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Environmental exposure to metals and male reproductive hormones: Circulating testosterone is inversely associated with blood molybdenum

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Abstract

Study Objective—To explore associations between exposure to metals and male reproductive hormone levels.

Design—Cross-sectional epidemiology study with adjustment for potential confounders.

Setting—Metal concentrations and reproductive hormone levels were measured in blood samples collected from 219 men. Patients: Men recruited through two Michigan, USA infertility clinics.

Interventions—None

Main Outcome Measures—Serum FSH, LH, inhibin B, testosterone, and SHBG.

Results—Cadmium, copper and lead were all significantly or suggestively positively associated with testosterone when modeled individually (p-values = 0.1, 0.03, and 0.07, respectively), findings that are consistent with limited previous human and animal studies. Conversely, molybdenum was associated with reduced testosterone (p-value for trend = 0.001). A significant inverse trend between molybdenum and testosterone remained when additionally considering other metals in the model, where a positive association between testosterone and zinc was also found. Finally, in exploratory analysis there was evidence for an interaction between molybdenum and zinc, where high

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molybdenum was associated with a 37% reduction in testosterone (relative to the population median level) among men with low zinc.

Conclusions—While reductions in testosterone and reproductive toxicity following molybdenum exposure have been previously demonstrated in animal studies, more research is needed to determine whether molybdenum poses a risk to human reproductive health.

Keywords

biomarkers; endocrine; epidemiology; exposure; fertility; metals

INTRODUCTION

The general population is exposed to metals at trace concentrations either voluntarily through supplementation or involuntarily through intake of contaminated food and water or contact with contaminated soil, dust, or air. Some metals, such as cadmium, lead, arsenic and mercury, are non-essential xenobiotics that are known to be harmful to human health (1–4). Several other metals, such as chromium, copper, manganese, molybdenum, selenium and zinc, are essential for good health but may be harmful above certain levels (5–9). A number of metals are reproductive toxicants and suspected endocrine disruptors. Although exposure to several of these toxic metals is prevalent, human studies of exposure to metals and altered hormone levels to date are quite limited. Alterations in reproductive hormone levels, even at degrees that are considered subclinical, may be associated with or lead to declined fertility and reproductive health, increased risk of endocrine-related cancers, or other adverse effects.

Cadmium and lead have been the most studied metals in relation to altered hormone levels. Cadmium exposure among humans is pervasive; environmental sources of cadmium include contaminated ambient air and soil from industrial pollution and other man-made or natural combustion sources such as cigarette smoke and volcanic activity. Cadmium ingested in foods and drinking water also plays an important role in aggregate human exposure (1). Cadmium is recognized as an endocrine disruptor but the mechanisms involved are not well understood (10). Several studies of occupationally exposed men have reported evidence of a positive association between cadmium and FSH (11) or testosterone (12,13,14) while others have not (15). More recently, studies focusing on cadmium exposure outside the workplace have reported positive associations between low-level cadmium exposure and increased testosterone, with some also reporting increased estradiol and/or FSH, in men (16,17,18) or postmenopausal women (19). Thus, though fairly limited in number, there has been some consistency with regard to the positive relationship between cadmium exposure and testosterone levels in human studies conducted to date.

Lead is one of the most studied occupational and environmental contaminants among human populations. Although lead exposure levels have been declining in industrialized nations for the past few decades, health effects from low exposure levels remain a concern. Several studies have investigated the relationship between exposure to lead and hormone levels among occupationally exposed men, but results have been conflicting (14,20–24). Among men with no occupational exposure, studies of Croatian men have reported positive associations between blood lead concentrations and testosterone and estradiol levels (16,17).

Studies of exposure to metals other than cadmium or lead and altered hormone levels are more limited. Among male welders, studies have reported an inverse association between exposure to stainless steel welding fume and testosterone levels, and a positive dose-related relationship between mild steel welding fume exposure and FSH (25). Metal exposures of concern include hexavalent chromium, nickel and molybdenum in stainless steel welding, and manganese in mild steel welding. Conversely, another European study found no associations between

welding and FSH, LH or testosterone levels (26). However, the study did not stratify by type of metal being welded in the statistical analysis of hormone levels. A Korean study of manganese exposure reported higher levels of FSH and LH among male welders compared to age-matched office workers (27). Mercury, a transition metal and common environmental contaminant, was recently found to be associated with increased estradiol levels in both males and females from a small residential population in Cambodia (28), which was in agreement with a previous study among women with repeated miscarriages (29). However, slight to no relationships were reported in earlier studies of occupational exposure to mercury and hormone levels (30–32). Finally, a recent study of exposure to arsenic, a metalloid with both manmade and natural environmental sources of contamination, and erectile dysfunction suggested that arsenic may impart increased risk through a reduction of circulating testosterone levels (33).

In summary, the existing evidence for a relationship between metals exposure and hormone levels are inconsistent, and human studies of hormone alterations in relation to metals at exposure levels found among the general population are lacking. The present study was conducted to assess the relationship between metal concentrations measured in blood and circulating reproductive hormone levels in men recruited through U.S. infertility clinics.

