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Research paper

Environmental doses of arsenic exposure are associated with increased reproductive-age male urinary hormone excretion and *in vitro* Leydig cell steroidogenesis

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ABSTRACT

Humans are ubiquitously exposed to arsenic from multiple sources, and chronic arsenic exposure may be associated with male reproductive health. Although association regarding arsenic exposure and sex hormone secretion in blood has been reported, sex hormone excretion in urine studies is lacking. Urinary sex hormone excretion has emerged as a complementary strategy to evaluate gonadal function. Herein, we determined the associations between environmental exposure to arsenic and urinary sex hormone elimination and *in vitro* Leydig cell steroidogenesis. Concentrations of arsenic and testosterone (T), estradiol (E_2) and progesterone (P) in repeated urine samples were determined among 451 reproductive-age males. Moreover, an *in vitro* Leydig cell MLTC-1 steroidogenesis experiment was designed to simulate real-world scenarios of low human exposure. Multivariable linear regression models were used to assess the associations of urinary arsenic levels with urinary hormones. Urinary arsenic concentrations were positively associated with urinary sex hormone (T, E_2, and P) levels. An *in vitro* test further demonstrated that a population-based environmental exposure range (0.01–5 μM) of arsenic induced Leydig cell steroidogenesis potency. Our results indicate that low-dose arsenic exposure exhibits an endocrine disrupting effect by stimulating Leydig cell steroidogenesis and accelerating urinary steroid excretion, which extends previous knowledge of the inverse association of high-dose arsenic exposure with sexual steroid production that is assumed to be anti-androgen.

1. Introduction

Arsenic is a metalloid element found ubiquitously in the environment in either an organic or inorganic state. Water with arsenic above the World Health Organization guideline of 10 ppb has been discovered in many countries, especially in Asia, including China (Hubaux et al., 2013; Rodríguez-Lado et al., 2013). Meanwhile, arsenic can naturally accumulate in rice and seaweed (Molin et al., 2017) and is one of important toxicants in PM2.5 air pollution (Huang et al., 2014); therefore, general populations are widely exposed to arsenic *via* digestion and inhalation. Usually, arsenic methylation occurs predominantly in the liver, which enhances its clearance from the human body (Drobná et al., 2010). Multidrug resistance proteins (MRPs) are responsible for the transport of hepatic arsenic metabolites into sinusoidal blood, ultimately for urinary elimination (Banerjee et al., 2014). Consequently, arsenic is monitored in most of the general population, and urinary arsenic metabolites are extensively used as exposure biomarkers (Zorimar, 2009).

Environmental arsenic exposure is associated with multiple adverse health outcomes, including male reproductive toxicity (Renu et al., 2018). Accumulated epidemiological and animal toxicological data have demonstrated that arsenic exposure can directly disrupt male

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steroidogenesis and ultimately impact testicular organ development and impair spermatogenesis (Zubair et al., 2017; Renu et al., 2018). Increasing evidence indicates that exposure to arsenic can result in inhibited blood sex hormones by changing steroidogenesis-related proteins and genes (Zeng et al., 2013, 2018; Hsieh et al., 2008; Ommati et al., 2020). In contrast to the decline in blood hormones, a previous Polish population study indicated that low-level exposure to arsenic was positively associated with urinary androgen hormone excretion in both males and females (Kozłowska et al., 2019). In line with this observation, our previous urinary metabolomics results also revealed that environmentally relevant arsenic exposure was associated with increased urinary testosterone in a Chinese male population (Zhang et al., 2014). Regarding these inconsistent observations, the difference in sex hormone secretion in blood and excretion in urine in response to arsenic exposure needs to be clarified. One of most plausible explanations for the inconsistent results is that sex hormones have distinct metabolic pathways in blood and urine (Schiffer et al., 2019).

