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# Joint effects of tobacco smoke exposure and heavy metals on serum sex hormones in adult males

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## Abstract

**Objective** This study aimed to explore the associations of tobacco smoke exposure (TSE) and heavy metal exposure on sex hormones and the joint effects between them in adult males.

**Methods** The study used data of 2244 adult males from the National Health and Nutrition Examination Survey (NHANES, 2013–2016). Weighted linear regression models were used to calculate their beta (β) coefficients and corresponding confidence interval (95% CI), which assessed the joint effects of TSE and heavy metals on sex hormones.

**Results** Sex hormone-binding globulin (SHBG) showed a positive association with increased per standard deviation (SD) for cotinine (β=0.024 [0.004, 0.043]; $P$<0.001), lead (β=0.021 [0.002, 0.039]; $P$=0.028), and cadmium (β=0.034 [0.015, 0.053]; $P$<0.001). Manganese was positively associated with estradiol (E2) (β=0.025 [0.009, 0.042]; $P$=0.002). The subjects with higher cadmium levels were more likely to have higher total testosterone (TT) (β=0.042 [0.023, 0.062]; $P$<0.001). TSE and lead exerted synergistic effects on TT ($p$ for interaction = 0.015) and E2 (p for interaction = 0.009), as also did TSE and cadmium on SHBG (p for interaction = 0.037). Compared with the reference group, TSE participants who were exposed to high concentrations of lead, cadmium, mercury, and manganese had significantly elevated TT levels, but these high levels presented no significant association with E2 levels. A significantly higher level of SHBG among TSE participants was detected in high concentrations for lead, cadmium, and mercury.

**Conclusion** TSE exacerbated sex hormone imbalances when combined with high levels of metal exposure. Smoking cessation is crucial, especially in the case of high levels of occupational exposure to heavy metals.

**Keywords** Joint effects · Heavy metal · Smoke exposure · Serum sex hormones · Adult males

## Introduction

Sex hormones are well known to be vital substances supporting good health in adult males, including total testosterone (TT), estradiol (E2) [1, 2], an imbalance of sex hormones in men being associated with diabetes [3], low bone mineral density [4], cardiovascular disease [5], and prostate cancer [6]. Sex hormone-binding globulin (SHBG) is a plasma glycoprotein synthesized in the liver which, along with albumin, binds to free sex hormones to maintain their balance [7].

Tobacco smoke exposure (TSE) is a serious environmental health threat responsible for reproductive toxicity given that the smoke contains thousands of toxic chemical substances that are harmful to humans. Sources of TSE include cigarette smoking, secondhand smoke, and thirdhand smoke residues attached, for example,to clothing, floors, and furniture [8]. Cigarette smoking may be a substantial source

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of TSE in adult males due to the increasing prevalence of smoking. Exposure to tobacco smoke may impair male reproductive function by influencing hormone levels, which further reduces the concentration and number of sperm as well as altering the morphology of sperm [9, 10]. Through measurement of the male reproductive hormone profile, a study found that current smokers had significantly higher levels of TT, while ex-smokers had sex hormone levels similar to those of never-smokers [11]. One study enrolled 4862 men aiming to determine whether TSE among adult males was associated with the concentrations of reproductive hormones. In the latter study, smokers also had higher TT than non-smokers, but no obvious discrepancies were found as regards SHBG and E2 [12].

Heavy metals are a class of pollutants that are found in tobacco, filters, cigarette smoke, and cigarette paper, which result in DNA damage, oxidative stress, and carcinogenicity [13]. Tobacco smoke contains toxic metals such as lead, cadmium, and mercury which are converted by burning into mainstream cigarette smoke. They eventually reach deep into the lungs and are deposited and absorbed into the blood. It is widely recognized that cigarette smoke inhalation is one of the main routes of cadmium exposure [14]. Many studies in both humans and animals have demonstrated that exposure to cadmium can disrupt the male endocrine system that affects sex hormone synthesis and homeostasis, although test results have not to date reached a consensus [15–17]. Apart from cadmium, it has additionally been confirmed that other heavy metal pollutants, such as arsenic, lead, and mercury, also induce reproductive toxicity [18, 19]. Previous studies have suggested that smoking increases the concentrations of heavy metals in the blood [20, 21]. A recent publication showed that blood concentrations of lead and cadmium in smokers are higher than in nonsmokers [22]. However, another recent study found a significant association between blood cadmium and non-smoker exposure to secondhand smoke and highlighted the importance of taking into serious consideration the severe harmful effects of passive smoking [23]. In addition, an earlier study which examined whether thirdhand smoke residue contributed to lead and cadmium in settled house dust showed that lead and cadmium were indeed significantly correlated with nicotine and accumulated in indoor dust [24].

