Urinary Cadmium Excretion Is Associated With Increased Synthesis of Cortico- and Sex Steroids in a Population Study

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Context: Urinary cadmium (Cd) excretion is associated with cancer and cardiovascular morbidity. A potential mechanism could be disturbance of steroidogenesis in gonads and adrenal glands.

Objective: We tested whether urinary excretion of Cd is correlated with that of cortico- and sex steroid metabolites in the general adult population.

Setting: The Swiss Kidney Project on Genes in Hypertension is a multicentric, family-based population study.

Measures: Urinary excretions of steroid hormone metabolites and Cd were measured with separate day and night collections. Associations were analyzed by mixed linear models.

Results: Urinary Cd and testosterone excretions in men were significantly correlated (respective day and night β values [standard error (SE)], 1.378 [0.242], $P < 0.0005$; and 1.440 [0.333], $P < 0.0005$), but not in women [0.333(0.257), $P = 0.2$; and 0.674 (0.361), $P = 0.06$]. Urinary Cd and cortisol excretions were positively associated in both sexes [day: $\beta = 0.475$ (SE, 0.157), $P = 0.0025$, and 0.877 (SE, 0.194), $P < 0.0005$, respectively; night: $\beta = 0.875$ (SE, 0.253), $P < 0.0005$ and 1.183 (SE, 0.277), $P = 0.00002$, respectively]. Cd excretion was correlated with mineralocorticoid metabolites excretion, except tetrahydroaldosterone, in both sexes ($P < 0.01$). There was an independent effect of Cd on sex hormone and corticosteroid synthesis and an interdependent effect on gluco- and mineralcorticoid production.

Conclusion: Our findings provide evidence for a global stimulating effect on steroid synthesis already at low-dose Cd exposure. These findings might explain the association of Cd with diseases such as steroid-sensitive cancers or metabolic disorders. (***J Clin Endocrinol Metab*** **103: 748–758, 2018)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 7 July 2017. Accepted 17 October 2017.
First Published Online 25 October 2017

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Abbreviations: 5α-THB, 5α-tetrahydrocorticosterone; 5α-THF, 5α-tetrahydrocortisol; 11β-OH-An, 11β-hydroxyandrosterone; An, androsterone; BMI, body mass index; BP, blood pressure; Cd, cadmium; eGFR, estimated glomerular filtration rate; Et, etiocholanolone; HHG, hypothalamic-pituitary-gonadal; THA, tetrahydrodehydrocorticosterone; THALDO, tetrahydroaldosterone; THB, tetrahydrocorticosterone; THF, tetrahydrocortisol; THS, 11-deoxycortisol.

Cadmium (Cd) is a health hazard for people working in the industry (especially iron, steel, nonferrous metals, and cement production) and in general populations with high exposure. There is a widespread, low-level Cd contamination of agricultural soil in many areas of the world and, because Cd is easily taken up by crops such as rice, wheat, potatoes, and vegetables, the exposure to Cd from foods in many areas is high enough to be of importance to human health (1). Cd accumulates mainly in the kidneys, and the concentration of Cd in the kidneys is reflected in its concentration in urine. Thus, urinary Cd concentration is commonly used as a surrogate for the body burden in health risk assessment (2). Low iron stores seem to increase gastrointestinal absorption of Cd and, because Cd in tobacco smoke is effectively absorbed in the lungs, current smoking habits are important determinants of Cd uptake (3).

The toxic effects of Cd were initially considered to be limited to kidney and bone damage, renal disease being the most studied Cd-induced pathology (4). Then the International Agency for Research on Cancer confirmed there is sufficient evidence of Cd being a human carcinogen, a conclusion based mainly on lung cancer studies of workers exposed to Cd (5). Some of the cancers associated with Cd exposure are hormone related (*e.g.*, prostate cancer) (6). Because Cd has also been proposed as an endocrine-disrupting chemical (7), one could speculate that this increased risk of hormone-related cancers could be due to stimulated hormone synthesis in gonadal glands. Population-based studies in China found an association between urinary Cd and increased serum levels of testosterone in male workers occupationally exposed to Cd, as well as in healthy male volunteers (8, 9). There was also an association of Cd with increased serum levels in other male populations of European origin, but this relation was confounded by lead (10, 11). In postmenopausal women, a Japanese study found a moderate association between urinary Cd and increased testosterone levels (12). However, in a second study, the associations between urinary Cd and serum levels of testosterone and estradiol were inversely related (13). To our knowledge, no population-based study has looked at the relation between the urinary excretion of Cd and of testosterone and other androgen metabolites.

Several studies in populations with low Cd exposure found an association between urinary Cd levels and an increased risk for cardiovascular diseases, an effect that was independent of the smoking habits of the study participants (14–16). Urinary Cd has also been associated with prediabetes (17). One of the several potential mechanisms that might explain cardiovascular morbidity associated with Cd could be an increase in blood pressure (18). Because arterial hypertension is a well-known adverse effect of the overproduction of corticosteroids (*i.e.*, glucocorticoids and mineralocorticoids), we hypothesized that urinary Cd was also associated with an increased synthesis of corticosteroids. Increased production of glucocorticoids could also contribute to insulin resistance and the development of prediabetes. To our knowledge, the association of urinary Cd and the excretion of gluco- and mineralocorticoid metabolites has not been explored previously in a population-based study.