Testosterone is the major sex hormone for male reproduction that regulates the processes underlying sexual behavior. Testicular Leydig cells are the primary synthesis organ in males. Testicular steroidogenesis utilizes cholesterol as a biosynthesis precursor and is governed by two enzyme, 3β-HSD and 17β-HSD (Sanderson, 2006), which are subsequently converted into intermediates of 17-α-hydroxypregnenolone (17-OHP) and androstenedione (ASD) before production of the end-product of testosterone (Bochud et al., 2018). Usually, in human blood, most androgens, including testosterone, are bound to the plasma proteins sex hormone binding globulin (SHBG) and albumin, and only a small fraction (1–2%) of androgens are free and accessible to target tissues and exert biological activity (Schiffer et al., 2019). Before urinary excretion, blood androgen is initially subjected to glucuronidation phase II conjugation metabolism by UDP-glucuronosyltransferase superfamily (UGTs) enzymes in the liver (Bélanger et al., 2003). Multidrug resistance proteins (MRPs) are involved in conjugated androgen efflux, eliminating conjugated androgen from hepatocytes to urine (Järvinen et al., 2020). Interestingly, exposure to arsenic was accompanied by increased UGT mRNA expression in mouse and human islets (Carmean et al., 2019). Meanwhile, MRPs mediate the detoxification and elimination of arsenic (Banerjee et al., 2016). Given that arsenic detoxification and elimination processes may interact with sex hormone production and metabolism, the relationships between blood sex hormones and arsenic exposure have been intensively investigated, but their impact on sex hormone urinary excretion has been insufficiently addressed. Due to relatively high hormone concentrations in urine and greater accessibility of urine compared with blood, measurements of urinary steroid excretion have emerged as methods to evaluate gonadal function (Singh et al., 2015). Clarifying the urinary hormone excretion response to arsenic exposure will offer optional approaches for arsenic reproductive toxicity assessment.

Given that single measurements of urinary arsenic potentially vary over time in a population study, which may result in a moderate degree of exposure misclassification (Wang et al., 2016a), and that toxicological *in vitro* arsenic exposure doses beyond environmentally relevant levels may lead to uncertainty when extrapolating data from *in vitro* to humans (Hughes et al., 2007), we explored the association between arsenic exposure and urinary hormone excretion among 451 reproductive-age men with repeated collection samples to overcome single measurement arsenic variations over time. We also applied an *in vitro* Leydig cell steroidogenesis experiment with low-dose arsenic exposure to simulate real-world scenarios of human exposure.

2. Methods

2.1. Study population

The men included in this study were a subset of participants in a larger study who came to the Wuhan Reproductive Medicine Center for fertility assessment without prior knowledge of infertility status (Wang et al., 2016b). This work has received approval for research ethics from Tongji Medical College (Wuhan, China) and proof of approval is available upon request, was undertaken from April to June 2013. After signing informed consent, participants were requested to have a blood sample drawn and provide two spot-urine specimens (at least 2 h apart). A face-to-face interview was also conducted to collect information on demographic characteristics [age, body mass index (BMI)], lifestyle factors (smoking status and drinking habit), medical history, and occupational exposures. We excluded participants who had self-reported urogenital or metabolic disease (testis injury, orchitis, vasectomy, epididymitis, diabetes and adrenal disorder) and occupational exposure. Finally, a total of 1040 participants were recruited for the entire study. Of them, 511 were measured for blood reproductive hormones in a previous study (Wang et al., 2016c). We further excluded 60 subjects for inadequate sample volumes, leaving 451 participants in our current investigation.

2.2. Urinary arsenic measures

Repeated spot-urine samples from each participant were collected in trace element-free polyethylene specimen tubes and stored at − 40 °C immediately until analysis was carried out. The determination of urinary arsenic concentrations has been described in our previous study (Wang et al., 2016c). In brief, a 3.0 mL aliquot of urine was acidified with 15 μL of 67% (v/v) HNO_3. After acidification in a refrigerator at 4 °C for at least 24 h, the urine samples were brought to room temperature, and 1.0 mL of each sample was diluted to 5.0 mL with 1.2% (v/v) HNO_3, which was then detected using an Agilent 7700x inductively coupled plasma mass spectrometer with an octopole-based collision/-reaction cell (Agilent Technologies, Waldbronn, USA). Quality control samples, spiked pooled urine samples, blank urine negative samples (containing deionized water) and two sets of standards were analyzed together with the unknown samples in each analytical batch. The limit of quantification (LOQ) of arsenic was 0.013 μg/L. The spike recoveries for arsenic ranged from 82% to 117%. To correct for urinary dilution, urinary creatinine (Cr) concentrations were measured using an automated clinical chemistry analyzer (Wang et al., 2016d).