Nowadays, the increasing number of smokers is placing ever more people at risk of active and/or passive exposure to tobacco smoke, which in turn makes it virtually impossible to avoid the dual effects of tobacco smoke and heavy metals. Separately estimating the effects of heavy metals, on the one hand, and of TSE, on the other hand, on sex hormones is, however, an ineffective approach. Cotinine, a metabolite of nicotine, is commonly used as a marker of TSE in both active [25] and passive smokers [23], which has been found to be associated with heavy metals such as chromium and lead. Therefore, the present study used cotinine as a marker reflecting the degree of TSE to determine whether adult males were exposed to tobacco smoke. In the present study, we not only explore the association between heavy metals and sex hormones in individuals with TSE and individuals with non-TSE, but also focus on the combined effects of TSE and heavy metals.

## Methods

### Study participants

The study used data from the National Health and Nutrition Examination Survey (NHANES) (https://www.cdc.gov/nchs/nhanes/index.htm) from 2013-2016. Study procedures were approved by the National Center for Health Statistics Research Ethics Review Board and informed consent was obtained from all participants [26]. NHANES has a unique advantage in that it enables utilization of a large, cross-sectional, multi-stage design for assessment of the health and nutritional status of a nationally representative sample. The following individuals were excluded: (1) age <18 years or females; (2) did not have available data on cotinine, heavy metals, or sex hormones; (3) use of sex hormones; and (4) covariates missing. Finally, a total of 2244 adult males were included in the final analysis (Fig. 1).

### Smoke exposure

Within the NHANES 2013-2016 cycles, the concentration of serum cotinine was quantified using an isotope-dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric (ID HPLC-APCI MS/MS) method. During a medical examination at a mobile medical examination center (MEC), blood samples were taken from the participants and properly processed, followed by storage in the appropriate frozen (-20°C) conditions. Cotinine is a reliable biomarker of TSE with a half-life of 20 h, This procedure is more representative than a self-reported questionnaire. Thus, TSE among males is defined as serum cotinine concentration higher than 10.0 ng/mL [27]. Heavy metals including lead, cadmium, mercury, and manganese, and sex hormone assessments have been meticulously illustrated in a previous study [28].

### Covariates

After retrieving relevant articles and carefully evaluating them, we selected the following confounding covariates: age, education (<high school, high school, and >high school), race (Mexican American, non-Hispanic white, non-Hispanic black, other Hispanic, or other), family poverty-income

