Reproductive Toxicology 41 (2013) 109–114

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Associations of urinary metal concentrations and circulating testosterone in Chinese men

Qiang Zeng[a,b,c,1], Bin Zhou[d,1], Wei Feng[a,b,c], Yi-Xin Wang[a,b,c], Ai-Lin Liu[a,b,c], Jing Yue[e], Yu-Feng Li[e,**], Wen-Qing Lu[a,b,c,*]

[a] Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[b] MOE (Ministry of Education) Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[c] State Key Laboratory of Environment Health (Incubation), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China
[d] College of Public Health, University of South China, Hengyang, Hunan, PR China
[e] Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China

ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 20 May 2013
Accepted 8 June 2013
Available online 17 June 2013

Key words:
Men
Reproductive health
Testosterone
Urinary metal

ABSTRACT

Toxicological studies have shown that metals directly or indirectly influence testosterone (T) production, but the data from humans is limited and inconsistent. The aim of this study was to examine the associations between urinary metal concentrations and circulating T in Chinese men. Urinary concentrations of 13 metals (arsenic, cadmium, cobalt, chromium, copper, iron, lead, manganese, molybdenum, mercury, nickel, selenium and zinc) and serum levels of T were analyzed in 118 men from an infertility clinic. Multivariable linear regression was used to assess the effect of metals exposure on T. Among the measured metals, the median urinary Zn (359.36 μg/g creatinine) and Co (0.16 μg/g creatinine) concentrations were the highest and the lowest, respectively. Significant dose–response relationships were found between decreased T and urinary Mn and Zn, even when considering multiple metals (both *P* for trend <0.05). Our results indicate that elevated Mn and Zn are inversely associated with T production.

1. Introduction

Several studies have reported that circulating testosterone (T) is declining in men [1–4]. These findings lead a number of studies to explore potential risk factors for the declining trend, as low concentrations of T may contribute to diabetes and prediabetic conditions, reduced bone and muscle mass, impaired sexual function and decreased quality of life [5]. Age has been considered as one of the primary contributors to the declining T trend. However, environmental factors, such as lifetime exposure to certain metals, may also contribute to the decreased T trend [6].

Metals, including essential and nonessential elements, are present in various environmental media such as air, diet, water, soil and dust. Humans can be widely exposed to metals at low concentrations either voluntarily through supplementation or involuntarily through intake of contaminated food and water or contact with contaminated dust, air or soil [7]. Consequently, metals have been detected in most of the general population's biological samples (e.g., urine, blood and seminal plasma) [7–9]. Potential human health risk of metals exposure at environmental levels has become a great concern worldwide.

Toxicological studies have suggested that some metals (e.g., As, Cr, Cu, Hg, Mn, Pb and Zn) can reduce T production in in vitro and in vivo [10–13]. The potential mechanism of metals-induced decrease in T production is that metals directly affect Leydig cells function or indirectly cause hypothalamic–pituitary–testicular axis disruption [11,12]. Epidemiological studies have also suggested that some metals (e.g., Cu, Cr, Mn and Mo) in blood and seminal plasma are inversely associated with T production [14–17]. However, the data regarding some metals are still inconsistent (e.g., Hg, Pb and Zn) and limited (e.g., Cu, Cr, Cd, Mn and Mo), and there is also a lack of data regarding most of the other metals (e.g., As, Co, Fe, Ni and Se). Previous studies primarily used metals measured in blood and seminal plasma as biomarkers to evaluate the exposure levels. Because many of the chemical forms of metals (e.g., Cd, Hg, Mn

* Corresponding author at: Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, PR China. Tel.: +86 27 83610149; fax: +86 27 83657765.
** Co-corresponding author. Tel.: +86 27 83662534; fax: +86 27 83662534.
E-mail addresses: yufengli64@yahoo.com.cn (Y.-F. Li), luwq@mails.tjmu.edu.cn (W.-Q. Lu).
[1] These two authors equally contributed to this work.

0890-6238/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.reprotox.2013.06.062

and Mo) are water soluble, measurement of these metals in urine is considered as better and sensitive biomarkers for the general population bio-monitoring [18,19].

Animal and human evidence suggests that exposure to metals may have an adverse impact on T production. The aim of the present study was to examine whether the metal exposure at environmental levels adversely affected circulating T in Chinese men. We assessed the metal exposure levels by measuring their concentrations in urine, including essential and nonessential elements. Because some essential and nonessential metals may additively, synergistically or antagonistically affect the T production [6,20], we also simultaneously examined the effects of multiple metals on T. To our best knowledge, this is the first and comprehensive study using urinary metal concentrations as biomarkers to assess the effects of metal exposure at environmental levels on circulating T.

2. Materials and methods

2.1. Study participants and data collection

We recruited study participants from men who participated in an ongoing cross-sectional study of exposure to environmental contaminants and male reproduction health [21]. Briefly, male partners in sub-fertile couples presenting to the Reproductive Center of Tongjing Hospital in Wuhan, China, to seek infertility examination were invited to participate in the study. During March to May 2012, a total of 148 men agreed to participate in the study. We excluded 30 men who had endocrine diseases (e.g., diabetes, thyroid or adrenal disorders) and other medical conditions associated with infertility (e.g., azoospermatism, orchiditis, epididymitis, vesiculitis, undescended testicle, injury of testis and hernia repair complicated by testicular atrophy). Thus, a total of 118 men, including fertile and sub-fertile men, were retained for the current analysis. In addition, only 9 men from all of the study subjects had the serum T below or above the reference value of T. All of the study participants completed a face-to-face questionnaire. The collected information included demographics, lifestyle habits, occupational exposures and medical characteristics. The study was approved by the Ethics Committee of Tongji Medical College, and informed consent was obtained from each participant at enrollment.