2.3. Hormonal analysis

All urine samples were transported on dry ice to the analytical laboratory in Zhoushan Hospital (Zhoushan, China) after collection. E_2, T, and progesterone (P) concentrations were determined by an automatic immunoassay system (BECKMAN COULTER UniCel™ DxI 800, Access®, USA) according to the manufacturer's instructions (Wang et al., 2017). To eliminate intra-individual fluctuations in hormone excretion, repeated urine samples were pooled with equal volumes before determination. The LODs of E_2, T, and P were 20 pg/mL, 0.1 ng/mL and 0.1 ng/mL, respectively. A value equal to half the LOD was set for the samples with concentrations below the LOD. The QC procedure for E_2, T, and P measurement was in accordance with the guidelines of Zhoushan Hospital, and the coefficient of variance was $< 10\%$. Serum T, E_2, follicle stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone-binding globulin (SHBG) levels were published in our previous study (Wang et al., 2016d). In brief, serum hormones were measured within 1 h of blood collection by direct chemiluminescence assay using available commercial test kits (Siemens Healthcare Diagnostics Inc.) in the Reproductive Center of Tongji Hospital.

2.4. MLTC-1 cell culture and arsenic exposure

To investigate the steroidogenic effects of arsenic at the exposure range (0.1–315 ng/mL) in the present study population, related low-dose (0.01 μM, 0.5 μM and 5 μM indicating human minimum, median and maximum human exposure levels, respectively) arsenic exposure

mouse testicular Leydig MLTC-1 cell experiments were conducted. Because arsenite is the main arsenic species that exerts reproductive toxicity compared with other arsenic chemical species, sodium arsenite was selected for *in vitro* cell exposure experiments. The MLTC-1 cell line was obtained from the Cell Institute of Shanghai (Shanghai, China) and cultured in RPMI-1640 medium (Gibco BRL, Grand Island, NY) supplemented with 100 unit/mL penicillin, 100 unit/mL streptomycin and 10% (v/v) fetal bovine serum (HyClone, USA). The cells were grown at 37 °C with 5% CO_2 in a humidified incubator (SANYO, Japan). MLTC-1 cells were seeded in 6-cm petri dishes and cultured for 24 h prior to treatment. Cells were exposed to 0 μM, 0.01 μM, 0.5 μM or 5 μM sodium arsenite for 48 h, and three replicates for each dose of arsenic exposure were performed. After arsenic exposure, the cells were washed with PBS and serum-free medium. Subsequently, cells were stimulated for 4 h with hCG in serum-free medium supplemented with 0.1% BSA. The medium was collected for sex hormone determination and cells for gene expression analysis.

2.5. Cell culture medium hormone analysis

The steroid hormones androstenedione (ASD), 17-α-hydroxypregnenolone (17-OHP) and testosterone were detected in the cell culture medium by LC-ESI-MS/MS, as previously described (Tian et al., 2018). In brief, an aliquot of 1 mL of medium was diluted with 3 mL of ammonium acetate buffer (1 mol/L) and spiked with 20 μL of 100 ng/mL D3-testosterone and D3–17-OHP as internal standards (ASD was semi-quantified). Then, the diluted samples were extracted three times with 3 mL of ethyl acetate. The three extracts were combined and washed with 5 mL of water, and then the combined extracts were evaporated under a gentle stream of nitrogen gas at 40 °C. The residue was reconstituted with 200 μL of methanol/water (50:50, v:v) by vortexing vigorously for 15 s and then transferred into an HPLC vial for LC-MS-MS analysis. Two quality control samples and two sets of standards were analyzed together with unknown samples in each analytical batch. The LODs of 17-OHP, ASD, and T were 0.05 ng/mL, 0.05 ng/mL and 0.05 ng/mL, respectively. The recoveries for 17-OHP, ASD, and T at the three spiked levels (*i.e.*, 0.5, 1.0, and 10.0 ng/mL) ranged from 74.7% to 112%.