METHODS

Subject Recruitment

Participants were recruited at two Michigan infertility clinics without knowledge of male factor infertility. Since couples may present at the clinics for problems relating to either male or female fertility problems (or both), the study population includes fertile men and men with a range of fertility problems. After obtaining informed consent, men between 18 and 55 years of age currently attempting to conceive a pregnancy with their partner were enrolled. Men with diabetes, thyroid or adrenal disorders, genetic disorders related to fertility, testicular cancer, unilateral orchiectomy, or taking hormone therapy were excluded. The protocols of the study were approved by the committees on research ethics at all participating institutions.

Reproductive Hormones

Blood samples were collected from participants during a morning clinic visit. Most of the hormone assays were performed by the Immulite Assay System (Diagnostic Products Corp., Los Angeles, CA). FSH, LH, and sex hormone binding globulin (SHBG) were measured by solid phase, two site, chemiluminescent enzyme immunometric assay. For testosterone (T) measurement a solid phase, competitive chemiluminescent enzyme immunoassay was employed. The intra- and inter-assay variabilities, expressed as coefficient of variations, are as follows: FSH 3.0 and 5.9; LH 5.7 and 9.5; SHBG 6.5 and 8.7; T 8.7 and 10.5. The sensitivities of the assays are as follows: FSH and LH 0.1 mIU/mL; SHBG 0.2 nmol/L; and T 15 ng/dL. Circulating inhibin B levels were measured using a two site ELISA assay (Diagnostic Systems Laboratories) which utilizes two monoclonal antibodies, one directed to the A and the other to the B subunit of inhibin (34). The assay does not cross-react with alpha-2-macroglobulin, follistatin or activin. Inhibin B intra-assay CV based on 3 quality control pools averaging 103.2, 183.7 and 345.5 pg/mL averaged 5.4±1.5, 2.9±0.5 and 3.4±1.0%, respectively. Interassay CV for inhibin B for the same quality control pools averaged 19.7, 16.8, 12.5 %, respectively. The free androgen index (FAI) was calculated as the molar ratio of total testosterone to sex hormone binding globulin (SHBG). The testosterone:LH ratio (T:LH ratio), a measure of Leydig cell function, was calculated by dividing testosterone (nmol/L) by LH (IU/L).

Measurement of Metals

Whole venous blood was collected using stainless steel needles into 2 mL plastic tubes containing EDTA (prescreened for mercury, cadmium and lead) and stored at −20°C. Samples

were assayed for arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), total mercury (Hg), molybdenum (Mo), selenium (Se), thallium (Tl) and zinc (Zn). Controls for all metals included human or bovine blood spiked with known quantities of each metal. In addition, cadmium, lead and mercury were analyzed with regard to standardized human reference blood containing known quantities of each metal. All samples were analyzed using a Perkin Elmer Elan DRC plus ICP Mass Spectrometer using the Centers for Disease Control and Prevention (CDC) methodology. The limits of detection (LOD) for the blood metal levels were as follows: As, 4.0 μg/L; Cd, 0.2 μg/L; Cr, 0.5 μg/L; Pb, 0.3 μg/dL; Mn, 1.0 μg/L; Hg, 0.2 μg/L; Mo, 1.0 μg/L; Se, 5 μg/L; Tl, 0.1 μg/L; and Zn, μg/L.

Statistical Analysis

Data analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics on subject demographics were calculated, along with the distributions of blood metal concentrations and hormone levels. Bivariate analysis was conducted between all hormone, metal concentration, and demographic variables to investigate differences between distributions or categories and the potential for confounding. Correlations or differences between groups were tested statistically using parametric or non-parametric methods where appropriate.

Due to the high proportion of samples below the LOD for a number of the metals, these variables were categorized into groups. At least 3 groups were formed for each metal to investigate dose-dependent relationships. Metals with greater then 75% of samples greater than the LOD were categorized into quartiles. For the other metals grouping cutpoints were determined by the percentage of samples above the LOD. The low group consisted of values below the LOD for each metal, while the medium and high groups were made up of equal-sized bins among the detected values.

The association between exposure categories for each metal and hormone levels were assessed by multiple linear regression. Age, body mass index (BMI), race, season and smoking were considered as covariates. FSH, LH, SHBG, FAI and T:LH ratio were transformed using the natural logarithm, whereas inhibin B and testosterone followed a normal distribution and were modeled untransformed. Statistical and biological factors were considered when selecting which covariates to include in adjusted models. Since it may be appropriate to consider the impact of multiple metals on reproductive function simultaneously (16,17,35), full linear regression models were also constructed for each hormone when considering all metals and covariates together. The backward elimination procedure was utilized and alpha was set at 0.10 for variables to be retained in the model. Covariates not retained in the final model were then added individually to further explore evidence of confounding (i.e. causing >10% change in effect estimates for metals in the final model). In secondary analyses the testosterone models were additionally adjusted for potential confounding by sperm concentration, motility or morphology measures among the men. Methods for assessing semen quality measures and their relationships with metal concentrations have been previously reported (35–37).