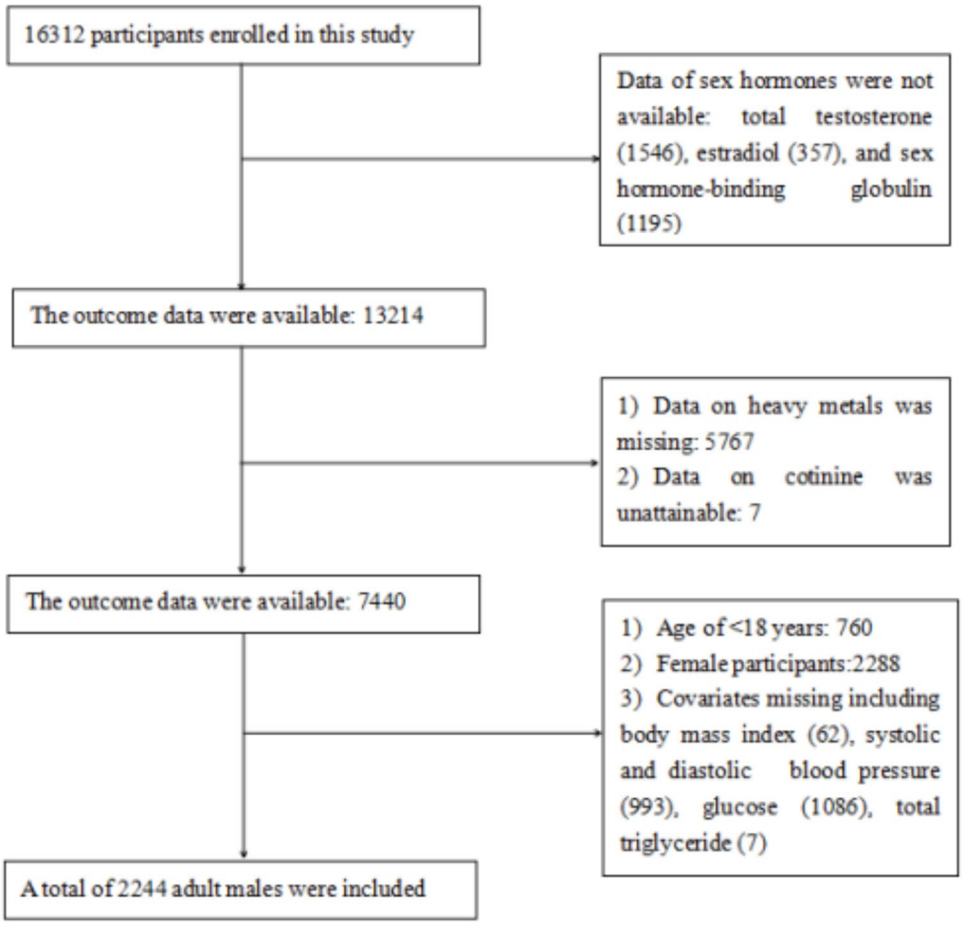


**Fig. 1** Flow chart presenting in detail the process of study selection

ratio (PIR), and alcohol consumption (0 drink/week, 1-6 drinks/week, or ≥7 drinks/week). Body mass index (BMI) was categorized as underweight (BMI<18.5 kg/m$^2$), normal weight (18.5 kg/m$^2$≤BMI<25 kg/m$^2$), overweight (25 kg/m$^2$≤BMI<30 kg/m$^2$) and obesity ( BMI≥30 kg/m$^2$). Hypertension was defined in patients with systolic blood pressure (SBP) as ≥140 mm Hg and/or diastolic blood pressure (DBP) ≥ 90 mm Hg. Diabetes was defined as fasting plasma glucose (Glu) ≥ 7 mmol/L. Patients with total cholesterol (TC) ≥ 5.72 mmol/L or total triglyceride (TG) ≥ 1.70 mmol/L were defined as having hyperlipidemia. Poverty was defined as a cutoff PIR of <1.

## Statistical analyses

In this study, none of the continuous variables passed the normal distribution check. Participants were divided into a TSE group and a non-TSE group. The Rao-Scott chi-square test and Mann-Whitney U test were used to test for differences between the two groups. The characteristics of the subjects were expressed by the median ($Q_{25}$, $Q_{75}$) for continuous variables, or unweighted frequencies (%) for categorical variables.

Outcome variables (TT, E2, and SHBG) were log-transformed and met the assumption of normality. Due to the non-significant association between original scale of heavy metals and outcome variables, the heavy metals were also log-transformed. Next, weighted linear regression models were used to calculate the β coefficients and corresponding confidence interval (95% CI) with adjustments for assumed covariates that assessed the effect of the relationship of cotinine and heavy metal exposure on sex hormones. Subjects were divided into four groups based on cotinine or metal quartiles [grouped into quartiles as follows: Q1 (reference), Q2, Q3, and Q4], and the lowest quartile was considered as the reference group. The median of each quartile group of cotinine or metals was included as a continuous variable in the model to test the potential linear trend.

Incorporated cross-product terms of heavy metals and TSE were used to identify their joint effects. Heavy metals were also divided into "high concentration" and "low concentration" groups based on their median and combined with linear modelling of TSE. The following four groups were generated: non-TSE with low concentration (reference group), non-TSE with high concentration, TSE with low concentration, and TSE with high concentration.

All analyses were performed using R software version 3.4.1 (http://www.R-project.org). A two-tailed *P* value of < 0.05 was considered as statistically significant.

## Results

### General characteristics

There were 733 males with TSE and 1511 males with non-TSE. A comparison of the general characteristics of the two groups is summarized in Table 1. TSE participants were younger compared with non-TSE participants. The results showed that TSE participants had higher levels of lead and cadmium but lower levels of mercury and manganese than non-TSE participants. In addition, pronounced differences were observed in race, BMI, smoking status, and poverty. Higher levels of TT and SHBG in TSE participants compared to non-TSE participants were also found.

### Association of cotinine and heavy metals with sex hormones

After adjustment for all the assumed covariates, SHBG showed a positive association with increased SD for cotinine (β=0.024 [0.004, 0.043]; *P*<0.001), lead (β=0.021 [0.002, 0.039]; *P*=0.028), and cadmium (β=0.034 [0.015, 0.053]; *P*<0.001). Manganese with per SD increment was positively associated with E2 (β=0.025 [0.009, 0.042]; *P*=0.002). Increased SD for cadmium was more likely to lead to higher TT (β=0.042 [0.023, 0.062]; *P*<0.001) (Table 2). Comparison was also carried out using quartiles. When the first quartile of metals or cotinine was used as a reference, a significantly higher level of TT was found in quartile 2 to 4 for lead and in quartile 2 and 4 for cadmium. E2 had lower levels in quartile 2 for mercury. An increased level of SHBG was detected in quartile 4 for cotinine and lead, and in quartile 2 and 3 for cadmium. However, quartile 4 for manganese showed decreased levels of SHBG (Table 2). The median of each quartile was substituted for the original data to seek the linear trend association. Cotinine and TT, cadmium and TT, cotinine and SHBG, and each metal and SHBG were linear trend associations (Table 2).

### Association between heavy metals and sex hormones stratified by TSE

In addition, the participants were also divided into two groups based on whether they experienced TSE, the results of which were used to explore relationship of metals on sex hormones. Among the TSE participants (Table S1), no metal was significantly associated with SHBG, while cadmium with per SD increment was positively associated with E2. The β-coefficients of relationship between manganese and E2 were higher than those of the participants overall. A significantly higher level of E2 was found in quartile 3 and 4 for manganese, the β-coefficients of relationship between lead and TT being lower than those of the participants overall. Among non-TSE participants (Table S2), E2 was positively linked to increased SD for manganese, but inversely associated with increased SD for lead and mercury. The negative associations were observed between lead and TT, as well as manganese and SHBG.