2.6. RNA extraction and real-time RT-PCR analysis

To investigate mRNA expression alterations, a quantitative real-time PCR assay was performed, as reported in our previously published study (Tian et al., 2019). Total RNA was extracted from cells using an RNA extraction kit (Promega, USA) following the manufacturer's protocol. Extracted RNA samples were stored at − 80 °C for subsequent analysis. A NanoDrop spectrophotometer (NanoDrop Technologies Inc., USA) was used to measure RNA concentration and purity. Reverse transcription of cDNA synthesis was performed using oligo dT primer, in which 1 μg of total RNA was applied using PrimeScript® RT Reagent Kit together with gDNA Eraser cDNA Synthesis Kits (Takara, Japan). Real-time PCR was carried out in a final volume of 20 μL and performed in triplicate using SYBR Green Master Mix reagents (Roche, USA) in an LC 480 system (Roche Applied Science, Germany) according to the manufacturer's protocol. The primer sets and product sizes used for amplification PCR analysis are shown in Table S1 (see SI). The conditions for quantitative PCR were as follows: 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 30 s. Gene expression levels were normalized to *GAPDH* expression levels. Three replicates of quantitative PCR were performed for each sample. Three replicates for each dose of sodium arsenite exposure were performed. The fold-changes of the tested genes were analyzed by the $2^{-\Delta\Delta Ct}$ method, where ΔΔCt = (Ct Target gene – Ct *GAPDH*) arsenic exposure – (Ct Target gene– Ct *GAPDH*) blank control.

2.7. Data analysis

Distributional plots and descriptive statistics were evaluated for population characteristics and for the distribution of arsenic levels and hormone concentrations. The average creatinine-adjusted arsenic levels in the repeated sample were modeled both as natural log-transformed continuous variables and categorized quartile variables. To achieve variance in homogeneity and normality in the distributions of the residuals, the concentrations of serum total T, free T, bioavailable T, E_2, LH, FSH, SHBG, and creatinine-adjusted urinary P, T and E_2 were also modeled as natural log-transformed values. Correlations between urine and serum hormones were estimated by Spearman's rank correlation.

The associations between quartiles of urinary arsenic levels and urinary hormone levels were assessed using multivariate linear regression models. Tests for trends across quartiles in the regression models were conducted by using creatinine-adjusted arsenic quartiles as continuous and categorized variables using integer values (*i.e.*, 1–4). Urinary arsenic levels (ln-transformed creatinine-adjusted arsenic) were also modeled as a continuous variable. The corresponding regression coefficients were back-transformed to obtain percent changes, which were calculated as [exp (β)−1] × 100% for categorized variables and $[(2)^{\beta}-1] \times 100\%$ for continuous variables (β was the coefficient of arsenic in the linear regression models). Confounders were included in the multivariable model based on biological and statistical factors. In the final multivariable models, age and body mass index (BMI) were treated as continuous variables; alcohol use (yes *vs.* no) was included as a dichotomous variable; and smoking status (current and former smoker *vs.* never smoker) was retained as a dummy variable.

The measured continuous variables of *in vitro* study gene expression and steroid hormone levels are presented as the means with their standard errors of the mean (means ± SEM). *In vitro* study data were subjected to analysis by one-way ANOVA followed by Tukey's multiple comparison testing to identify significant differences between groups when three or more groups were calculated. For all tests, p-values < 0.05 were considered significant criteria.

3. Results

3.1. Characteristics of participants

The characteristics of selected participants in our study are summarized in Table 1. Subjects included in the present study (n = 451) were similar to the entire population (n = 1040) with respect to demographic characteristics. The mean age and body mass index (BMI) were 32 ± 5.4 years old and 23.2 ± 3.1 kg/m^2, respectively. The majority of subjects were of Han ethnic background. More than half of the participants had never fathered a pregnancy (60%), had education levels of high school and above (61%), and had household income of over 3000 Yuan per month (56%). The rates of current smoking and alcohol consumption were 48% and 42%, respectively.

