Research article

The association of urinary cadmium with sex steroid hormone concentrations in a general population sample of US adult men

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Abstract

Background: Studies investigating the association of cadmium and sex steroid hormones in men have been inconsistent, but previous studies were relatively small.

Methods: In a nationally representative sample of 1,262 men participating in the morning examination session of phase I (1998–1991) of the third National Health and Nutrition Examination Survey, creatinine corrected urinary cadmium and serum concentrations of sex steroid hormones were measured following a standardized protocol.

Results: After adjustment for age and race-ethnicity, higher cadmium levels were associated with higher levels of total testosterone, total estradiol, sex hormone-binding globulin, estimated free testosterone, and estimated free estradiol (each p-trend < 0.05). After additionally adjusting for smoking status and serum cotinine, none of the hormones maintained an association with urinary cadmium (each p-trend > 0.05).

Conclusion: Urinary cadmium levels were not associated with sex steroid hormone concentrations in a large nationally representative sample of US men.

Background

Cadmium is a widespread toxic and carcinogenic metal with numerous adverse health effects in humans [1]. In the general population, environmental exposure to cadmium occurs primarily through smoking, the consumption of contaminated food and water, and inhalation of contaminated air [2,3].
Cadmium shows androgen and estrogen-like activities in vitro and in vivo, and it disrupts the male endocrine system in animal models. There is conflicting evidence regarding whether cadmium increases or decreases testosterone production in experimental models [4-6], with decreases more commonly seen after a single, large dose injection of cadmium and increases more commonly seen after chronic oral cadmium exposure [7]. Studies investigating the association of cadmium and sex steroid hormones in men have been inconsistent, finding either no association [8-10], or a positive association between cadmium and testosterone levels [11-13]. Inconsistencies in these studies may be due to small sample sizes, to differences in study design, or to inadequate control of confounders such as tobacco use, a major source of cadmium [14] that is also associated with higher testosterone levels in men [15,16].

The purpose of the current analysis was to evaluate the association of urinary cadmium levels, a biomarker of long term and ongoing cadmium exposure, with serum concentrations of sex steroid hormones (total testosterone, total estradiol, androstenediol glucuronide [AAG], estimated free testosterone, and estimated free estradiol) and sex hormone-binding globulin (SHBG) in the Third National Health and Nutrition Examination Survey (NHANES III).

Methods

Study Population

NHANES III was a stratified, multistage probability survey designed to select a representative sample of the civilian non-institutionalized US population [17]. NHANES III included two phases (phase I: October 1988 – October 1991 and phase II: September 1991 – October 1994), each capable of independently producing unbiased national estimates. Within each phase, participants were randomly assigned to either a morning or afternoon/evening examination session of phase I (N = 1,967). The study was restricted to participants in the morning session to reduce extraneous variation due to diurnal production of sex hormones. Serum for hormone assays was available for 1,470 participants (75%). After excluding 25 participants missing data for urinary cadmium, 11 participants missing data for urinary creatinine, 7 participants missing data for serum testosterone, 5 participants missing data for serum SHBG, 9 participants missing data for serum albumin, 11 participants missing data for serum cotinine, and 140 participants missing data for other covariates, the final sample included 1,262 men. The protocol for NHANES III was approved by the National Center for Health Statistics of the Center for Disease Control and Prevention Institutional Review Board. All participants gave written informed consent. The assay of stored serum specimens for the Hormone Demonstration Program was approved by the Institutional Review Boards at the Johns Hopkins Bloomberg School of Public Health and the National Center for Health Statistics, Centers for Disease Control and Prevention.

Data Collection

Demographic, household income, physical activity, cigarette smoking, and alcohol consumption data were collected using standardized questionnaires during an in-home interview [17]. Whole body electrical resistance was measured using a Valhalla Scientific Body Composition Analyzer (model 1990B; Valhalla Scientific, Inc., San Diego, CA) and prediction equations were used to predict percent body fat [18]. Serum cotinine was measured using liquid chromatography/atomospheric pressure ionization tandem mass spectrometry [19]. Atomic absorption spectrometry was used to measure blood lead as described by Sassa and colleagues [20] and serum selenium as described by Lewis and colleagues [21].