2.2. Blood sample collection and analyses

We collected 5-ml peripheral blood from each participant in the morning using blood sampling tubes containing the coagulants. After centrifugation of the blood sample at 1000 rpm for 10 min, T concentration in serum was measured on the day of collection by the direct chemiluminescence assay with commercial test kits (Siemens Healthcare Diagnostics Inc., East Walpole, USA). Control samples adding the low and high levels of T to the serum sample were also analyzed according to the same analysis procedure each day. The reference value of T ranged from 241 to 827 ng/dL and the limit of detection (LOD) was 10 ng/dL. The recovery and the coefficient of variance (interday variation) ranged from 83.0% to 107.5% and5.4% to 8.2%, respectively.

2.3. Urine sample collection and analyses

We collected a single-spot urine sample into a conical polyethylene container from each participant to minimize background contamination [22]. After collection, all urine samples were packed into coolers with ice packs and sent to the laboratory, then stored at −40 °C until analysis. Metal concentrations in urine were analyzed according to the method described in detail by Heitland and Köster [22]. Briefly, the frozen urine samples were allowed to equilibrate to room temperature. A 3-ml aliquot of urine was transferred to a polypropylene tube (Jiayu Co., Ltd., Haimen, China) containing 15-μl 67% (v/v) HNO_3 (Optima™ grade, Fisher, Belgium) and stored in a refrigerator at 4 °C. After vigorously shaking, 1-ml sample was pipetted into a 10-ml disposable polypropylene tube and then filled up to 5 ml with 1.2% (v/v) HNO_3. The samples were allowed to stand for 30 min before inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies, Waldbronn, Germany) analysis.

Urinary creatinine was determined by the picric acid assay with commercial test kits purchased from Jiancheng Bioengineering Ltd. (Nanjing, China). The creatinine-adjusted urinary metal concentrations were expressed as μg/g creatinine to control for the effect of variation in urine diluteness.

2.4. Quality assurance/quality control

Control samples of urine standard reference material (SRM) 2670a and SRM 1640a (National Institute of Standards and Technology, Gaithersburg, Maryland, USA), as well as a pooled urine ($n = 100$) prepared at the laboratory were used for internal quality assurance. SRM 2670a and spiked urine were analyzed at two different concentrations for each element after calibration procedure to evaluate analytical accuracy. SRM 1640a was analyzed after every 20 sample to assess the instrument performance. If their concentrations were significantly different from the certified value of SRM 1640a, the instrument was recalibrated using multi-element standards and the previous 20 samples were reanalyzed. The LOD of urinary metals concentrations were as follows: 0.01 μg/L for As; 0.002 μg/L for Cd; 0.001 μg/L for Co; 0.01 μg/L for Cr; 0.23 μg/L for Cu; 0.29 μg/L for Fe; 0.01 μg/L for Hg; 0.02 μg/L for Mn; 0.004 μg/L for Mo; 0.03 μg/L for Ni; 0.18 μg/L for Pb; 0.12 μg/L for Se; 0.001 μg/L for Zn.

Table 1
Demographic characteristics of study participants ($n = 118$[a]).

Characteristics	Mean ± standard deviation
Age, years	30.8 ± 5.6
Body mass index (BMI), kg/m^2	23.9 ± 4.4
Smoking status	
Never	51 (43.2)
Former	23 (19.5)
Current	44 (37.3)
Alcohol use	
Yes	89 (75.4)
No	29 (24.6)
Education level	
Less than high school	51 (43.2)
High school and above	67 (56.8)
Income, RMB yuan/month	
≤2000	30 (25.9)
2000–6000	70 (60.3)
≥6000	16 (13.8)

[a] 3 missing age and 2 missing income.

2.5. Statistical analyses

We performed data analysis using the Statistical Package for the Social Sciences (SPSS), version 18.0 (SPSS Inc., Chicago, IL, USA). We calculated descriptive statistics for demographic characteristics, urinary metal concentrations and circulating T of study participants. We also used Spearman correlation coefficients to examine the associations between demographic variables or T and creatinine-adjusted urinary metal concentrations.

We used univariable and multivariable linear regression models to assess the dose–response relationships between quartiles of creatinine-adjusted urinary metal concentrations and T. Age, body mass index (BMI), smoking status, alcohol use, education and income were considered as covariates. We used the change-in-effect estimate method to determine whether the covariates should be included in the multivariable model [23]. Potential confounders were entered into the multivariable model if they changed the effect estimates by 10% or greater for at least one metal exposure. We finally included age and BMI as continuous variables, alcohol use (yes vs. no) as a dichotomous variable, smoking status (current and former vs. never smoker) and income (2000–6000 and >6000 vs. <2000 yuan per month) as dummy variables in the model.

We also performed full linear regression model to simultaneously examine the effects of multiple metals on T. We used the backward elimination procedure and set alpha at 0.10 for variables to be retained in the final model. We then added covariates not retained in the final model individually to further explore evidence of confounding if they changed the effect estimates for metals by 10% or greater. We performed the trend test by entering the quartiles of creatinine-adjusted urinary metal concentrations into the model as an ordinal categorical variable using integer values (0, 1, 2 and 3). We defined statistical significance as a P value <0.05. Because of the exploratory nature of the analysis, we also defined P-values <0.10 as statistically suggestive [24].

3. Results

3.1. Descriptive statistics of study participants

The demographic characteristics of study participants are shown in Table 1. The study participants aged from 22 to 47 with a mean age of 30.8 (±5.6) years and BMI of 23.9 (±4.4) kg/m^2. There were 44 (37.3%) current smokers, 23 (19.5%) former smokers and 51 (43.2%) never smokers. Eighty-nine men (75.4%) were alcohol users. Fifty-one men (43.2%) reported their education level as less