3.2. Distribution of arsenic and hormone levels in urine

The distributions of arsenic and hormone levels are presented in Table 2. All subjects' arsenic levels were beyond the LOD values. The average concentration ranges of arsenic and creatinine-adjusted arsenic in repeated urine samples were 2.8–308 ng/mL and 6.1–194 μg/g creatinine, respectively. The geometric mean concentrations of urinary T, P, and E_2 were 4.2, 5.5 and 0.31 ng/mL, respectively, and creatinine-adjusted arsenic values were 4.0, 5.2 and 0.29 μg/g creatinine, respectively. Urinary hormones were significantly correlated with each other (Spearman correlation coefficients = 0.68–0.81), while no obvious correlation was found between serum and urinary hormones (Fig. 1). Serum total T positively correlated with serum E_2 and SHBG but negatively correlated with the serum percentage of free T (Fig. 1).

3.3. Associations between arsenic and hormones

Multivariable linear regression models were used to assess the

Table 1
The demographics of study participants (n = 451).

Characteristic	Included participants (n = 451)[a]	Whole participants (n = 1040)[b]
Age (years)	32 ± 5.4	32 ± 5.4
BMI (kg/m^2)	23.2 ± 3.1	23 ± 3.2
Daily cigarette consumption	7.0 ± 8.3	7.3 ± 8.4
Race		
Han	438 (97%)	1013 (97%)
Other	13 (3.0%)	27 (3%)
Ever fathered a child		
Yes	179 (40%)	419 (40%)
No	272 (60%)	615 (60%)
Education level		
Less than high school	177 (39%)	390 (38%)
High school and above	274 (61%)	641 (62%)
Smoking status		
Never-smoker	184 (41%)	406 (39%)
Former	51 (11%)	113 (11%)
Current	216 (48%)	521 (50%)
Alcohol use		
Yes	188 (42%)	405 (39%)
No	263 (58%)	635 (61%)
Income, RMB yuan/ month		
< 3000	199 (44%)	456 (44%)
3000–6000	177 (39%)	397 (38%)
6000	74 (17%)	185 (18%)

[a] One participant had missing information on household income.
[b] A total of 2 men had missing information on age, 6 on ever fathered a pregnancy, 9 on education, and 2 on household income.

Table 2
Distribution of arsenic and sex hormone concentrations in urine and serum samples (n = 451).

Analytes	> LOD	GM	Min	25th	50th	75th	Max
Unadjusted urinary hormone and arsenic levels (ng/mL)							
First sample As	100%	34	2.8	22	35	55	301
Second sample As	100%	29	0.10	18	30	55	315
T	–	4.2	0.33	3.0	4.5	6.5	16
P	–	5.5	0.22	3.9	6.0	8.4	23
E_2	–	0.31	0.02	0.23	0.31	0.43	1.9
Urinary Creatinine levels (g/L)							
First sample Creatinine	–	1.5	0.1	1.0	1.5	2.3	4.7
Second sample Creatinine	–	1.6	0.06	1.2	1.8	2.6	6.4
Creatinine-adjusted urinary hormone levels (μg/g creatinine)							
First sample As	100%	30	6.1	22	30	40	182
Second sample As	100%	29	0.13	21	29	39	197
T	–	4.0	1.1	3.0	4.0	5.0	16
P	–	5.2	0.96	3.9	5.1	6.8	20
E_2	–	0.29	0.03	0.22	0.28	0.39	1.8

associations between average urinary creatinine-adjusted arsenic and urinary hormones (Fig. 2 and Table 3). We found significant dose-dependent trends between urinary quartiles of arsenic and urinary hormones (T, P and E_2) (all P trend < 0.05). After adjusting for confounding variables [age and body mass index (BMI), alcohol use and smoking status] in the model, dose-dependent trends remained significant. Compared with men in the lowest quartile, men in the highest quartile of urinary arsenic had an increase in urinary levels of 38.7% (CI: 24.4%, 54.7%), 39.1% (CI: 24.6%, 55.4%) and 44.6% (CI: 30.1%, 60.8%) for T, E_2 and P, respectively. Similarly, arsenic was positively associated with urinary hormones when urinary arsenic level was treated as a continuous variable (Fig. 2 and Table 3). Additionally, in the present study, urinary arsenic was associated with an increased ratio of urinary hormones T, to serum hormones T and E_2, while no significance was found for the ratio of urinary T to E_2 (Fig. 2 and Table 3). Consistent with the previous overlapping whole population, there was no significant association between urinary arsenic and serum hormones in the subset population (data not shown).