All materials used for collecting and processing urinary cadmium specimens were screened for possible cadmium contamination [22]. A spot urine specimen was collected and shipped at -20°C to the NHANES laboratory at the National Centers for Environmental Health at the Centers for Disease Control and Prevention in Atlanta, GA. Urinary cadmium was measured by graphite furnace atomic absorption with Zeeman background correction, using the CDC modification of the method of Pruszkowska and colleagues [23]. Since NHANES III only collected spot urine samples, all analyses were performed using creatinine corrected urinary cadmium values (urinary cadmium divided by urinary creatinine concentrations, expressed as µg/g) to account for between participant differences in urine dilution. Urinary creatinine was measured using the method of Jaffe with a Beckman ASTRA automated analyzer [24].

Sex Steroid Hormones

Participants in the morning examination session fasted overnight before having blood drawn. After centrifugation, serum was aliquotted and stored at -70°C. Serum concentrations of testosterone, estradiol, AAG (a metabolite of dihydrotestosterone), and SHBG were measured in 2005 at the laboratory of Dr. Nader Rifai at Children’s Hospital in Boston, MA. Sex steroid hormone levels and SHBG are stable after multiple freeze-thaw cycles [25,26]. Testosterone, estradiol, and SHBG levels were quantified using competitive electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN). AAG was measured by an enzyme immunoassay (Diagnostics Systems Laboratories, Webster, TX). The detection limits of the assays were 0.02...
ng/mL for testosterone, 5 pg/mL for estradiol, 0.33 ng/mL for AAG, and 3 nmol/L for SHBG. The coefficients of variation (CV%) for quality control specimens included during the analyses of the NHANES III samples ranged from 5.8 to 5.9% for testosterone, 2.5 to 6.7% for estradiol, 5.0 to 9.5% for AAG, and 5.3 to 5.9% for SHBG. Free testosterone concentrations were estimated from measured testosterone, SHBG, and albumin, while free estradiol concentrations were estimated from measured estradiol, SHBG, and albumin [27,28].

Statistical Analysis
Participants were categorized into quartiles of urinary cadmium based on the weighted population distribution. Age and race-ethnicity adjusted means and percentages were calculated by linear regression for continuous covariates and logistic regression for dichotomous covariates.

Due to skewed distributions of sex steroid hormones, we calculated adjusted geometric means by quartile of cadmium using multiple linear regression on log-transformed hormone levels. We also present analyses for molar ratios of estradiol to testosterone, testosterone to SHBG, and estradiol to SHBG. Initial models adjusted for age and race-ethnicity. Subsequent models further adjusted for smoking status and serum cotinine. Additional models further adjusted for household income, physical activity, alcohol consumption, percent body fat, blood lead, and serum selenium. Tests for linear trend across quartiles of cadmium were computed by including an ordinal variable with the median of each quartile of cadmium in the linear regression models. Due to the importance of smoking in determining cadmium levels and the possibility of overadjustment, we additionally present models separately for never, former, and current smokers.

Data were analyzed using SUDAAN (version 9.0; Research Triangle Institute, Research Triangle Park, NC) to account for the complex NHANES sampling design, including unequal probabilities of selection, over-sampling, and non-response.

Results
The median level (range) of urinary cadmium in the study sample was 0.34 µg/g creatinine (0.003–4.22 µg/g). Participants with higher cadmium levels were more likely to be older, to be non-Hispanic black, to be current smokers, to have household incomes < $20,000, not to exercise, and to have higher blood lead levels (Table 1). The median levels (range) of urinary cadmium among never (n = 436), former (n = 416), and current (n = 410) smokers were 0.21 µg/g (0.003–4.22 µg/g), 0.42 µg/g (0.004–2.32 µg/g), and 0.56 µg/g (0.004–4.02 µg/g), respectively. After adjustment for age and race-ethnicity, higher cadmium levels were associated with higher levels of total testosterone, total estradiol, SHBG, estimated free testosterone, and estimated free estradiol (each p-trend < 0.05), but not with AAG or any of the molar ratios (Table 2). After additionally adjusting for smoking status and serum cotinine, the associations of urinary cadmium levels with sex steroid hormones and their molar ratios were small and not statistically significant (each p-trend > 0.05). After stratifying by smoking status, the associations of urinary cadmium levels with sex steroid hormones and their molar ratios were small and not statistically significant (each p-trend > 0.05; Table 3). However, among never smokers there was a marginally significant, negative association between urinary cadmium and total testosterone (p-trend = 0.06) and among current smokers there was a marginally signif-