Steroid hormone production starts with cholesterol and mainly occurs in the adrenal glands, the testes, and the ovaries (19). Steroidogenesis in the adrenal gland is commonly divided into the synthesis of mineralocorticoids, glucocorticoids, and sex hormones. Testicular synthesis of testosterone follows a pathway similar to androgen synthesis in the adrenal glands, with the notable exception that the stimulus to the Leydig cells is transduced by the luteinizing hormone receptor. By measuring the metabolites of hormone synthesis, urinary steroid profiling covers steroidogenesis both in testes and adrenal glands, and has been a part of the diagnosis of disorders of steroidogenesis for 40 years (20).

We had the chance to measure Cd and the steroid profile in the same urine of each of our study participants and to investigate the association between urinary Cd and the excretions of sex steroid and corticosteroid metabolites.

Subjects and Methods

Study population

Swiss Kidney Project on Genes in Hypertension is a family-based, cross-sectional study exploring the role of genes in blood pressure (BP) regulation and renal function in the general population. A detailed description of the methods is provided elsewhere; they are briefly described here (21). From December 2009 to March 2013, adult participants were recruited in two regions (Bern and Geneva) and one city (Lausanne) of Switzerland. A random sample of the inhabitants was drawn using different strategies. Inclusion criteria were (1) age $\geq$18 years, (2) being of European ancestry, (3) having at least one first-degree family member willing to participate, and (4) providing written, informed consent. Pregnant or breastfeeding women were not included. The general participation rate was 25.6%. The Swiss Kidney Project on Genes in Hypertension study conformed with the 2008 Declaration of Helsinki of the World Medical Association and has been approved by the ethics committees of each participating university hospital.

Measurements and definitions

Participants arrived at the hospital in the morning after an overnight fast for venipuncture and physical examination. Body weight was measured in kilograms to the nearest 100 g, using electronic scales; height was measured to the nearest 0.5 cm,

using a height gauge (both from Seca, Hamburg, Germany). Body mass index (BMI) was calculated as weight (in kilograms) divided by height in square meters. BP was measured with a validated, nonmercury, manual auscultatory sphygmomanometer (A&D, Tokyo, Japan). Each participant's conventional BP measured in the clinic (office BP) was the mean of the five consecutive readings, and hypertension was defined as a mean office BP ≥140/90 mm Hg. Diabetes was considered present when reported or treated, or when the fasting blood glucose level was ≥7 mmol/L. Separate daytime and nighttime urine samples were collected, covering 24 hours. To take potential incomplete urine collection into account, participants with a 24-hour urine volume <300 mL were excluded and urinary creatinine excretion per kilogram of body weight was added as covariate (22). Creatinine was measured using isotope dilution mass spectrometry-traceable methods and the Chronic Kidney Disease Epidemiology Collaboration formula was used to calculate the estimated glomerular filtration rate (eGFR). Ferritin, and serum and urinary electrolytes were analyzed by standard clinical laboratory methods at each center. Cd level was measured using an inductively coupled plasma–mass spectrometer with a hexapole collision cell (Thermo Fisher Scientific, Loughborough, UK). Sample preparation comprised the dilution of urine samples with HNO_3 1% as well as the addition of an enriched ^{111}Cd spike to each sample to calibrate by isotope dilution. The isotope ratio $^{114}Cd/^{111}Cd$ and ^{118}Sn were measured for Cd determination; additional isotopes were measured for control purposes only. Detailed information concerning the analytical method is specified in a previous report (23).

Gas chromatography–mass spectrometry of steroid metabolites

Steroid hormone synthesis starts with cholesterol and leads to the synthesis of mineralo- and glucocorticoid hormones, as well as sex steroids (Supplemental Fig. 1). The metabolites of the intermediate and the end products of this synthesis are excreted in the urine. These were measured by gas chromatography–mass spectrometry in day and night urine samples according to the method described by Shackleton (24).

The analyses comprised four metabolites per steroid-hormone category. Androgens were testosterone, with its metabolites androsterone (An) and etiocholanolone (Et), as well as the major metabolite of the testosterone precursor androstenedione, 11β-hydroxyandrosterone (11β-OH-An). As a corollary, An and Et were also markers of androstenedione synthesis. For the quantification of glucocorticoid synthesis, the cortisol precursor 11-deoxycortisol (THS) and its major metabolites tetrahydrocortisol (THF) and 5α-tetrahydrocortisol (5α-THF) were used. For the category of mineralocorticoids, tetrahydroaldosterone (THALDO), the major metabolite of aldosterone, and the metabolites of its principal precursor corticosterone, tetrahydrodehydrocorticosterone (THA), tetrahydrocorticosterone (THB), and 5α-tetrahydrocorticosterone (5α-THB) were analyzed.

Statistical analyses

All the continuous variables with normal distribution (assessed graphically) are expressed as mean ± standard deviation or as median with 25th to 75th interquartile ranges whenever distribution was skewed. Categorical variables are expressed as numbers and frequencies. Student t tests or Mann-Whitney U tests, as appropriate, and χ^2 tests were performed to compare baseline characteristics for continuous and categorical variables, respectively. Pearson tests were conducted to obtain correlations for continuous variables. We used principal component analysis to reduce the dimensionality of the steroid data and more adequately capture an overall effect for each of the functional subgroups (*i.e.*, androgens, glucocorticoids, and mineralocorticoids).