In TSE participants, cadmium and manganese showed a linear trend with E2 (Table S1). However, in non-TSE participants, only manganese showed a linear trend relationship with SHBG (Table S2).

### Joint effects between TSE and heavy metals on sex hormones

Table 3 and Fig. 2 show the results of joint effects between each heavy metal and TSE on sex hormones. There were statistically significant synergistic effects between TSE and lead on TT (*p* for interaction = 0.015) and E2 (*p* for interaction = 0.009), as well as TSE and cadmium on SHBG (*p* for interaction = 0.037).

Among TSE participants, a significantly higher level of TT was detected at a high concentration for each metal when compared with the reference group (Fig. 3). However, the β-coefficients for E2 were not significant at a high concentration for each metal. TSE participants had significantly elevated SHBG levels when exposed to high concentrations of lead, cadmium, and mercury. In addition, TT levels were higher at low concentrations for mercury and manganese. E2 levels were lower at low concentrations for lead, cadmium, and manganese. SHBG levels were higher at low concentrations for lead, mercury, and manganese. For non-TSE participants, a low concentration of manganese was inversely associated with SHBG.

## Discussion

This study provides novel insights into the effects of TSE and heavy metals on sex hormones in adult males. The main findings of the study were that synergistic effects were detected between TSE and lead and TSE and cadmium on TT, and were also observed between TSE and cadmium on SHBG. Moreover, we observed that cadmium and TT, manganese and E2, lead and SHBG, and cadmium and SHBG were positively associated. However, among TSE participants, there was no significant association between each metal and TT and SHBG. Cadmium and manganese showed a positive association with E2.

**Table 1** Baseline characteristics of the 2244 participants

| Characteristics | TSE | non-TSE | *P* |
|---|---|---|---|
| Age, y, median ($Q_{25}$, $Q_{75}$) | 42 (29; 58) | 51 (35; 65) | <0.001 |
| Lead, ug/dL, median ($Q_{25}$, $Q_{75}$) | 1.30 (0.85; 2.05) | 1.04 (0.68; 1.65) | <0.001 |
| Cadmium, ug/L, median ($Q_{25}$, $Q_{75}$) | 0.63 (0.32; 1.08) | 0.20 (0.14; 0.30) | <0.001 |
| Mercury, ug/L, median ($Q_{25}$, $Q_{75}$) | 0.64 (0.36; 1.21) | 0.84 (0.42; 1.76) | <0.001 |
| Manganese, ug/L, median ($Q_{25}$, $Q_{75}$) | 8.50 (6.95; 10.55) | 8.95 (7.25; 11.20) | 0.001 |
| TT, ng/dL, median ($Q_{25}$, $Q_{75}$) | 436.00 (324.00, 579.50) | 382.00 (294.00, 488.50) | <0.001 |
| E2, pg/mL, median ($Q_{25}$, $Q_{75}$) | 23.00 (17.80, 29.95) | 23.70 (18.50, 29.10) | 0.908 |
| SHBG, nmol/L, median ($Q_{25}$, $Q_{75}$) | 39.73 (28.15, 57.46) | 37.91 (26.99, 54.60) | 0.013 |
| Education, n (%) | | | 0.270 |
| Below high school | 208 (28.4) | 383 (25.3) | |
| High school | 206 (28.1) | 427 (28.3) | |
| Above high school | 319 (43.5) | 701 (46.4) | |
| Race, n (%) | | | <0.001 |
| Mexican American | 91 (12.4) | 254 (16.8) | |
| Other Hispanic | 52 (7.1) | 185 (12.2) | |
| Non-Hispanic White | 304 (41.5) | 570 (37.7) | |
| Non-Hispanic Black | 204 (27.8) | 229 (15.2) | |
| Other race | 82 (11.2) | 273 (18.1) | |
| Smoker, n (%) | | | <0.001 |
| Current smoker | 414 (56.5) | 213 (14.1) | |
| Past smoker | 120 (16.4) | 198 (13.1) | |
| Never | 199 (27.1) | 1100 (72.8) | |
| Alcohol intake n (%) | | | 0.353 |
| 0 drink/week | 477 (65.1) | 1024 (67.8) | |
| 1–6 drinks/week | 175 (23.9) | 321 (21.2) | |
| ≥ 7 drinks/week | 81 (11.1) | 166 (11.0) | |
| Poverty, n (%) | | | <0.001 |
| No | 527 (71.9) | 1289 (85.3) | |
| Yes | 206 (28.1) | 222 (14.7) | |
| Body mass index, n (%) | | | 0.017 |
| Underweight | 15 (2.1) | 35 (2.3) | |
| Normal weight | 277 (37.8) | 587 (38.8) | |
| Overweight | 325 (44.3) | 721 (47.8) | |
| Obesity | 116 (15.8) | 168 (11.1) | |
| Hypertension , n (%) | | | 0.498 |
| No | 323 (44.1) | 643 (42.6) | |
| Yes | 410 (55.9) | 868 (57.4) | |
| Hyperlipidemia, n (%) | | | 0.050 |
| No | 452 (61.7) | 866 (57.3) | |
| Yes | 281 (38.3) | 645 (42.7) | |
| Diabetes, n (%) | | | 0.150 |
| No | 649 (88.5) | 1305 (86.4) | |
| Yes | 84 (11.5) | 206 (13.6) | |