Finally, evidence of interaction between metals in the association with hormone levels was explored. Since we lacked the sample size and statistical power to include interaction terms in the models, this exploratory analysis was performed by qualitatively comparing effect estimates from statistical analyses on a metal of concern that was retained in the final regression model (e.g. cadmium or lead) while stratifying on a metal that may be protective against metal-related adverse effects on reproductive function (e.g. zinc, copper or selenium) (38).

RESULTS

A total of 219 men were recruited into the study and provided data on serum hormone levels and blood metals concentrations. As previously described (35–37), participants were mostly white (76%), with a median (25^{th}, 75^{th} percentile) age of 34 (30,38) years and a median BMI of 28 (25,33). Most men (76%) were non-smokers at the time of the study. Distributions of metal concentrations measured in blood and serum hormone levels are presented in Table 1. As, Cu, Pb, Hg, and Zn were above the limit of detection for most samples. Fifty percent of samples had cadmium and thallium above the LOD, while 30% of samples were above the LOD for chromium and molybdenum.

Preliminary comparisons between variables were conducted using parametric or non-parametric correlations or hypothesis tests, depending on the detection rate and distribution of the individual variables (all reported associations had a p-value < 0.05). Age was positively associated with FSH and SHBG, but inversely associated with inhibin B and FAI. BMI was inversely associated with inhibin B, testosterone, and SHBG but positively associated with FAI. For metal concentrations in blood, age was positively associated with lead and mercury, while BMI was positively associated with copper but inversely associated with molybdenum. Current smoking was associated with increased cadmium and copper concentrations in blood, and with decreased zinc. Current smoking was also associated with increased inhibin B levels. Non-white race was positively associated with cadmium, copper, and lead, but inversely associated with selenium. Non-white race was also associated with higher testosterone levels (median 402 ng/dL vs. 381 ng/dL). Finally, income was inversely associated with cadmium, copper, and lead, but positively associated with mercury.

Results from crude linear regression models (not shown) were similar to those adjusted for age, BMI and current smoking (Table 2). The main difference from the crude models was that the positive associations of cadmium, copper and lead with testosterone became stronger in the adjusted models. Elevated arsenic (the highest quartile) was inversely associated LH in both the crude and adjusted models. There were suggestive positive relationships between arsenic and inhibin B and T:LH ratio. Cadmium and thallium concentrations above the limit of detection were associated with non-monotonic increases in inhibin B, while chromium was associated with a non-monotonic decrease in inhibin B. Monotonic patterns were observed for the positive association between manganese and inhibin B, the inverse association between manganese and SHBG, and the inverse association between selenium and LH. Among the comparisons in Table 2, the strongest trend was found for molybdenum with testosterone. Compared to men with molybdenum levels below the LOD, the middle and high molybdenum groups (70^{th} to 85^{th} percentile, and $>85^{th}$ percentile, respectively) were associated with 23 ng/dL (95% CI −58, 12) and 60 ng/dL (−97, −23) reductions in testosterone levels, respectively (p for trend = 0.001). Molybdenum was also associated with a significant inverse trend in FAI (p for trend = 0.004).

Because it may be appropriate to consider the impact of multiple metals on hormone levels simultaneously (16,17), all metals and covariates were considered together in linear regression models for each hormone. In the final linear regression models, molybdenum remained as a predictor of both testosterone and FAI (Table 3). When adjusting the testosterone and FAI models for other metals and covariates, the regression coefficients for the middle and high molybdenum groups did not change appreciably from those presented in Table 2. Zinc and race were also retained in the testosterone model, where zinc was positively associated with testosterone. The final testosterone model remained unchanged when additionally adjusting for sperm concentration, motility or morphology measures among the men (results not shown). In addition to the inverse association with molybdenum, FAI was also positively associated with chromium and copper. Copper and selenium were retained in the final LH model, where

both had monotonic inverse relationships with LH. For inhibin B, cadmium and chromium were retained with opposing relationships that were consistent with the results when the metals were modeled independently (Table 2). There was a suggestive inverse association between manganese and T:LH ratio when also adjusting for copper and selenium, which were both positively associated with the Leydig cell function indicator.

Evidence for interaction was explored by repeating the multiple linear regression models involving testosterone or FAI and molybdenum (adjusted for age, BMI, current smoking and race) while stratifying on potentially protective metals. There was evidence of effect modification by zinc in the association between molybdenum and testosterone. When analysis was restricted to men with zinc concentrations in the lowest quartile, the middle and high molybdenum groups (70^{th} to 85^{th} percentile, and $>85^{th}$ percentile, respectively) were associated with 41 ng/dL (95% CI −132, 49) and 142 ng/dL (−230, −54) reductions in testosterone levels, respectively (p for trend = 0.02), compared to men with molybdenum below the LOD. For the high molybdenum/low zinc group, this represents a 37% decline in testosterone level relative to the population median (389 ng/dL). The evidence for interaction when stratifying by copper or selenium was not as strong (results not shown). There was also no evidence for modification of the association between molybdenum and FAI, or in the association between manganese and T:LH ratio, when stratifying by copper, selenium or zinc (results not shown).