Association analyses

We took the square-root–transformed Cd excretion and log-transformed steroid metabolite excretion data, separately for day and night urine, for statistical analyses. Mixed linear models were used, taking familial correlations into account by way of a random effect. To examine the associations of each steroid metabolite (dependent variable of interest, taken one at a time) with Cd excretions in day and night urine samples, multivariable analyses were performed, including the following covariates: age, center attended, smoking, diabetes, BMI, antihypertensive treatment, office BP, ferritin level, eGFR, urinary flow rate, and 24-hour urinary excretion levels of sodium, potassium, calcium, magnesium, and creatinine for model 1. In women, menopausal status was added in model 2. We also conducted similar analyses using the first principal component of each of the three categories of steroids as the dependent variable of interest. Statistical significance was considered for a two-sided $P < 0.0005$, when applying a conservative Bonferroni correction for 100 tests. Comparison of day and night coefficients was done by a z-score, calculated by the following equation: $(\beta_1-\beta_2)/\sqrt{(SE_1{}^2+SE_2{}^2)}$.

For graphical illustration, we performed separate analyses for day and night urine samples with square-root or log-transformed Cd and steroid metabolite excretions and adjusted for age, center attended, smoking, diabetes, antihypertensive treatment, BMI, office BP, ferritin level, eGFR, urinary flow rate, and urinary excretion levels of sodium, potassium, calcium, magnesium and creatinine. All statistical analyses were conducted using STATA 14.0 (StataCorp, College Station, TX).

Results

Characteristics of the participants (473 men, 527 women) are summarized in Table 1. Although men differed from women in smoking status and ferritin levels, urinary Cd excretion during day and night were the same in both sexes. In men and women, smokers tended to have higher urinary Cd excretion in unadjusted analyses (Fig. 1; Supplemental Fig. 2), but the higher level of Cd excretion in smokers became clearly significant when accounting for important confounding factors (Supplemental Table 1). Participants with a higher BMI had also higher day and night urinary Cd excretions; however, this association was not statistically significant (Supplemental Table 1).

Urinary volumes and the amounts of excreted steroid metabolites, separated by sex and by day or night samples, are listed in Table 2. The urinary volumes of men and women were the same. Men excreted significantly higher amounts of androgens and corticosteroid metabolites during

Table 1. Study Population

Variables	Men (n = 473)	Women (n = 527)
Age, years	47 (17.7)	47.6 (17)
Smoking, no. (%)	131 (27.7)	109 (20.7)
Use of contraceptive pill, no. (%)		97 (18.4)
Menopause, no. (%)		48 (9)
Diabetes, no. (%)	7 (1.5)	1 (0.2)
Antihypertensive treatment, no. (%)	29 (6)	13 (2.4)
SBP, mm Hg	121.2 (15.4)	114.3 (17)
DBP, mm Hg	78.1 (9.4)	72.3 (9.5)
BMI, kg/m^2	26 (4.2)	24.1 (4.6)
Ferritin, μg/L	191.1 (163.4)	100 (78.3)
eGFR, mL/min/1.73 m^2	97.9 (18.5)	95.1 (17)
Creatinine excretion, mg/kg BW/24 h	22.4 (5.6)	18.3 (4.4)
Urinary electrolyte excretion, mmol/24 h		
Na	163.6 (66)	124.4 (49.9)
K	71.5 (23.2)	58 (21.2)
Ca	4.39 (2.39)	3.79 (2.07)
Mg	4.48 (1.65)	3.73 (1.39)
Daytime urinary Cd excretion, median (IQR), μg/d	0.165 (0.105–0.275)	0.163 (0.092–0.246)
Nighttime urinary Cd excretion, median (IQR), μg/night	0.083 (0.049–0.141)	0.080 (0.048–0.13)

Data are given as mean (standard deviation), unless otherwise specified.

Abbreviations: BW, body weight; Ca, calcium; DBP, diastolic BP; IQR, interquartile range; K, potassium; Mg, magnesium; Na, sodium; SBP, systolic blood pressure.

day and night than women. Only tetrahydroaldosterone excretion levels were equal in both sexes.

Cd and androgens

Androgens are sex steroid hormones that interact with vertebrate androgen receptor. The most important human derivative is testosterone. Natural androgens are made by the gonads (*i.e.*, testes in men) and adrenal glands; therefore, men and women synthesize both androgens, although in different amounts. Anand Et are metabolites of testosterone and of the common precursor of testosterone, androstenedione. 11β-OH-An is thought to be the major metabolite of adrenal androstenedione production.