*TSE* tobacco smoke exposure; *TT* total testosterone; *E2* estradiol; *SHBG* sex hormone-binding globulin

Continuous variables were expressed as the median ($Q_{25}$, $Q_{75}$) and categorical variables are presented as unweighted frequencies (%). The Rao-Scott chi-square test and Mann-Whitney U test were used to compare categorical variables and continuous variables between the TSE group and the non-TSE group, respectively

**Table 2** Association of cotinine and heavy metals with sex hormones

| Cotinine and metals | Sex hormones (β) (95%CI) | | | |
|---|---|---|---|---|
| | N (%) | TT | E2 | SHBG |
| Cotinine* | | | | |
| Per SD increment | 2244 | 0.018 (-0.002, 0.038) | -0.009 ( -0.026, 0.009) | **0.024 (0.004, 0.043)** |
| Quartiles 1 | 621 (27.7) | 0 (Ref) | 0 (Ref) | 0 (Ref) |
| Quartiles 2 | 504 (22.4) | -0.010 (-0.062, 0.043) | -0.017 (-0.062, 0.027) | 0.028 (-0.023, 0.078) |
| Quartiles 3 | 558 (24.9) | -0.004 (-0.057, 0.048) | -0.001 (-0.045, 0.044) | 0.032 (-0.018, 0.083) |
| Quartiles 4 | 561 (25.0) | 0.080 (-0.023, 0.138) | -0.027 (-0.076, 0.023) | **0.100 ( 0.044, 0.156)** |
| *P* value for trend | | 0.001 | 0.537 | <0.001 |
| Lead | | | | |
| Per SD increment | 2244 | -0.003 ( -0.022, 0.016) | -0.009 (-0.026, 0.006) | **0.021 (0.002, 0.039)** |
| Quartiles 1 | 574 (25.6) | 0 (Ref) | 0 (Ref) | 0 (Ref) |
| Quartiles 2 | 552 (24.6) | **0.058 (0.005, 0.111)** | -0.006 (-0.051, 0.039) | 0.046 (-0.005, 0.097) |
| Quartiles 3 | 560 (25.0) | **0.075 (0.020, 0.130)** | -0.019 (-0.065, 0.028) | 0.041 (-0.012, 0.094) |
| Quartiles 4 | 558 (24.8) | **0.071 (0.013, 0.128)** | -0.014 (-0.063, 0.035) | **0.095 (0.039, 0.150)** |
| *P* value for trend | | 0.063 | 0.534 | 0.003 |
| Cadmium | | | | |
| Per SD increment | 2244 | **0.042 (0.023, 0.062)** | 0.015 (-0.002, 0.032) | **0.034 (0.015, 0.053)** |
| Quartiles 1 | 601 (26.8) | 0 (Ref) | 0 (Ref) | 0 (Ref) |
| Quartiles 2 | 548 (24.4) | **0.097 (0.045, 0.149)** | 0.014 (-0.030, 0.058) | **0.086 (0.036, 0.136)** |
| Quartiles 3 | 536 (23.9) | 0.051 (-0.003, 0.105) | -0.040 (-0.086, 0.006) | **0.087 (0.034, 0.140)** |
| Quartiles 4 | 559 (24.9) | **0.132 (0.076, 0.188)** | 0.034 (-0.014, 0.082) | 0.129 (- 0.074, 0.183) |
| *P* value for trend | | <0.001 | 0.123 | <0.001 |
| Mercury | | | | |
| Per SD increment | 2244 | 0.005 (-0.014, 0.025) | -0.017 (-0.033, 0.002) | -0.005 (-0.024, 0.013) |
| Quartiles 1 | 569 (25.3) | 0 (Ref) | 0 (Ref) | 0 (Ref) |
| Quartiles 2 | 558 (24.9) | -0.015 (-0.067, 0.037) | **-0.051 (-0.095, -0.007)** | -0.013 (-0.063, 0.037) |
| Quartiles 3 | 557 (24.8) | 0.024 (-0.029, 0.076) | -0.007 (-0.052, 0.037) | -0.015 (-0.036, 0.066) |
| Quartiles 4 | 560 (25.0) | 0.002 (-0.053, 0.056) | -0.088 (-0.063, 0.004) | -0.020 (-0.073, 0.032) |
| *P* value for trend | | 0.951 | 0.102 | 0.044 |
| Manganese | | | | |
| Per SD increment | 2244 | 0.003 (-0.016, 0.022) | **0.025 (0.009, 0.042)** | -0.032 (-0.051, 0.014) |
| Quartiles 1 | 563 (25.1) | 0 (Ref) | 0 (Ref) | 0 (Ref) |
| Quartiles 2 | 565 (25.2) | -0.018 (-0.070, 0.034) | 0.012 (-0.032, 0.056) | -0.048 (-0.098, 0.002) |
| Quartiles 3 | 556 (24.8) | -0.024 (-0.076, 0.029) | 0.041 (-0.004, 0.086) | -0.095 (-0.146, -0.045) |
| Quartiles 4 | 560 (24.9) | -0.018 (-0.072, 0.036) | 0.046 (-0.001, 0.092) | **-0.108 (-0.161, -0.056)** |
| *P* value for trend | | 0.389 | 0.409 | <0.001 |