DISCUSSION

The present study is one of the largest and most comprehensive to date to assess relationships between metal exposure and reproductive hormone levels, and we reported a number of statistically significant or suggestive associations. At least a portion of the significant or suggestive results are likely due to chance since a large number of comparisons were made. Some of our findings have not been reported elsewhere, such as the relationships between cadmium and chromium with inhibin B or the inverse relationships between copper and selenium with LH. However, some of the results are consistent with previous animal and human studies in support of our findings. For example, the suggestive positive associations between testosterone and cadmium (12,16–19), copper (39), and lead (16,17) are consistent with other studies. In addition, our finding of a significant inverse association between testosterone and molybdenum is consistent with studies in animals (40).

To our knowledge this is the first human study to investigate the association between molybdenum and reproductive hormone levels. One case study of a suspected molybdenum poisoning exists, where a male in his late thirties ingested higher than recommended doses of molybdenum supplements (a total of 13.5 mg ingested over 24 days) and experienced psychosis, loss of libido, and low testosterone levels (41). The reproductive toxicity of molybdenum has been described in several animal studies as early as the 1950's where severe testicular degeneration in bulls and decreased fertility in male rats were reported after being fed molybdenum (9,42–44). A study of rams reported reduced testosterone levels and lower semen volume and sperm concentration, motility, and normal morphology in animals given molybdenum and sulphate compared to a control group which was given copper supplementation (40). More recently studies of rats have also reported that tetrathiomolybdenum (TTM) caused a reduction in epididymal weights, sperm concentration, motility and normal morphology at high dose levels (45), and oral administration of sodium molybdate caused degeneration of testicular morphology and function, dose-dependent declines in sperm concentration, motility and normal morphology, and evidence of male-mediated embryotoxicity (e.g. reduced implantation, increased pre/post implantation losses, and reduced fetal growth) (46). In a recent study of catfish from polluted waters in the Vietnamese Mekong Delta area, Yamaguchi and colleagues (47) found significant inverse

associations between tissue molybdenum, lead, and arsenic concentrations and gonadosomatic index (gonad weight/body weight × 100). Endocrine effects and reduced fertility in females following molybdenum exposure have also been reported in animal studies (48,49).

The biological mechanisms that may be involved in the association between molybdenum and reduced testosterone are not fully known. Molybdenum was found to accumulate in the testes of rats administered sodium molybdate (46) and in the brain (including hypothalamus), adrenal, pituitary, and potentially the testes of sheep treated with TTM (50,51), suggesting that these may be target organs following exposure to molybdenum. In the latter study this discovery was accompanied by the observation of organ structural changes (testes, adrenal and pituitary) and significant hormone depletion among exposed sheep compared to non-exposed controls (51). In addition, it is known that molybdate interacts directly with steroid receptors, and it has been used to stabilize steroid receptors and prevent receptor inactivation or transformation in experimental studies (52). However, if and how this interaction relates to the association between molybdenum exposure and declined testosterone levels in men or other adverse male reproductive health outcomes remains unclear.

Molybdenum is a ubiquitous trace element found in food and drinking water, and is present in multivitamin/multimineral supplements. Among foods, molybdenum is found at higher concentrations in leafy vegetables and legumes (44). Molybdenum concentrations in food, especially plants, are greatly dependent on species and soil characteristics. Concentrations of molybdenum in drinking water also likely vary. It was present at detectable levels in a majority (62%) of groundwater or surface water samples tested in the US over the last 15 years, collected mostly during land use surveys, with concentrations ranging from less than 0.05 μg/l to over 4500 μg/l (53). Human exposure may be elevated in areas involved in the mining of molybdenum ore, or can also result from certain industrial operations (54). Molybdenum is used in the manufacture of electronic parts, polyurethane foam, glass, ceramics, and lubricants, in the production of catalysts and pigments, in stainless steels and other steel alloys, and in chemical reagents found in hospital laboratories (46,54,55). Molybdenum compounds are also used in agriculture in fertilizers for leguminous plants (55,56) and have been proposed for use as a fungicide (57). The United States is the world's largest producer of molybdenum with large natural reserves in the western mountain regions, and global market demand has been increasing rapidly over the past five years (58).

The results of this study are consistent with our previous findings among this cohort where molybdenum was inversely associated with semen quality parameters (37). However, the inverse relationship between molybdenum and testosterone appears to be independent of semen quality, as the association remained when additionally adjusting the regression models for sperm concentration, motility or morphology. This independence is not surprising given that testosterone is a poor predictor of these semen quality parameters in men recruited from infertility clinics (59). The suggestive inverse association between manganese and T:LH ratio is also interesting in light of our recent findings, as it may provide evidence for a role of declined Leydig cell function in the previously observed relationship between manganese and declined semen quality (36). Manganese has also been reported to impact Leydig cell function in animal studies (60,61)

Our finding of a positive association between zinc and testosterone is also consistent with previous research, as zinc is known to play a role in testicular development, sperm maturation and steroidogenesis (38,62). We also found evidence for an interaction between molybdenum and low zinc in exploratory analysis, where testosterone levels were considerably lower among men with high molybdenum and lower copper (or zinc) concentrations in blood. Animal studies suggest that an interaction of molybdenum with other minerals is likely, but most discussion of these interactions involve copper since molybdenum has a chelating effect on copper and is

associated with impaired copper utilization (9,40,45). We previously reported evidence for an interaction involving low copper on the association between molybdenum and reduced semen quality (37) but did not find evidence of an interaction between molybdenum and low copper on circulating testosterone levels in the present analysis.