The associations of urinary androgen metabolites with Cd excretion are shown in Table 3 and Supplemental Fig. 3. Multivariate regression analyses revealed a strong positive association between Cd and testosterone excretion in men (day: $\beta = 1.378 \pm 0.242$; night: $\beta = 1.44 \pm 0.333$; both $P < 0.0005$), but not in women (day: $\beta = 0.333 \pm 0.257$, $P = 0.2$; night: $\beta = 0.674 \pm 0.361$, $P = 0.06$). Excretions of An were positively, but not significantly, associated with Cd excretion in both sexes [men: $\beta = 0.639 \pm 0.184$, $P = 0.0005$ (day), $\beta = 0.374 \pm 0.283$, $P = 0.19$ (night); women: $\beta = 0.237 \pm 0.243$, $P = 0.33$ (day), $\beta = 1.195 \pm 0.345$, $P = 0.0005$ (night)]. When only premenopausal women were included in the model, the association became significant for nocturnal An excretions ($\beta = 1.271 \pm 0.347$; $P < 0.0005$). In women, urinary Cd was also associated with the nightly excretion of 11β-OH-An in both models. There were positive correlations between urinary Cd and urinary Et excretions, but these associations did not reach statistical significance.

To differentiate increased androgen synthesis between gonads and adrenal glands, the model was adjusted for corticosteroids (*e.g.*, cortisol as a marker of glucocorticoids and THB as a marker of mineralocorticoid synthesis; Supplemental Tables 2 and 3). Adjustment for cortisol and THB excretions led to a minor attenuation of the β-coefficient between urinary Cd and testosterone excretions in men (day: $\beta = 1.116 \pm 0.230$, night: $\beta = 1.166 \pm 0.330$ both $P < 0.0005$ for the adjustment with cortisol; day: $\beta = 1.238 \pm 0.238$, night: $\beta = 1.310 \pm 0.337$, both $P < 0.0005$ for the adjustment with THB). With these adjustments, the nocturnal correlation of urinary Cd, An, and 11β-OH-An lost its statistical significance (An: $\beta = 0.955 \pm 0.341$, $P = 0.005$ for cortisol adjustment, $\beta = 0.768 \pm 0.335$, $P = 0.022$ for THB adjustment; 11β-OH-An: $\beta = 0.864 \pm 0.257$, $P = 0.0008$ for cortisol adjustment, $\beta = 0.702 \pm 0.242$, $P = 0.004$ for THB adjustment).

To capture the influence of the steroid hormone groups (*e.g.*, sex hormones, glucocorticoids, and mineralocorticoids) on each other, principal component analysis was done. The model was recalculated for the first principal components of all the three steroid groups (Supplemental Table 4). The first principal component capturing sex steroids was positively associated with urinary Cd excretion in men (day: $\beta = 2.758 \pm 0.576$, $P < 0.0005$; night: $\beta = 2.289 \pm 0.812$, $P = 0.003$), but not in women (day: $\beta = -0.929 \pm 0.774$, $P = 0.23$, night: $\beta = 2.169 \pm 0.810$, $P = 0.007$). Upon adjustment with the first glucocorticoid and the first mineralocorticoid

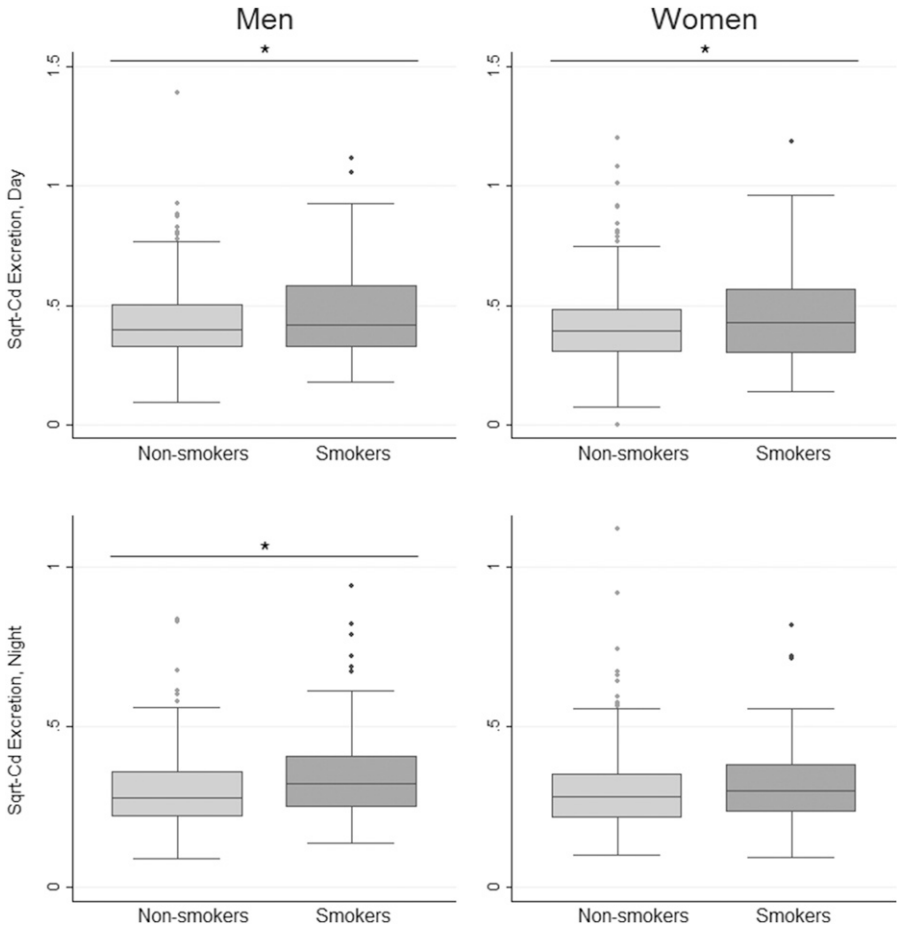

Figure 1. Box plots of urinary Cd excretion (square-root transformed) in men and women separated by day and night. Significant differences for a two-sided *t* test ($P < 0.05$, no multiple testing correction) are indicated with a bar with asterisk. Sqrt, square root.

component, the β-coefficients in males lost their statistical significance.