*β* beta coefficients; *SD* standard deviation; *CI* confidence interval; *TT* total testosterone; *E2* estradiol; *SHBG* sex hormone-binding globulin

Model adjusted for age, education, race, body mass index, smoking status, alcohol intake, poverty, hypertension, hyperlipidemia, and diabetes. *Model adjusted for assumed covariates excluding smoking status

It has been reported that second and thirdhand exposure to tobacco smoking could contribute as one source of exposure to heavy metals [2]. Compared with calculation of smoking habits (smoking status or level of tobacco consumption) for estimations, using cotinine as a marker in adult males exposed to tobacco smoke more efficaciously assesses oxidative damage caused by heavy metals as related to TSE. Willers et al. [29] have demonstrated that lead and cadmium concentrations are associated with cotinine in children's exposure to environmental tobacco smoke (ETS). Strong associations between the ETS exposure index and cotinine were also observed. Most of the existing studies used data from self-reported smoking habits rather than confirmation based on cotinine. For example, several studies have found that lead and cadmium accumulate in the blood as smoking increases [30, 31]. Another study which investigated the effects of smoking on blood lead and cadmium levels in residents living close to a mining and smelting

**Table 3** Joint effects exerted by TSE and heavy metals on sex hormones

| Sex hormones | Heavy metals | β (CI: 95%) | *P* |
|---|---|---|---|
| TT | Lead | 0.098 (0.018,0.177) | **0.015** |
| | Cadmium | 0.089 (-0.006,0.185) | 0.068 |
| | Mercury | 0.004 (-0.073,0.083) | 0.901 |
| | Manganese | 0.018 (-0.059,0.097) | 0.636 |
| E2 | Lead | 0.090 (0.022,0.157) | **0.009** |
| | Cadmium | 0.072 (-0.009, 0.155) | 0.081 |
| | Mercury | 0.037 (-0.029, 0.104) | 0.268 |
| | Manganese | 0.062 (-0.004, 0.128) | 0.068 |
| SHBG | Lead | 0.030 (-0.046,0.108) | 0.431 |
| | Cadmium | 0.098 (0.005,0.191) | **0.037** |
| | Mercury | -0.028 (-0.104,0.047) | 0.463 |
| | Manganese | 0.021(-0.054,0.097) | 0.576 |

*β* beta coefficients; *CI* confidence interval; *TSE* tobacco smoke exposure. *TT* total testosterone; *E2* estradiol; *SHBG* sex hormone-binding globulin

β and 95% CI were calculated in linear regression models with adjustments for age, education, race, body mass index, alcohol intake, poverty, hypertension, hyperlipidemia, and diabetes

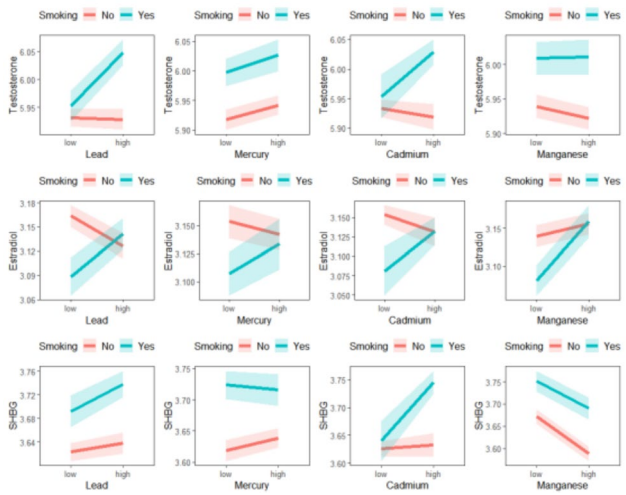


**Fig. 2** Joint effects exerted by each heavy metal and smoke exposure on sex hormones

area in China who were exposed to high levels of lead and cadmium showed that smokers had significantly higher blood lead and cadmium levels than non-smokers [22]. Our researchers, unlike those of the previous study, separated the participants into TSE and non-TSE groups based on the cutoff values of serum cotinine. It was revealed that TSE participants had higher levels of lead and cadmium than non-TSE participants, further confirming that exposure to tobacco smoke increases the accumulation of cadmium and lead in the human body. Interestingly, lower levels of mercury and manganese were observed in TSE participants as compared with the non-TSE participants. On the other hand, previous studies have demonstrated that exposure to ETS may be one source of increased exposure to mercury [2] and manganese [32]. The discrepancies could be attributed to the fact that the TSE participants were younger than non-TSE participants. Mercury and manganese in the blood were mainly absorbed from food or water by the gut and gradually accumulated in the body with increasing age.