3.4. Arsenic exposure associated with steroidogenic effects in MLTC-1 Leydig cells

Steroidogenic results for arsenic-exposed mouse testicular Leydig MLTC-1 cells are illustrated in Fig. 3. For the 0.01 μM arsenic exposure group, 17-OHP, ASD, and T were not significantly altered compared with controls. The contents of ASD following 0.5 μM arsenic exposure were above control levels (1.17-fold of control), but no significant difference was found, whereas T was significantly induced ($p = 0.001$) to 1.36-fold of the control level. Interestingly, 5 μM arsenic exposure significantly decreased 17-OHP production (0.76-fold of control, $p = 0.003$) and slightly decreased ASD secretion (0.89-fold of control, $p = 0.19$), whereas the induction of T production remained significant (1.24-fold of control, $p = 0.01$).

Meanwhile, the relevant steroidogenic pathway genes responding to arsenic exposure were also investigated. Steroidogenesis pathway genes, including *SR-B1*, *StAR*, *CYP17α* and *3β-HSD,* were significantly inhibited (0.24–0.78-fold) in all arsenic treatment groups compared to the vehicle controls (Fig. 3, Table S2), while *P450SCC* (catalyzes cholesterol side-chain cleavage to pregnenolone) expression was upregulated (1.44–1.47-fold) significantly ($p < 0.01$) in the 0.01 μM and 0.5 μM arsenic-treated groups. Similarly, *17β-HSD* (catalyzes androstenedione to testosterone) mRNA expression was significantly ($p < 0.01$) increased in 0.5 μM arsenic treatments (Fig. 3, Table S2).

4. Discussion

In the present study, we integrated epidemiological and *in vitro* toxicological studies to evaluate the association of arsenic exposure and sex hormone levels. Although there was no significant association between arsenic and serum hormones, we observed that environmental arsenic exposure was positively associated with urinary sex hormone excretion in the male population. Furthermore, the results of low-dose arsenic-induced steroidogenesis stimulation in a toxicological experiment supported our epidemiological observations. Collectively, our results indicate that arsenic exposure tends to exhibit multiple endocrine disrupting modes in the context of male reproduction.

Sex hormones have been demonstrated to play an essential role in spermatogenesis and male fertility (Schiffer et al., 2019). Arsenic is considered to be an endocrine disruptor that may affect steroidogenic pathway gene expression and subsequently cause steroidogenic dysfunction (Reddy et al., 2011). Nonetheless, previous epidemiological evidence of an association between arsenic exposure and hormone concentrations remains limited and inconclusive. A significantly positive relationship between the second trimester blood arsenic levels and serum testosterone was previously reported in 918 Chinese pregnant women cohort studies, with an approximately 9.14% increase in testosterone in the highest tertile (range: 5.84–27.3 μg/L) compared with the lowest tertile (range: 1.02–2.39 μg/L) (Luo et al., 2020). Within the nonoccupational general population, no significant association was found between urinary arsenic and serum sex hormones (T, E_2, LH, FSH) in 133 male adolescents from Spain (Castiello et al., 2020). The arsenic levels reported in the aforementioned previous studies (median = 2.1 μg/L, range: 1.04–942 μg/L) are much lower than those in our study (median = 31.4 μg/L). A study in a Chinese male population involving 118 men reported a negative association between urinary arsenic exposure and serum testosterone in the highest tertile, with an approximately 99.32 ng/dL (95% CI: −189.87, −8.77) decrease in testosterone in the fourth quartile (range: 55.35–195.26 μg/L) compared with the