Because Cd is also toxic for the hypothalamic-pituitary-gonadal (HHG) axis, regression coefficients of the associations between Cd and androgen metabolites were compared by z-score. There was no such difference for day and night in both sexes (data not shown).

Cd and glucocorticoids

Cortisol in humans has a short half-life and is rapidly metabolized into its hydroxylated metabolites THF and 5α-THF. Therefore, the amounts of excreted THF and 5α-THF are much larger than that of cortisol. THS is a precursor of cortisol and a marker of glucocorticoid synthesis.

The associations of glucocorticoid metabolites with urinary Cd excretion are shown in Table 4 and Supplemental Fig. 4. Urinary Cd and cortisol excretions were positively associated in men (day: $\beta = 0.475 \pm 0.157$, $P = 0.003$, night: $\beta = 0.875 \pm 0.253$, $P = 0.0005$) and women (day: $\beta = 0.877 \pm 0.194$, $P < 0.0005$, night: $\beta = 1.183 \pm 0.277$, $P < 0.0005$), but only reached significance in women. In both sexes, all four correlations were significant for the excretion of the cortisol precursor THS and the major cortisol metabolite THF ($P < 0.0005$ for all).

To differentiate between increased testicular and adrenal steroid synthesis, the androgens testosterone (in men) and An (in women) were introduced as a parameter in the model (Supplemental Table 5). These adjustments attenuated the correlations between cortisol and Cd in both sexes and rendered the nightly correlation in women not significant ($\beta = 0.854 \pm 0.277$; $P = 0.002$). The correlations between daily THS and THF excretions in women and nightly THS and THF excretions in men resisted further adjustment ($P < 0.0005$ for all). To investigate the common toxic effect of Cd on adrenal glands (*i.e.*, glucocorticoids and mineralocorticoids), the model was adjusted for the mineralocorticoid THB (Supplemental Table 6). With this adjustment, all significant correlations between the excretion of Cd and of glucocorticoid metabolites disappeared.

The association between Cd and the principal component analysis is shown in Supplemental Table 7. There was a strong correlation between Cd excretion and the first principal glucocorticoid component in both sexes

Table 2. Descriptive Data for Day and Night Urine for Volume and Excretion of Steroid Hormone Metabolites

Variable	Men		Women		*P* Value
Day					
Urinary volume, mL	1014 (700–1506)	473	1100 (760–1535)	527	0.12
Urinary flow rate, mL/min	1.08 (0.76–1.58)	473	1.21 (0.81–1.69)	527	0.02
Androsterone, μg	1186 (783–1784)	415	428 (242–748)	501	<0.01
11β-OH-An, μg	608 (432–833)	457	293 (194–414)	521	<0.01
Et, μg	1053 (618–1519)	416	576 (315–909)	499	<0.01
Testosterone, μg	30.3 (18.1–45.6)	461	4.4 (2.6–8.1)	519	<0.01
THS, μg	46.6 (34– 62)	464	35.9 (24.3– 49.6)	524	<0.01
Cortisol, μg	79.2 (57–111)	464	60.5 (41.5– 91.2)	524	<0.01
THF, μg	1310 (1026–1704)	400	817 (562–1054)	498	<0.01
5α- THF, μg	1076 (758–1523)	408	379 (236–605)	511	<0.01
THA, μg	74.8 (50.8–100.8)	459	52 (36.4–75.2)	524	<0.01
THB, μg	96.7 (68.6–137.2)	464	73.1 (49.2–101.6)	524	<0.01
5α-THB, μg	244.9 (171.8– 342)	464	120.7 (78.3– 183)	524	<0.01
THALDO, μg	13.4 (8.1–23.1)	464	12.7 (7.6–21.9)	524	0.31
Night					
Urinary volume, mL	454 (300–650)	473	487 (300–700)	527	0.34
Urinary flow rate, mL/min	0.95 (0.66–1.41)	473	0.95 (0.58–1.34)	527	0.85
Androsterone, μg	592 (367–859)	408	217 (120–360)	497	<0.01
11β-OH-An, μg	221 (148–322)	460	113 (78–168)	523	<0.01
Et, μg	515 (332–790)	419	303 (168–473)	491	<0.01
Testosterone, μg	16.1 (10–24.1)	460	2.3 (1.38–3.87)	507	<0.01
THS	17.9 (12.5– 27)	465	14.4 (9.6– 20.9)	524	<0.01
Cortisol, μg	30.7 (21–45.1)	465	22.8 (15.7–34.5)	523	<0.01
THF, μg	415 (294–583)	426	253 (174–366)	501	<0.01
5α- THF, μg	354 (232–499)	431	123 (75.4–198)	515	<0.01
THA, μg	27.3 (18.4–41)	464	20 (12.6–32.2)	524	<0.01
THB, μg	41.8 (27.6–63.4)	465	31.3 (20.4–47.6)	524	<0.01
5α- THB, μg	74.7 (51.8– 108.5)	465	36.2 (23.1 – 59.2)	524	<0.01
THALDO, μg	5.51 (3.39–9.39)	464	5.47 (2.95–9.28)	522	0.9

Data are given as median (IQR). The numbers of analyzed participants are indicated after the parentheses.