| Table 1: Age and race-ethnicity adjusted participant characteristics* by quartile of creatinine corrected urinary cadmium |
|---------------------------------------------------------------|
| Quartile 1 < 0.18 µg/g | Quartile 2 0.18–0.33 µg/g | Quartile 3 0.34–0.62 µg/g | Quartile 4 ≥ 0.63 µg/g | p-trend |
| Age, years | 31.7 (0.8) | 37.1 (0.7) | 44.6 (1.3) | 54.3 (1.2) | < 0.001 |
| Non-Hispanic white, % | 83.9 (2.9) | 80.3 (3.4) | 74.5 (5.6) | 74.3 (5.2) | 0.07 |
| Non-Hispanic black, % | 6.5 (1.5) | 10.9 (2.2) | 8.1 (1.1) | 11.1 (1.7) | 0.03 |
| Mexican-American, % | 4.2 (1.0) | 5.6 (1.0) | 4.5 (0.9) | 4.7 (0.8) | 0.99 |
| Current smokers, % | 11.2 (2.1) | 20.4 (3.7) | 42.9 (4.2) | 73.6 (2.4) | < 0.001 |
| Former smokers, % | 29.3 (4.3) | 33.9 (4.2) | 35.8 (3.7) | 25.7 (3.2) | 0.06 |
| Serum cotinine, ng/mL | 26.4 (8.4) | 39.6 (8.4) | 107.2 (12.9) | 231.0 (12.1) | < 0.001 |
| Low income, % | 24.1 (3.0) | 20.4 (2.7) | 31.0 (2.9) | 42.1 (4.0) | < 0.001 |
| Consume alcohol, % | 75.6 (4.1) | 71.5 (3.4) | 74.8 (3.2) | 72.7 (3.8) | 0.76 |
| No exercise, % | 40.6 (6.1) | 30.1 (3.5) | 41.0 (4.4) | 55.2 (4.2) | 0.005 |
| Percent body fat, mean percent | 25.8 (0.5) | 26.1 (0.5) | 25.4 (0.6) | 24.7 (0.5) | 0.05 |
| Blood lead, µg/dL† | 3.2 (2.8, 3.6) | 3.6 (3.2, 4.0) | 4.3 (3.9, 4.8) | 5.5 (4.9, 6.3) | < 0.001 |
| Serum selenium, ng/mL | 126.5 (2.7) | 126.7 (1.1) | 127.3 (1.3) | 121.7 (1.3) | 0.05 |

*Mean or percentage (standard error)  
†Geometric mean (95% confidence interval)
significant, positive association between urinary cadmium and free testosterone concentrations (p-trend = 0.09).

**Discussion**

In this large, representative sample of US adult men, urinary cadmium levels were not associated with sex steroid hormone levels after adjustment for confounders. In our analysis, smoking was an important confounder of the association between cadmium and sex steroid hormone levels. The lack of association between cadmium and sex steroid hormones was also evident after stratifying by smoking status, although we identified a marginally significant association between cadmium and total testosterone among never smokers and free testosterone among current smokers. These findings may be explained as chance findings or a result of residual confounding, although we cannot exclude a modest association.

Four previous studies have evaluated the association of urine or blood cadmium with sex steroid hormone levels in men [10-13]. In a cross-sectional study of Chinese men with environmental exposure to cadmium, blood and urine cadmium did not meet the criteria for inclusion in stepwise models predicting testosterone levels [10]. Blood cadmium was positively associated with testosterone in two separate studies of Croatian men who had never been occupationally exposed to cadmium [11,12] and in a study of Chinese male smelter workers [13]. These studies...
Our findings pertain to low-level chronic environmental cadmium exposure and may not be generalizable to environmental or occupational settings involving higher doses of cadmium. Indeed, differences in cadmium levels may account for the inconsistent results in previous research. Additionally, urinary cadmium reflects predominantly cumulative long-term exposure and we cannot rule out short-term effects of cadmium exposure on sex steroid hormone levels. Due to measurement error. Consequently, we cannot exclude the existence of a modest association between urine cadmium and sex steroid hormone levels. However, the inconsistent findings in experimental models and the lack of a clearly delineated biological mechanism that could explain an effect of chronic environmental cadmium exposure on sex steroid hormone levels. Our analysis was based on single measures of sex steroid hormones in plasma and of cadmium in spot urine samples, all of which were subject to substantial within person variability and laboratory measurement error. Consequently, we cannot exclude the existence of a modest association between urine cadmium and sex steroid hormone levels. However, the inconsistent findings in experimental models and the lack of a clearly delineated biological mechanism that could explain an effect of chronic environmental cadmium exposure on sex steroid hormone levels.