The high percentage of blood samples with concentrations below the limit of detection for some metals (e.g. molybdenum and cadmium) was a limitation of the present study, and restricted our ability to further investigate dose-response relationships and metal-metal interactions. Molybdenum and cadmium have been found to accumulate in the kidneys of animals and humans and are excreted in urine (44,54). Thus, more sensitive biomarkers and assays, such as the measurement of molybdenum and cadmium in urine which has been able to quantify exposure in a high percentage of samples from the US general population (54), should be implemented in future studies of molybdenum or cadmium exposure and endocrine or reproductive measures.

Another potential limitation of the present study was the collection of only a single blood sample from each participant for measurement of metals and hormone levels. This limits our ability to explore temporal (i.e. cause and effect) relationships between exposure to metals and hormone levels. It may also introduce exposure measurement error since the biological half-lives for the metals measured in the present study vary considerably. However, a single measure may represent exposure over a longer period of time even for those with short half-lives. For metals that are stored in the body, such as lead, a single measure is likely a reliable marker of exposure over months or years (63). For metals that are rapidly excreted but enter the body primarily through diet (including drinking water and mineral supplements), such as molybdenum, levels are also likely relatively stable over time due to consistent dietary patterns and drinking water sources (64). Despite the potential for diurnal and pulsatile fluctuations in hormone levels measured in serum, a single measure has been shown to provide a reliable measure of circulating hormone levels (testosterone, FSH and LH) in population studies (65–67). In addition, requiring multiple blood samples from participants would likely result in a reduced participation rate and lower statistical power.

As an indication of whether the concentrations of metals measured in blood in the present study were representative of those found among adult men in the wider general population, we compared the distribution of metal concentrations with those reported for adults in the Third National Report of Human Exposure to Environmental Chemicals (54). Of the metals measured in the present study, only lead and mercury were measured in blood in the Third National Report. The distribution of blood lead in the present study was similar to, though slightly lower than, those reported in the Third National Report. The median and 90^{th} percentile values were 1.6 and 3.6 μg/dL among adults in the Third National Report compared to 1.5 and 3.2 μg/dL in the present study. Mercury levels among adults from the Third National Report were only measured in females, but the distribution of mercury values was similar to those in the present study of men. Recent population concentration distributions of blood molybdenum could not be located, though the maximum value in the present study (5.4 μg/L) was equal to the 70^{th} percentile value reported in a 1968 US study (68). Results of that study suggested high regional variability where a maximum value of 410 μg/L was measured in a blood sample from Missoula, Montana. More data on the sources, distribution and time trends in molybdenum exposure are needed.

In conclusion, we found a number of suggestive or significant relationships between metals and hormone levels in the present study. The strongest and most consistent evidence was found for an inverse association between molybdenum and testosterone or FAI. These associations were robust when considering potential confounding variables and when simultaneously considering other potentially harmful and/or beneficial metals. We also found suggestive

evidence for an interaction between molybdenum and zinc, where low zinc levels strengthened the inverse relationship between molybdenum and testosterone. More research is needed to determine whether molybdenum poses a risk to human reproductive health.

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Table 1

Distribution of metal concentrations in blood (in μg/L, unless otherwise noted) and serum hormone levels. n = 219.

Metal	Selected Percentiles						
	10th	25th	50th	75th	90th	95th	Max
Arsenic	2.83	5.80	8.10	10.0	11.5	12.4	25.5
Cadmium	<0.2	<0.2	0.20	0.40	0.90	1.30	2.80
Chromium[a]	<0.5	<0.5	<0.5	0.60	1.00	2.40	16.6
Copper	754	817	887	974	1052	1112	1397
Lead (μg/dL)	0.80	1.10	1.50	2.00	3.20	4.20	16.2
Mercury	0.30	0.60	1.10	2.30	3.90	5.40	14.5
Molybdenum[b]	<1.0	<1.0	<1.0	1.10	1.60	2.00	5.40
Thallium	<0.1	<0.1	0.10	0.12	0.14	0.18	0.45
Zinc	5706	6169	6770	7294	7979	8250	9251
Hormone							
FSH (mIU/mL)	1.60	2.15	3.30	4.80	7.90	11.3	37.3
LH (mIU/mL)	1.55	2.25	3.35	4.90	6.15	7.00	21.8
Inhibin B (pg/mL)[c]	44.2	63.3	105	141	184	203	326
Testosterone (ng/dL)	233	288	389	479	570	633	753
SHBG (nmol/L)	12.7	18.0	26.0	35.5	43.4	51.8	70.5
FAI[d]	0.34	0.40	0.50	0.64	0.81	1.00	1.39
T:LH ratio	1.94	2.59	3.88	5.74	9.01	11.6	32.0

[a] For chromium exposure groups used in statistical analysis: 70th percentile = 0.6 μg/L; 85th percentile = 0.9 μg/L.