[men: $\beta = 3.274 \pm 0.662$, $P < 0.0005$ (day), $\beta = 3.309 \pm 0.856$, $P < 0.0005$ (night); women: $\beta = 2.210 \pm 0.636$, $P < 0.0005$ (day), $\beta = 4.596 \pm 0.939$, $P < 0.0005$ (night)]. On adjustment for the first principal component of sex steroids, the associations between Cd and the first principal glucocorticoid component were no longer significant in men but remained significant in women. The significance of all correlations disappeared only after the adjustment with the first principal mineralocorticoid component.

Cd and mineralocorticoids

Aldosterone is the main mineralcorticoid in humans, but because its quantification in urine is very difficult, its hydroxylated metabolite THALDO is considered a standard of aldosterone production. Corticosterone is a precursor of aldosterone with mineralocorticoid properties and the metabolites of corticosterone (THA, THB, and 5α-THB) are considered markers of mineralocorticoid synthesis.

The associations of Cd and mineralocorticoid metabolites are summarized in Table 5. Urinary excretion of Cd and THALDO were not correlated in either men nor women. There was an association between Cd and the nightly excretion of the corticosterone metabolite THB in men ($\beta = 1.0001 \pm 0.266$; $P < 0.0005$) and day and night in women (respectively, $\beta = 0.914 \pm 0.208$, $P < 0.0005$; $\beta = 1.298 \pm 0.292$, $P < 0.0005$). There was also a significant correlation between excretion of Cd and the corticosterone metabolites 5α-THB and THA during day and night in women ($P < 0.0005$ for all four). Of seven significant correlations, five resisted further adjustment with one of the androgens (testosterone in men and An in women; Supplemental Table 8). All significant correlations disappeared only upon further adjustment with cortisol (Supplemental Table 9).

The first principal component capturing mineralocorticoids was positively associated with urinary Cd excretion in men during the day ($\beta = 2.779 \pm 0.651$; $P < 0.0005$) and in women during the night ($\beta = 4.197 \pm 0.815$; $P < 0.0005$; Supplemental Table 10). On adjustment for the first principal component of sex steroids, the association was no longer significant in men, but remained significant in women. Significance disappeared after the adjustment with the first principal glucocorticoid component.

Discussion

To our knowledge, this is the first population-based study to find a strong positive association between Cd exposure

Table 3. Association of Androgens (Dependent Variable) With Day and Night Urinary Cd Excretion (Independent Variable), by Sex

	Men				Women				
	β	SE	*P* Value[a]	No.[b]	β	SE	*P* Value[a]	No.[b]	*P* Interaction
Day									
Model 1[c]									
Androsterone	0.639	0.184	0.00051	378	0.237	0.243	0.32921	459	0.05
11β-OH-An	0.606	0.157	0.00011	421	0.369	0.187	0.04845	479	0.15
Et	0.564	0.187	0.00258	380	0.423	0.240	0.07783	458	0.21
Testosterone	1.378	0.242	<0.0005	424	0.333	0.257	0.19646	477	0.13
Model 2[d]									
Androsterone					0.297	0.246	0.22652	454	
11β-OH-An					0.352	0.187	0.06039	472	
Et					0.423	0.243	0.08163	451	
Testosterone					0.272	0.259	0.29382	470	
Night									
Model 1[c]									
Androsterone	0.374	0.283	0.18622	372	1.195	0.345	0.00053	458	0.32
11β-OH-An	0.759	0.239	0.00147	423	1.220	0.265	<0.0005	483	<0.01
Et	0.819	0.268	0.00221	384	1.139	0.346	0.00101	453	0.87
Testosterone	1.440	0.333	<0.0005	423	0.674	0.361	0.06214	467	0.82
Model 2[d]									
Androsterone					1.271	0.347	<0.0005	452	
11β-OH-An					1.266	0.267	<0.0005	476	
Et					1.190	0.351	0.00069	447	
Testosterone					0.742	0.364	0.04166	460	

[a]Multiple testing corrected *P* value cutoff = 0.0005 (accounting for 100 tests).

[b]No. of analyzed participants.

[c]Model 1 was adjusted for age, center attended, smoking, diabetes, antihypertensive treatment, BMI, office BP, ferritin, eGFR, and urinary flow rate, as well as urinary Na, K, Ca, Mg, and creatinine excretion.

[d]Model 2 was adjusted for menopausal status.

and increased excretion of urinary metabolites of all three kinds of steroid hormones (*i.e.*, androgens, glucocorticoids, and mineralocorticoids). The observed relation between the excretion of Cd and steroid metabolites was robust and concerned predominantly testosterone synthesis in men (day, β = 1.378; night, β =1.440) and glucocorticoid synthesis in both sexes. There, Cd was predominantly associated with the major cortisol metabolite tetrahydrocortisol [men: β = 0.560 (day) and β =0.942 (night);, women: β =0.871 (day) and β =1.306 (night)]. There was also a statistically significant association between Cd and THB excretions. These associations are supported by principal component analysis. Other population-based studies also found increased testosterone levels in men (8, 10, 11) and women (12). However, none of these studies looked at the production of androgens in the urine. The increased corticosteroid synthesis upon Cd exposure has only been described in animals before, to our knowledge.