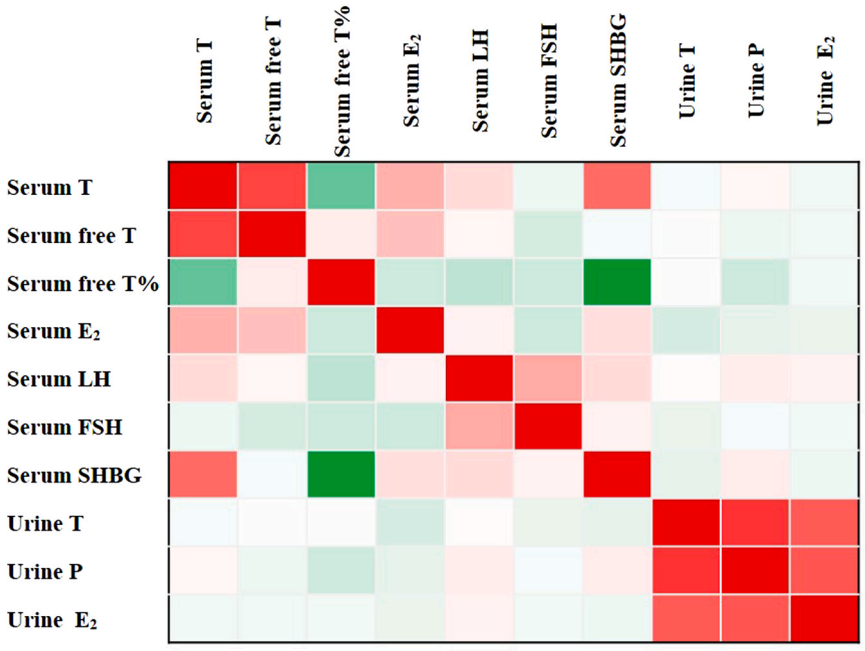

Fig. 1. Spearman correlation coefficients between serum hormones and urinary hormones. The color intensity of the quadrangle indicates the magnitude of the correlation. Green indicates a negative correlation, and red indicates a positive correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