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Table 3: Adjusted* geometric means (95% confidence interval) by quartile of creatinine corrected urinary cadmium and smoking status.

| Total testosterone, ng/mL | Quartile 1 < 0.18 µg/g | Quartile 2 0.18–0.33 µg/g | Quartile 3 0.34–0.62 µg/g | Quartile 4 ≥ 0.63 µg/g | p-trend |
|---------------------------|------------------------|---------------------------|---------------------------|------------------------|---------|
| Never smokers             | 0.102 (0.094, 0.111)    | 0.099 (0.088, 0.111)      | 0.084 (0.063, 0.112)      | 0.085 (0.064, 0.114)   | 0.14    |
| Former smokers            | 0.083 (0.075, 0.091)    | 0.084 (0.071, 0.099)      | 0.086 (0.079, 0.093)      | 0.084 (0.076, 0.093)   | 0.97    |
| Current smokers           | 0.109 (0.097, 0.123)    | 0.114 (0.106, 0.123)      | 0.129 (0.121, 0.137)      | 0.126 (0.117, 0.136)   | 0.09    |
| SHBG, nmol/L              |                        |                           |                           |                        |         |
| Never smokers             | 0.81 (0.73, 0.90)       | 0.86 (0.79, 0.94)         | 0.89 (0.78, 1.00)         | 0.77 (0.60, 1.00)      | 0.92    |
| Former smokers            | 0.84 (0.75, 0.94)       | 0.86 (0.80, 0.92)         | 0.82 (0.78, 0.87)         | 0.84 (0.77, 0.91)      | 0.87    |
| Current smokers           | 1.00 (0.90, 1.10)       | 0.99 (0.92, 1.05)         | 1.16 (1.09, 1.23)         | 1.09 (1.01, 1.17)      | 0.39    |
| Androstaneol glucuronide, ng/mL | 6.67 (6.67, 8.03) | 7.37 (6.80, 9.10) | 1.16 (6.75, 11.50) | 7.54 (6.20, 9.17) | 0.16 |
| Never smokers             | 8.08 (6.96, 9.37)       | 8.41 (7.36, 9.60)         | 7.62 (7.02, 8.28)         | 7.89 (7.10, 8.77)      | 0.71    |
| Current smokers           | 7.41 (6.75, 8.14)       | 7.15 (6.62, 7.72)         | 7.71 (7.25, 8.19)         | 7.16 (6.58, 7.79)      | 0.51    |
| Total testosterone/SHBG   |                        |                           |                           |                        |         |
| Never smokers             | 0.539 (0.490, 0.593)    | 0.520 (0.470, 0.575)      | 0.457 (0.337, 0.619)      | 0.453 (0.332, 0.619)   | 0.25    |
| Former smokers            | 0.416 (0.364, 0.474)    | 0.448 (0.382, 0.525)      | 0.420 (0.380, 0.464)      | 0.412 (0.370, 0.459)   | 0.59    |
| Current smokers           | 0.507 (0.456, 0.563)    | 0.552 (0.491, 0.621)      | 0.628 (0.575, 0.685)      | 0.588 (0.535, 0.646)   | 0.31    |
| Estradiol*1000/SHBG       |                        |                           |                           |                        |         |
| Never smokers             | 3.60 (3.09, 4.19)       | 3.83 (3.37, 4.36)         | 4.03 (3.29, 4.93)         | 3.42 (2.62, 4.45)      | 0.88    |
| Former smokers            | 3.35 (2.70, 4.15)       | 3.77 (3.37, 4.20)         | 3.20 (2.90, 3.53)         | 3.25 (2.86, 3.70)      | 0.52    |
| Current smokers           | 3.75 (3.30, 4.27)       | 3.95 (3.44, 4.53)         | 4.84 (4.35, 5.38)         | 4.22 (3.66, 4.86)      | 0.72    |
steroid hormone concentrations support our findings of no association.

Conclusion
Cadmium binds with high affinity to the androgen receptor, where it inhibits the binding of androgens and induces androgen-like effects [29]. While cadmium itself may exert androgen-like effects, our analysis indicates that urinary cadmium is not an important determinant of sex steroid hormone levels in adult men at the range of exposure evaluated in the general US population.

Abbreviations
AAG – androstanediol glucuronide; SHBG – sex hormone-binding globulin; NHANES III – Third National Health and Nutrition Examination Survey

Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
AM, EG, MSS, and EAP were responsible for the conception and design, analysis and interpretation of data. AM drafted the manuscript, that was edited by EG, MSS, and EAP. SR, SB, NR, and WGN were responsible for interpreting the data and revising the manuscript. All authors read and approved the final manuscript.

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