With its global view on hormone production, the urinary steroid profile allows a special look at steroidogenesis. First, it allows analysis of the total production of intermediate and end products of sex steroid and corticosteroid hormone synthesis. Even if the end product is not affected, we could still capture an effect for intermediate products or metabolites of the end products. Second, by adjusting the multivariate regression model for certain steroid metabolites of the two other groups or for the first principal component of a group of steroid hormones, we found an independent effect of Cd on increased androgen and an interdependent effect on gluco- and mineralocorticoid synthesis. These data are in line with a dual endocrine effect of Cd: one on testes and the other on adrenal glands.

Urinary Cd excretion in our cohort is in a range that has been observed in other population-based studies that described an association of Cd with prostate cancer (6), cardiovascular diseases (14–16), or prediabetes (17). However, a recent meta-analysis found no clear evidence of an association between Cd exposure and risk of prostate cancer, although results were heterogeneous across studies (25). Another meta-analysis concluded that urinary Cd, even at low levels, is associated positively with all-cause, cardiovascular, and cancer mortality (26). Our data suggest an endocrinological toxic effect of Cd at this low level of exposure. Whether this explains the associations of urinary Cd with mortality in the aforementioned human studies remains to be demonstrated.

Table 4. Association of Glucocorticoid Metabolites (Dependent Variable) With Day and Night Urinary Cd Excretion (Independent Variable), by Sex

	Men				Women				
	β	SE	*P* Value[a]	No.[b]	β	SE	*P* Value[a]	No.[b]	*P* Interaction
Day									
Model 1[c]									
THS	0.601	0.154	<0.0005	427	0.727	0.184	<0.0005	482	0.46
Cortisol	0.475	0.157	0.00250	427	0.877	0.194	<0.0005	482	0.36
THF	0.560	0.146	<0.0005	367	0.871	0.188	<0.0005	457	<0.01
5α- THF	0.514	0.258	0.04619	375	0.680	0.245	0.00556	470	0.01
Model 2[d]									
THS					0.719	0.187	<0.0005	475	
Cortisol					0.886	0.195	<0.0005	475	
THF					0.864	0.190	<0.0005	451	
5α- THF					0.674	0.248	0.00656	463	
Night									
Model 1[c]									
THS	0.979	0.225	<0.0005	428	0.986	0.257	<0.0005	484	0.86
Cortisol	0.875	0.253	0.00053	428	1.183	0.277	<0.0005	483	0.71
THF	0.942	0.230	<0.0005	392	1.306	0.255	<0.0005	463	0.06
5α- THF	0.030	0.367	0.93557	398	0.989	0.324	0.00225	477	<0.01
Model 2[d]									
THS					0.972	0.256	<0.0005	477	
Cortisol					1.191	0.279	<0.0005	476	
THF					1.290	0.256	<0.0005	456	
5α- THF					0.980	0.326	0.00260	470	

[a]Multiple testing corrected *P* value cutoff = 0.0005 (accounting for 100 tests).

[b]No. of analyzed participants.

[c]Model 1 was adjusted for age, center attended, smoking, diabetes, antihypertensive treatment, BMI, office BP, ferritin, eGFR, and urinary flow rate, as well as urinary Na, K, Ca, Mg, and creatinine excretion.

[d]Model 2 was adjusted for menopausal status.

Interestingly, this endocrine effect does not appear to be disrupting steroid hormone production, but rather increasing it. Because it is thought that the half-life of absorbed Cd is 10 to 15 years in humans (2), one could speculate that with increased hormone levels over decades, this might partially explain the observed association of Cd exposure to prostate cancer, hypertension, or insulin resistance.

Cd excretion

Previous studies of the toxicity of Cd in humans concentrated preferentially on renal biomarkers for which the level of renal toxicity started only with a Cd excretion of >1 μg/g urinary creatinine (27). The observed levels in our study were well below this limit and in line with levels observed in other countries (28, 29).

It has been shown that urinary Cd excretion correlates well with Cd uptake (2). Because we measured total urinary Cd during day and night, an adjustment for urinary creatinine was not necessary. Cd excretions in men and women in our cohort were the same despite reports that Cd excretion was higher in women than in men owing to lower iron stores and consecutively increased gastrointestinal uptake (3). To exclude additional uptake by lower iron stores, we introduced also serum ferritin levels in the multivariate regression model.

For both men and women, we found day and night urinary Cd excretions to be higher in smokers and to be positively associated with BMI, which is consistent with the knowledge that smoking is a major source of Cd (23) and that Cd accumulates in white adipose tissue (30).

Cd and androgens

It is likely that Cd toxicity on gonadal function is multifactorial, mediated via its direct effects on Leydig cells and/or the HHG axis (31). Therefore, disruption of sex hormone synthesis by Cd should also take circadian rhythms into account. Until now, the toxic effect on the HHG axis has only been studied in male animals (32), to our knowledge. In the current study, Cd excretion during the night was associated with a steeper increase in sex hormone synthesis than during the day, indicating such an effect on the HHG axis. However, the difference of β-coefficients between Cd and diurnal and nocturnal sex hormone synthesis was not significant.