[b] For molybdenum exposure groups used in statistical analysis: 70th percentile = 1.0 μg/L; 85th percentile = 1.5 μg/L.

[c] For inhibin B, 5 samples were lost during analysis (n=214).

[d] FAI: free androgen index

Table 2

Adjusted[a] regression coefficients for change in serum hormone level associated with metal exposure groups.

	Hormone						
Metal Percentiles	**FSH[c]**	**LH[c]**	**Inhibin B**	**Testosterone[d]**	**SHBG[c]**	**FAI[c]**	**T:LH[c]**
Arsenic							
<25th	0	0	0	0	0	0	0
25th–50th	−0.09 (−0.33, 0.16)	0.01 −0.18, 0.24)	14.9 (−6.47, 36.2)	1.46 (−35.6, 38.6)	−0.03 (−0.20, 0.13)	0.000 (−0.13, 0.13)	−0.06 (−0.30, 0.17)
50th–75th	−0.07 (−0.31, 0.17)	0.01 (−0.19, 0.23)	14.7 (−6.25, 35.7)	−5.45 (−42.1, 31.2)	0.03 (−0.14, 0.19)	−0.04 (−0.17, 0.08)	−0.04 (−0.27, 0.19)
>75th	−0.15 (−0.39, 0.09)	−0.23 (−0.44, −0.02)	20.3 (−0.50, 41.2)	2.63 (−34.1, 39.4)	−0.002 (−0.17, 0.16)	−0.01 (−0.14, 0.12)	0.22 (−0.01, 0.45)
p for trend	0.28	**0.04**	0.07	0.99	0.83	0.70	0.07
Cadmium							
<50th	0	0	0	0	0	0	0
50th–75th	−0.14 (−0.35, 0.07)	−0.02 (−0.21, 0.16)	24.3 (6.21, 42.3)	16.3 (−15.8, 48.4)	0.11 (−0.03, 0.26)	−0.02 (−0.13, 0.10)	0.12 (−0.08, 0.33)
>75th	0.03 (−0.23, 0.30)	−0.02 (−0.25, 0.21)	17.5 (−5.07, 40.0)	27.8 (−12.0, 67.6)	0.09 (−0.08, 0.27)	0.01 (−0.12, 0.15)	0.13 (−0.13, 0.38)
p for trend	0.82	0.83	**0.03**	0.14	0.16	0.93	0.23
Chromium							
<70th	0	0	0	0	0	0	0
70th–85th	0.30 (0.07, 0.54)	0.13 (−0.08, 0.34)	−32.0 (−52.7, −11.5)	2.55 (−33.8, 38.9)	−0.15 (−0.31, 0.01)	0.11 (−0.01, 0.23)	−0.17 (−0.40, 0.06)
>85th	0.12 (−0.11, 0.36)	0.01 (−0.21, 0.22)	−23.1 (−43.5, −2.84)	18.5 (−18.0, 54.9)	−0.05 (−0.22, 0.11)	0.09 (−0.04, 0.21)	0.03 (−0.21, 0.26)
p for trend	0.09	0.66	**0.004**	0.35	0.23	0.08	0.79
Copper							
<25th	0	0	0	0	0	0	0
25th–50th	−0.28 (−0.51, −0.04)	−0.04 (−0.25, 0.18)	5.80 (−15.0, 26.6)	−11.9 (−47.9, 24.2)	−0.13 (−0.30, 0.03)	0.02 (−0.09, 0.16)	−0.06 (−0.29, 0.17)
50th–75th	−0.27 (−0.51, −0.03)	−0.13 (−0.35, 0.09)	13.5 (−8.17, 35.3)	6.23 (−30.5, 43.0)	0.004 (−0.16, 0.17)	0.01 (−0.12, 0.14)	0.14 (−0.10, 0.38)
>75th	−0.17 (−0.42, 0.09)	−0.11 (−0.34, 0.12)	3.19 (−19.4, 25.8)	41.9 (3.52, 80.3)	−0.000 (−0.17, 0.17)	0.10 (−0.04, 0.23)	0.21 (−0.04, 0.45)
p for trend	0.19	0.25	0.59	**0.03**	0.68	0.24	0.05