As components of tobacco smoke, heavy metals accumulate in the body and interfere with the endocrine system as exposure to tobacco smoke increases. A cross-sectional study which enrolled 2286 Chinese men reported that blood cadmium level was negatively associated with TT and SHBG [33]. Additionally, a study based on the NHANES data found that lead and TT, lead and SHBG, and cadmium and SHBG in blood were positively associated [7], this being in line with our results. A study conducted by Kresovich et al. found that blood lead levels and testosterone showed a significant positive association, but there were no statistically significant differences observed between lead and free testosterone or SHBG. In the latter investigation, cadmium was positively associated with SHBG, but not TT or E2 or the free levels of these hormones [7]. Additionally, they also observed that the relationship between blood lead level and TT showed a positive trend among current smokers. A meta-analysis [34] of observational studies concluded that smoking was associated with higher TT, which was consistent with other study findings [7] that current smokers had significantly higher levels of TT concentrations than never-smokers. What is difficult to determine is whether the effects of TSE on sex hormones are due to the tobacco smoke itself or to the heavy metals present in aerosolized smoke. Therefore, one issue that needs to be clarified is the difference between the effects of heavy metal exposure on sex hormones among TSE and non-TSE individuals, respectively.

In our study, the associations between lead concentrations and sex hormones were significantly different in TSE participants and non-TSE participants. Lead showed a positive trend with TT and E2 in the presence of TSE, but showed a negative relationship with TT and E2 in the case of non-TSE. It is noteworthy that SHBG levels increased in parallel with accumulation of lead and cadmium in overall participants. However, there were no significant associations of lead and cadmium with SHBG levels when participants were separated into the TSE group and the non-TSE group. One possible explanation for this finding is that the actual link between lead and cadmium and sex hormones is likely confounded by TSE. As is well known, SHBG is a liver-secreted protein that is involved in the regulation of sex steroid hormone bioavailability. TT and E2 exist in free form in the blood or bind to albumin and SHBG. Our investigation found that SHBG levels were increased in parallel with an increase in

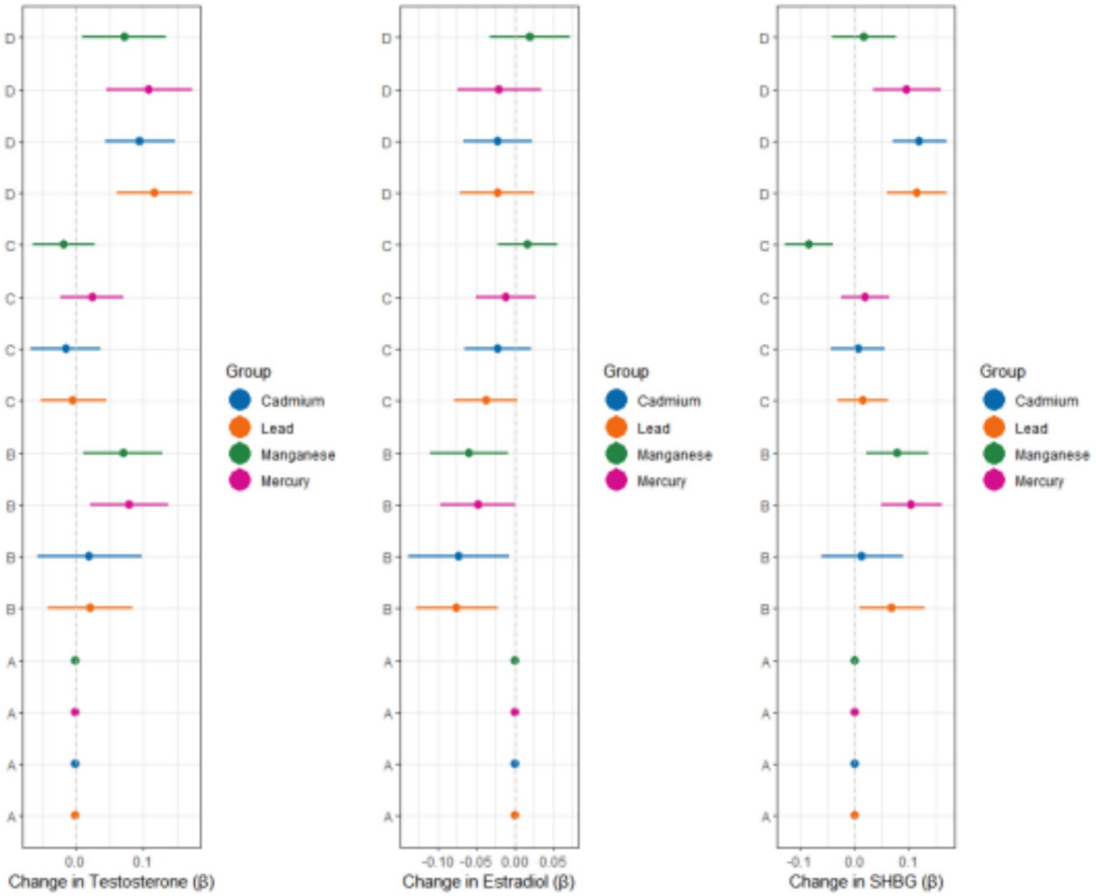


**Fig. 3** Change in sex hormones in (**B**); TSE participants with low concentration; (**C**) non-TSE participants with high concentration; (**D**) TSE participants with high concentration for each metals when compared with (**A**) non-TSE participants with low concentration

