults. *Cell*. 2021;184:1455–68.
- Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, et al. Endocrine-disrupting chemicals and public health protection: a statement of principles from the Endocrine Society. *Endocrinology*. 2012;153:4097–110.
- Carwile JL, Ye X, Zhou X, Calafat AM, Michels KB. Canned soup consumption and urinary bisphenol a: a randomized crossover trial. *Jama*. 2011;306:2218–20.
- Ehrlich S, Calafat AM, Humblet O, Smith T, Hauser R. Handling of thermal receipts as a source of exposure to bisphenol a. *Jama*. 2014;311:859–60.
- Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol a (BPA). *Reprod Toxicol*. 2007;24:139–77.
- Zalko D, Jacques C, Duplan H, Bruel S, Perdu E. Viable skin efficiently absorbs and metabolizes bisphenol a. *Chemosphere*. 2011;82:424–30.
- Jambor T, Knížatová N, Lukáč N. Men's reproductive alterations caused by bisphenol a and its analogues: a review. *Physiol Res*. 2021;70:5643–s56.
- Borman ED, Foster WG, Greenacre MK, Muir CC, deCatanzaro D. Stress lowers the threshold dose at which bisphenol a disrupts blastocyst implantation, in conjunction with decreased uterine closure and e-cadherin. *Chem Biol Interact*. 2015;237:87–95.
- Rochester JR, Bolden AL, Kwiatkowski CF. Prenatal exposure to bisphenol a and hyperactivity in children: a systematic review and meta-analysis. *Environ Int*. 2018;114:343–56.
- Rubin AM, Seebacher F. Bisphenols impact hormone levels in animals: a meta-analysis. *Sci Total Environ*. 2022;828:154533.
- Rezg R, El-Fazaa S, Gharbi N, Mornagui B. Bisphenol a and human chronic diseases: current evidences, possible mechanisms, and future perspectives. *Environ Int*. 2014;64:83–90.
- Ma Y, Liu H, Wu J, Yuan L, Wang Y, et al. The adverse health effects of bisphenol a and related toxicity mechanisms. *Environ Res*. 2019;176:108575.
- Eladak S, Grisin T, Moison D, Guerquin MJ, N'Tumba-Byn T, et al. A new chapter in the bisphenol a story: bisphenol S and bisphenol F are not safe alternatives to this compound. *Fertil Steril*. 2015;103:11–21.
- Chen D, Kannan K, Tan H, Zheng Z, Feng YL, et al. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity-a review. *Environ Sci Technol*. 2016;50:5438–53.

19. Barbagallo F, Condorelli RA, Mongioi LM, Cannarella R, Aversa A, et al. Effects of Bisphenols on testicular steroidogenesis. *Front Endocrinol*. 2020;11:373.
20. Choi JY, Lee J, Huh DA, Moon KW. Urinary bisphenol concentrations and its association with metabolic disorders in the US and Korean populations. *Environ Pollut (barking, Essex : 1987)*. 2022;295:118679.
21. Rochester JR, Bolden AL. Bisphenol S and F: a systematic review and comparison of the hormonal activity of Bisphenol a substitutes. *Environ Health Perspect*. 2015;123:643–50.
22. Ullah A, Pirzada M, Jahan S, Ullah H, Turi N, et al. Impact of low-dose chronic exposure to bisphenol a and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: a focus on the possible hormonal mode of action. *Food Chem Toxicol*. 2018;121:24–36.
23. Wang Y, Aimuzi R, Nian M, Zhang Y, Luo K, et al. Bisphenol a substitutes and sex hormones in children and adolescents. *Chemosphere*. 2021;278:130396.
24. Pollock T, Arbuckle TE, Guth M, Bouchard MF, St-Amand A. Associations among urinary triclosan and bisphenol a concentrations and serum sex steroid hormone measures in the Canadian and U.S. Populations *Environ Int*. 2021;146:106229.
25. Galloway T, Cipelli R, Guralnik J, Ferrucci L, Bandinelli S, et al. Daily bisphenol a excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. *Environ Health Perspect*. 2010;118:1603–8.
26. Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, et al. Urinary bisphenol a levels in young men: association with reproductive hormones and semen quality. *Environ Health Perspect*. 2014;122:478–84.
27. Zeng JY, Chen PP, Liu C, Deng YL, Miao Y, et al. Bisphenol a analogues in associations with serum hormone levels among reproductive-aged Chinese men. *Environ Int*. 2022;167:107446.
28. Woodward MJ, Obsekov V, Jacobson MH, Kahn LG, Trasande L. Phthalates and sex steroid hormones among men from NHANES, 2013–2016. *J Clin Endocrinol Metab*. 2020;105:e1225–34.
29. Tao C, Li Z, Fan Y, Li X, Qian H, et al. Independent and combined associations of urinary heavy metals exposure and serum sex hormones among adults in NHANES 2013–2016. *Environ Pollut (barking, Essex : 1987)*. 2021;281:117097.
30. Centers for Disease Control and Prevention (CDC) Lpm, https://www.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/EPHPP_H_MET. Accessed 10 June 2021.
31. Centers for Disease Control and Prevention (CDC). https://www.cdc.gov/Nchs/Nhanes/2013-2014/TST_H.htm; Accessed 10 June 2021.
32. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84:3666–72.
33. Newton CJ, Samuel DL, James VH. Aromatase activity and concentrations of cortisol, progesterone and testosterone in breast and abdominal adipose tissue. *J Steroid Biochem*. 1986;24:1033–9.
34. Bao W, Liu B, Rong S, Dai SY, Trasande L, et al. Association between Bisphenol a exposure and risk of all-cause and cause-specific mortality in US adults. *JAMA Netw Open*. 2020;3:e2011620.
35. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, et al. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect*. 2005;113:192–200.
36. Vesper HW, Wang Y, Vidal M, Botelho JC, Caudill SP. Serum Total testosterone concentrations in the US household population from the NHANES 2011–2012 study population. *Clin Chem*. 2015;61:1495–504.
37. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-a and the great divide: a review of controversies in the field of endocrine disruption. *Endocr Rev*. 2009;30:75–95.
38. Mendiola J, Jørgensen N, Andersson AM, Calafat AM, Ye X, et al. Are environmental levels of bisphenol a associated with reproductive function in fertile men? *Environ Health Perspect*. 2010;118:1286–91.
39. Adoamnei E, Mendiola J, Vela-Soria F, Fernández MF, Olea N, et al. Urinary bisphenol a concentrations are associated with reproductive parameters in young men. *Environ Res*. 2018;161:122–8.
40. Hu P, Pan C, Su W, Vinturache A, Hu Y, et al. Associations between exposure to a mixture of phenols, parabens, and phthalates and sex steroid hormones in children 6–19 years from NHANES, 2013–2016. *Sci Total Environ*. 2022;822:153548.
41. Le TN, Nestler JE, Strauss JF 3rd, Wickham EP 3rd. Sex hormone-binding globulin and type 2 diabetes mellitus. *Trends Endocrinol Metab*. 2012;23:32–40.
42. Laaksonen DE, Niskanen L, Punnonen K, Nyssönen K, Tuomainen TP, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care*. 2004;27:1036–41.
43. Mingrone G, Greco AV, Giancaterini A, Scarfone A, Castagneto M, et al. Sex hormone-binding globulin levels and cardiovascular risk factors in morbidly obese subjects before and after weight reduction induced by diet or malabsorptive surgery. *Atherosclerosis*. 2002;161:455–62.
44. Buttari B, Riganò R, Palmieri L, Lo Noce C, Blankenberg S, et al. Sex Hormone-Binding Globulin and Its Association to Cardiovascular Risk Factors in an Italian Adult Population Cohort. 2022;5:5.
45. Hyde Z, Flicker L, Almeida OP, Hankey GJ, McCaul KA, et al. Low free testosterone predicts frailty in older men: the health in men study. *J Clin Endocrinol Metab*. 2010;95:3165–72.
46. Tracz MJ, Sideras K, Boloña ER, Haddad RM, Kennedy CC, et al. Testosterone use in men and its effects on bone health. A systematic review and meta-analysis of randomized placebo-controlled trials. *J Clin Endocrinol Metab*. 2006;91:2011–6.
47. Narinx N, David K, Walravens J, Vermeersch P, Claessens F, et al. Role of sex hormone-binding globulin in the free hormone hypothesis and the relevance of free testosterone in androgen physiology. *Cell Mole Life Sci*. 2022;79:543.
48. Huang H, Leung LK. Bisphenol a downregulates CYP19 transcription in JEG-3 cells. *Toxicol Lett*. 2009;189:248–52.
49. Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol a is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology*. 2004;145:592–603.
50. Teeguarden JG, Waechter JM Jr, Clewell HJ 3rd, Covington TR, Barton HA. Evaluation of oral and intravenous route pharmacokinetics, plasma protein binding, and uterine tissue dose metrics of bisphenol a: a physiologically based pharmacokinetic approach. *Toxicol Sci*. 2005;85:823–38.
51. Takeuchi T, Tsutsumi O, Ikezaki Y, Takai Y, Taketani Y. Positive relationship between androgen and the endocrine disruptor, bisphenol a, in normal women and women with ovarian dysfunction. *Endocr J*. 2004;51:165–9.
52. Higashihara N, Shiraishi K, Miyata K, Oshima Y, Minobe Y, et al. Subacute oral toxicity study of bisphenol F based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". *Arch Toxicol*. 2007;81:825–32.
53. Yamasaki K, Takeyoshi M, Sawaki M, Imatanaka N, Shinoda K, et al. Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. *Toxicology*. 2003;183:93–115.
54. Fäys F, Hardy EM, Palazzi P, Haan S, Beausoleil C, et al. Biomonitoring of fast-elimination endocrine disruptors - results from a 6-month follow up on human volunteers with repeated urine and hair collection. *Sci Total Environ*. 2021;778:146330.
55. Morgan MK, Nash M, Barr DB, Starr JM, Scott Clifton M, et al. Distribution, variability, and predictors of urinary bisphenol a levels in 50 North Carolina adults over a six-week monitoring period. *Environ Int*. 2018;112:85–99.
56. Wang YX, Liu C, Shen Y, Wang Q, Pan A, et al. Urinary levels of bisphenol a, F and S and markers of oxidative stress among healthy adult men: variability and association analysis. *Environ Int*. 2019;123:301–9.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.