Cd probably has a biphasic dose toxicity on Leydig cells. Animal studies have concluded that decreases of testosterone are seen after a single, large, injected dose of

Table 5. Association of Mineralocorticoid Metabolites (Dependent Variable) With Day and Night Urinary Cd Excretion (Independent Variable), by Sex

	Men				Women				
	β	SE	*P* Value[a]	No.[b]	β	SE	*P* Value[a]	No.[b]	*P* Interaction
Day									
Model 1[c]									
THA	0.185	0.161	0.24823	422	0.771	0.193	<0.0005	482	<0.01
THB	0.443	0.169	0.00871	427	0.914	0.208	<0.0005	482	0.04
5α-THB	0.561	0.224	0.01241	427	0.819	0.227	<0.0005	482	0.02
THALDO	0.496	0.232	0.03274	427	0.420	0.236	0.07529	482	0.10
Model 2[d]									
THA					0.725	0.194	<0.0005	475	
THB					0.881	0.210	<0.0005	475	
5α- THB					0.821	0.229	<0.0005	475	
THALDO					0.414	0.238	0.08162	475	
Night									
Model 1[c]									
THA	0.428	0.273	0.11653	427	1.247	0.283	<0.0005	484	<0.01
THB	1.001	0.266	<0.0005	428	1.298	0.292	<0.0005	484	0.21
5α-THB	0.339	0.334	0.31066	428	1.343	0.324	<0.0005	484	<0.01
THALDO	−0.044	0.340	0.89719	427	0.589	0.358	0.10013	482	0.70
Model 2[d]									
THA					1.241	0.285	<0.0005	477	
THB					1.293	0.294	<0.0005	477	
5α- THB					1.363	0.325	<0.0005	477	
THALDO					0.596	0.362	0.09912	475	

[a]Multiple testing corrected *P* value cutoff = 0.0005 (accounting for 100 tests).

[b]No. of analyzed participants.

[c]Model 1 was adjusted for age, center attended, smoking, diabetes, antihypertensive treatment, BMI, office BP, ferritin, eGFR, and urinary flow rate, as well as urinary Na, K, Ca, Mg, and creatinine excretion.

[d]Model 2 was adjusted for menopausal status.

Cd and increases more commonly after chronic oral Cd exposure (33, 34). In the present investigation, we confirmed the correlation of urinary Cd excretion with increased production of testosterone during day and night in men. Because 11β-OH-An is thought to be the major metabolite of adrenal androstenedione production, our results also point to increased production of androgens of adrenal origin in women.

Cd and corticosteroids

So far, the toxic effect of Cd on adrenal glands has only been studied in animal models, for which contradicting results were found: After Cd treatment, glucocorticoid synthesis was increased in two studies (35, 36) and decreased in one (37). After Cd exposure, rats had increased plasma aldosterone levels and a decreased plasma level of its precursor corticosterone (38). Also *in vitro* studies of microsomes of the adrenal cortex of the guinea pig (39) and *in vivo* studies in an aquatic animal (40) found increased glucocorticosteroid synthesis after Cd exposure. Hence, toxic effects on the adrenal gland also seem to be biphasic.

Why Cd could stimulate corticosteroid synthesis remains an open question. Previous research has shown that Cd stimulates the activity of cholesterol monooxygenase (41), the first enzyme of steroid synthesis. One could speculate that low Cd exposure stimulates steroid synthesis *in toto* via this pathway.

Strengths and limitations

The strengths of the study are its multicentric, population-based design and the use of the same standardized protocol across centers. We could investigate the association between Cd and steroid hormone production over 24 hours and, therefore, eliminate oscillation of serum hormone levels. An important limitation is the cross-sectional design, which means it is not possible to infer causation in the phenotypic associations, and results are subject to potential type 1 statistical errors because of multiple testing. Our study results need to be confirmed in other population studies.

Conclusion

Our findings are consistent with a potential endocrine effect of chronic low-dose exposure of Cd in the general adult population. The positive associations between an increased excretion of androgens, corticosteroids, and Cd

are compatible with a stimulating effect on steroid hormone synthesis in testes and adrenal glands. Consequently, further policies aimed at reducing Cd exposure in the general population should be implemented.

Acknowledgments

We thank the study nurses Marie-Odile Levy, Guler Gök-Sogüt, Ulla Schüpbach, and Dominique Siminski for data collection; Pamela Hayoz for performing analysis of urinary steroids; and Sandrine Estoppey for invaluable help for logistics and database management.

Financial Support: This study was supported by a grant from the Swiss National Science Foundation [FN 33CM30-124087, Schweizerischer Nationalfonds (SNF), Wildhainweg 3, Postfach 8232, 3001 Bern, Switzerland].

Author Contributions: M. Bochud, M.P., B.P., I.G., G. Ehret, M.M., F.P., M. Burnier, A.P.-B., P.-Y.M., B.V., and D.A. planned the study and interpreted the data. Additional data interpretation was done by J.J.-B., D.P., V.D., M.H., G.E., and B.D. M. Bochud, B.V., and D.A. wrote the paper.

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Disclosure Summary: The authors have nothing to disclose.

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