	Hormone						
Metal Percentiles	**FSH[c]**	**LH[c]**	**Inhibin B**	**Testosterone[d]**	**SHBG[c]**	**FAI[c]**	**T:LH[c]**
Lead							
<25th	0	0	0	0	0	0	0
25th–50th	0.13 (−0.10, 0.37)	0.004 (−0.20, 0.21)	−6.45 (−27.2, 14.3)	28.6 (−6.82, 64.1)	−0.01 (−0.16, 0.15)	0.08 (−0.04, 0.20)	0.07 (−0.16, 0.30)
50th–75th	0.10 (−0.15, 0.35)	0.13 (−0.09, 0.35)	−4.62 (−26.6, 17.4)	15.8 (−21.8, 53.3)	0.04 (−0.12, 0.21)	0.03 (−0.10, 0.17)	−0.05 (−0.29, 0.19)
>75th	0.07 (−0.18, 0.31)	0.08 (−0.14, 0.29)	−7.79 (−29.0, 13.4)	39.9 (3.32, 76,4)	0.07 (−0.10, 0.23)	0.08 (−0.05, 0.21)	0.07 (−0.17, 0.31)
p for trend	0.65	0.32	0.52	0.07	0.34	0.35	0.80
Manganese							
<25th	0	0	0	0	0	0	0
25th–50th	0.06 (−0.16, 0.28)	0.004 (−0.19, 0.20)	13.2 (−5.58, 31.9)	−20.9 (−53.7, 11.9)	−0.03 (−0.18, 0.11)	−0.04 (−0.15, 0.08)	−0.07 (−0.28, 0.14)
50th–75th	−0.03 (−0.29, 0.23)	0.02 (−0.21, 0.25)	15.4 (−7.34, 38.2)	0.03 (−39.4, 39.5)	−0.06 (−0.23, 0.12)	0.01 (−0.13, 0.15)	−0.07 (−0.33, 0.18)
>75th	−0.09 (−0.34, 0.16)	−0.07 (−0.30, 0.15)	22.9 (0.89, 44.9)	−26.6 (−64.9, 11.6)	−0.17 (−0.33, 0.003)	−0.01 (−0.15, 0.12)	−0.11 (−0.35, 0.14)
p for trend	0.39	0.59	**0.04**	0.34	0.06	0.99	0.40
Mercury							
<25th	0	0	0	0	0	0	0
25th–50th	0.10 (−0.14, 0.34)	0.01 (−0.20, 0.23)	−12.8 (−33.8, 8.21)	12.1 (−24.9, 49.1)	−0.18 (−0.34, −0.02)	0.11 (−0.02, 0.23)	−0.09 (−0.32, 0.15)
50th–75th	−0.13 (−0.36, 0.10)	−0.01 (−0.22, 0.19)	10.2 (−10.0, 30.4)	7.61 (−27.5, 42.7)	0.04 (−0.11, 0.20)	−0.02 (−0.14, 0.10)	0.04 (−0.19, 0.26)
>75th	−0.14 (−0.38, 0.09)	−0.07 (−0.28, 0.14)	6.50 (−14.1, 27.1)	14.2 (−21.8, 50.2)	0.02 (−0.14, 0.18)	0.03 (−0.09, 0.15)	0.12 (−0.11, 0.35)
p for trend	0.12	0.54	0.28	0.48	0.42	0.99	0.26
Molybdenum							
<70th	0	0	0	0	0	0	0
70th – 85th	0.03 (−0.22, 0.26)	−0.02 (−0.23, 0.19)	−1.96 (−22.9, 20.0)	−23.0 (−58.3, 12.3)	−0.01 (−0.17, 0.15)	−0.07 (−0.20, 0.05)	−0.06 (−0.29, 0.17)
>85h	0.04 (−0.22, 0.28)	−0.12 (−0.34, 0.10)	7.18 (−14.6, 29.0)	−60.0 (−97.2, −22.8)	0.06 (−0.11, 0.23)	−0.19 (−0.32, −0.06)	−0.02 (−0.26, 0.23)
p for trend	0.79	0.33	0.61	**0.001**	0.58	**0.004**	0.78
Selenium							
<25th	0	0	0	0	0	0	0

Metal Percentiles	Hormone						
	FSH[c]	**LH[c]**	**Inhibin B**	**Testosterone[d]**	**SHBG[c]**	**FAI[c]**	**T:LH[c]**
25th–50th	0.02 (−0.22, 0.26)	−0.11 (−0.32, 0.10)	−18.6 (−39.3, 2.11)	−29.6 (−65.6, 6.51)	−0.14 (−0.30, 0.02)	−0.01 (−0.13, 0.12)	−0.04 (−0.27, 0.19)
50th–75th	0.05 (−0.19, 0.30)	−0.15 (−0.36, 0.06)	−15.9 (−37.2, 5.31)	−20.1 (−56.8, 16.5)	−0.01 (−0.17, 0.15)	−0.06 (−0.18, 0.07)	0.09 (−0.15, 0.32)
>75th	−0.03 (−0.27, 0.21)	−0.29 (−0.50, −0.08)	−3.43 (−24.2, 17.3)	−26.1 (−62.6, 10.5)	−0.17 (−0.32, −0.01)	0.003 (−0.12, 0.13)	0.12 (−0.11, 0.35)
p for trend	0.88	0.008	0.82	0.24	0.14	0.85	0.20
Thallium							
<50th	0	0	0	0	0	0	0
50th–75th	−0.31 (−0.52, −0.11)	−0.25 (−0.43, −0.07)	25.9 (8.15, 43.7)	−14.4 (−45.5, 16.8)	0.01 (−0.13, 0.15)	−0.05 (−0.16, 0.06)	0.20 (0.01, 0.40)
>75th	−0.07 (−0.28, 0.14)	−0.14 (−0.32, 0.05)	16.4 (−1.57, 34.4)	−2.25 (−34.1, 19.6)	−0.04 (−0.18, 0.10)	−0.01 (−0.12, 0.10)	0.09 (−0.11, 0.29)
p for trend	0.23	0.06	**0.03**	0.75	0.64	0.76	0.22
Zinc							
<25th	0	0	0	0	0	0	0
25th–50th	0.10 (−0.14, 0.34)	0.01 (−0.20, 0.22)	10.4 (−10.6, 31.5)	−21.7 (−57.5, 14.1)	−0.02 (−0.18, 0.14)	−0.03 (−0.16, 0.10)	−0.06 (−0.29, 0.17)
50th–75th	−0.09 (−0.33, 0.16)	−0.03 (−0.24, 0.19)	9.91 (−11.3, 31.1)	9.88 (−26.5, 46.3)	0.06 (−0.10, 0.23)	−0.01 (−0.14, 0.12)	0.08 (−0.15, 0.32)
>75th	0.04 (−0.20, 0.28)	−0.02 (−0.24, 0.19)	9.22 (−12.1, 30.5)	19.4 (−16.7, 55.6)	−0.01 (−0.17, 0.15)	0.05 (−0.08, 0.17)	0.06 (−0.17, 0.29)
p for trend	0.86	0.78	0.43	0.13	0.84	0.43	0.41