cotinine, while E2 and TT were not associated with cotinine. A metabolite of nicotine in cigarettes, such as cotinine, shares a disposal pathway with androgens and alters the feedback mechanism of the hypothalamic-pituitary-gonadal axis and thus increases androgens [35]. It is reported that one cigarette contains 1-2 μg of cadmium, of which about 10% is inhaled [33]. Cadmium level in the blood may serve as a good reflection of TSE [36]. Our study suggested that cadmium was positively associated with TT and SHBG in participants overall, but was associated with E2 only in TSE participants. Androstanedione is an intermediate product of the biochemical synthesis of testosterone and E2. Aromatase converts testosterone to E2 through the transformation of androstanedione to E2. Androstanedione glucuronide, as a metabolite of androstenedione, reflects the extent to which androstenedione is converted to E2. A previous study found that lead and cadmium were not associated with androstanedione glucuronide, hinting that these metals did not affect the synthesis of TT or E2 directly [7].

It could be hypothesized that in the case of TSE, the increased presence of these metals in E2 and TT was mainly due to the competitively stimulated feedback mechanism of cotinine. These mechanisms are outlined below. Influenced by TSE, SHBG increased significantly, resulting in increased TT levels and decreased free testosterone levels, which in turn weakened the inhibitory effect of testosterone on hypothalamic-pituitary negative feedback and increased pituitary luteinizing hormone (LH) secretion, which activated testicular leydig cells to produce more testosterone. Our current research found that TSE and lead exerted synergistic effects on TT and E2; meanwhile, TSE and cadmium showed synergistic effects on SHBG, which could be attributable to lead showing a positive trend with TT and E2 in TSE participants, while it had a significantly negative association in non-TSE participants. It appears that TSE and high metal concentration work synergistically thereby increasing TT and SHBG levels, although the underlying mechanism remains as yet unclarified. A 2021 study recruited 267 workers with lead exposure in order to evaluate oxidative stress by measuring thiobarbituric acid reactive substances (TBARS) in both smokers and nonsmokers. The results indicated that the increased serum levels of TBARS and

the ratio of peroxidation markers are driven by interaction between blood lead levels and smoking [37]. Future studies are required to focus on the joint effects of heavy metals and smoking on sex hormones and the role that oxidative stress plays in them.

Our research has a number of strengths. Of note, this was, to the best of our knowledge, the first study to comprehensively explore the interaction between TSE and blood heavy metals on sex hormones, the results potentially providing more precise estimates of their relationship. Additionally, participants were selected from the NHANES dataset, which reflected the overall situation in the USA, the results of which tend to be more persuasive. Furthermore, because of the inevitable information bias induced by self-reported questionnaires, TSE was defined based on the cutoff values of serum cotinine concentrations,which is more reliable.

Several potential limitations warrant attention in this study. Firstly, NHANES was a cross-sectional survey that temporally recorded the levels of heavy metals, cotinine, and sex hormones so that changes of the measurements could not be observed continuously. Therefore, it was not only impossible for us to determine causality, but it was also unlikely that we could exclude reverse causality. Secondly, although we adjusted for relevant covariates when evaluating the joint effects of heavy metals and TSE on sex hormones, the results of this study were likely confounded by the overall effect of the mixture of blood heavy metals.

## Conclusion

Our results indicated that there are synergistic effects between TSE and lead level on TT and E2, as well as TSE and cadmium on SHBG; they also underlined the fact that smoking exacerbates sex hormone imbalances under high levels of metal exposure. The latter findings may provide evidence of the importance of smoking cessation, especially in the case of concurrent high levels of occupational exposure to heavy metals.

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**Author contributions** Xiaoguo Hua performed the data analyses and wrote the manuscript. Rui Hu revised the final typescript. Cai Chen searched for and evaluated the retrieved articles. Xiqiu Feng and Jiangjie Sun contributed to the conception of the study. Xiujun Zhang were responsible for the communication of the paper.

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**Data availability** The data used to support the findings of this study are available from the corresponding author upon request.

## Declarations

**Ethics approval** The application of every technique followed all applicable rules and regulations. The US National Center for Healthcare Statistics (NCHS) granted permission for the implementation of NHANES for 2013–2016.

**Informed consent** Not applicable

**Registry and registration No. of the study/trial** Not applicable

**Animal studies** Not applicable

**Conflict of interest** The authors declare that they have no conflict of interest.

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