[a] Adjusted for age, BMI, and current smoking.

[b] N=214 for inhibin B (5 samples lost during analysis)

[c] ln-transformed

[d] testosterone model adjusted for lnSHBG

Table 3

Final linear regression models[a] for hormone levels when considering multiple metals and other potentially important covariates.

Variable	Regression coefficient (95%CI)	p-value (for trend where appropriate)
FSH[b]		
Age		
Year increase	0.02 (0.01, 0.04)	0.005
Smoking Status[b]		
Current smoker	−0.18 (−0.38, 0.02)	0.07
LH[b]		
Copper		
25th–50th	−0.07 (−0.28, 0.14)	
50h–75th	−0.16 (−0.37, 0.05)	
>75th	−0.21 (−0.42, 0.01)	0.04
Selenium		
25th–50th	−0.12 (−0.32, 0.09)	
50h–75th	−0.14 (−0.35, 0.07)	
>75th	−0.25 (−0.46, −0.04)	0.02
Race[c]		
White	−0.19 (−0.37, −0.01)	0.03
Inhibin B		
Cadmium		
50h–75th	22.6 (4.68, 40.6)	
>75th	26.5 (8.53, 44.5)	0.005
Chromium		
70th –85th	−32.2 (−52.7, −11.8)	
> 85th	−17.5 (−38.1, 3.00)	0.009
Age		
Year increase	−1.70 (−2.95, −0.44)	0.01
BMI		
Unit increase	−2.13 (−3.29, −0.96)	0.0006
Testosterone		
Molybdenum		
70th –85th	−18.5 (−53.3, 16.3)	
> 85th	−55.9 (−92.5, −19.3)	0.003
Zinc		
25th–50th	−14.7 (−49.1, 19.7)	

Variable	Regression coefficient (95%CI)	p-value (for trend where appropriate)
50h–75th	10.6 (−24.1, 45.2)	
>75th	27.9 (−7.00, 62.9)	0.05
SHBG		
ln-unit increase	179 (150, 208)	<0.0001
Age		
Year increase	−4.18 (−6.34, −2.02)	0.0002
BMI		
Unit increase	−3.05 (−5.25, −0.86)	0.006
Race[c]		
White	−43.7 (−72.3, −15.1)	0.002
Free Androgen Index[b]		
Molybdenum		
70th –85th	−0.10 (−0.22, −0.02)	
> 85th	−0.21 (−0.34, −0.08)	0.0009
Chromium		
70th –85th	0.11 (−0.01, 0.24)	
> 85th	0.10 (−0.02, 0.23)	0.04
Copper		
25th–50th	0.06 (−0.07, 0.18)	
50h–75th	0.06 (−0.06, 0.19)	
>75th	0.14 (0.02, 0.27)	0.02
Age		
Year increase	−0.02 (−0.03, −0.01)	<0.0001
T:LH Ratio[b]		
Manganese		
25th–50th	−0.11 (−0.33, 0.10)	
50h–75th	−0.17 (−0.43, 0.10)	
>75th	−0.18 (−0.44, 0.07)	0.09
Copper		
25th–50th	−0.03 (−0.27, 0.20)	
50h–75th	0.18 (−0.07, 0.42)	
>75th	0.26 (0.01, 0.51)	0.02
Selenium		
25th–50th	0.004(−0.23, 0.23)	
50h–75th	0.10 (−0.13, 0.34)	
>75th	0.17 (−0.06, 0.41)	0.10
BMI		

Variable	Regression coefficient (95%CI)	p-value (for trend where appropriate)
Unit increase	−0.03 (−0.04, −0.01)	0.0007

[a] Backward elimination; same results obtained with forward selection and stepwise procedures

[b] Variable ln-transformed

[c] Reference group = non-white