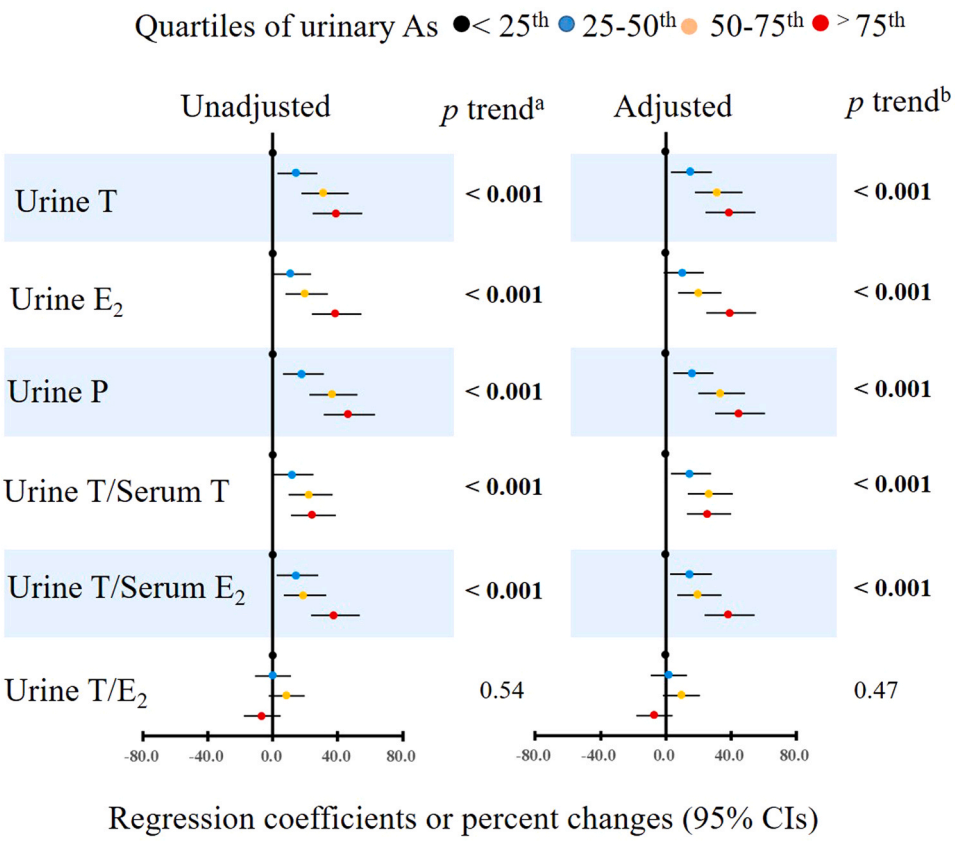

Fig. 2. Regression coefficients or percentage changes (95% CIs) in hormones associated with urinary arsenic exposure. [a]Tests for trends creatinine-adjusted across quartiles in the regression models. [b]Tests were adjusted for age, BMI, alcohol use and smoking status.

first quartile (range: 2.52–17.45 μg/L), but there was a lack of dose–response trends (Zeng et al., 2013). In the present study, urinary arsenic was associated with increased urinary hormone excretion in a linear dose-dependent manner, which is inconsistent with our previously published data on serum hormones as outcomes in an overlapping population (Wang et al., 2016c). The discrepancy between our studies

Table 3
The associations between arsenic exposure and hormone levels (reported as percentage change with 95% CI).

Arsenic	Urine T (%)[a]	Urine E_2 (%)	Urine P (%)	Urine T/Serum T (%)	Urine E_2/Serum E_2 (%)	Urine T/E_2 (%)
Unadjusted models						
Ln transformed As	**22.4 (15.1, 30.1)**	**20.8 (13.6, 28.5)**	**26.0 (18.5, 33.8)**	**14.2 (7.3, 21.7)**	**19.6 (12.4, 27.3)**	-0.9 (−3.7, 1.9)
< 25%	Reference	Reference	Reference	Reference	Reference	Reference
25–50%	14.5 (2.6, 27.6)	10.8 (−0.6, 23.7)	18.1 (6.1, 31.4)	11.9 (0, 25)	14.5 (2.5, 27.8)	0.2 (−11.1, 11.4)
50–75%	31.4 (17.8, 46.5)	20.1 (7.7, 34.0)	36.6 (22.8, 52.0)	22.4 (9.5, 36.9)	19.0 (6.7, 32.8)	8.5 (−2.8, 19.8)
> 75%	39.1 (24.7, 55.0)	38.5 (24.1, 54.5)	46.2 (31.4, 62.7)	24.2 (11.1, 38.8)	37.7 (23.4, 53.6)	-6.6 (−17.9, 4.7)
p trend	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	0.54
Adjusted models[b]						
Ln transformed As	**22.2 (14.8, 30.0)**	**21.3 (13.9, 29.1)**	**25.0 (17.7, 32.7)**	**15.0 (8.2, 22.4)**	**19.9 (12.7, 27.7)**	-1.0 (−3.8, 1.7)
< 25%	Reference	Reference	Reference	Reference	Reference	Reference
25–50%	15.0 (1.1, 26.5)	10.4 (−2.5, 22.4)	16.3 (4.6, 29.3)	14.8 (3.0, 27.9)	14.7 (2.7, 28.1)	1.7 (−9.4, 12.8)
50–75%	31.7 (17.9, 47.0)	20.0 (−1.1, 13.2)	33.4 (20.0, 48.4)	26.5 (13.4, 41.1)	19.8 (7.3, 33.9)	9.6 (−1.6, 20.8)
> 75%	38.7 (24.4, 54.7)	39.1 (24.6, 55.4)	44.6 (30.1, 60.8)	25.6 (12.7, 40.1)	38.1 (23.7, 54.3)	-6.9 (−18.1, 4.2)
p trend	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	0.47

[a] The corresponding regression coefficients were back-transformed to obtain percent changes.
[b] Tests were adjusted for age, BMI, alcohol use and smoking status.

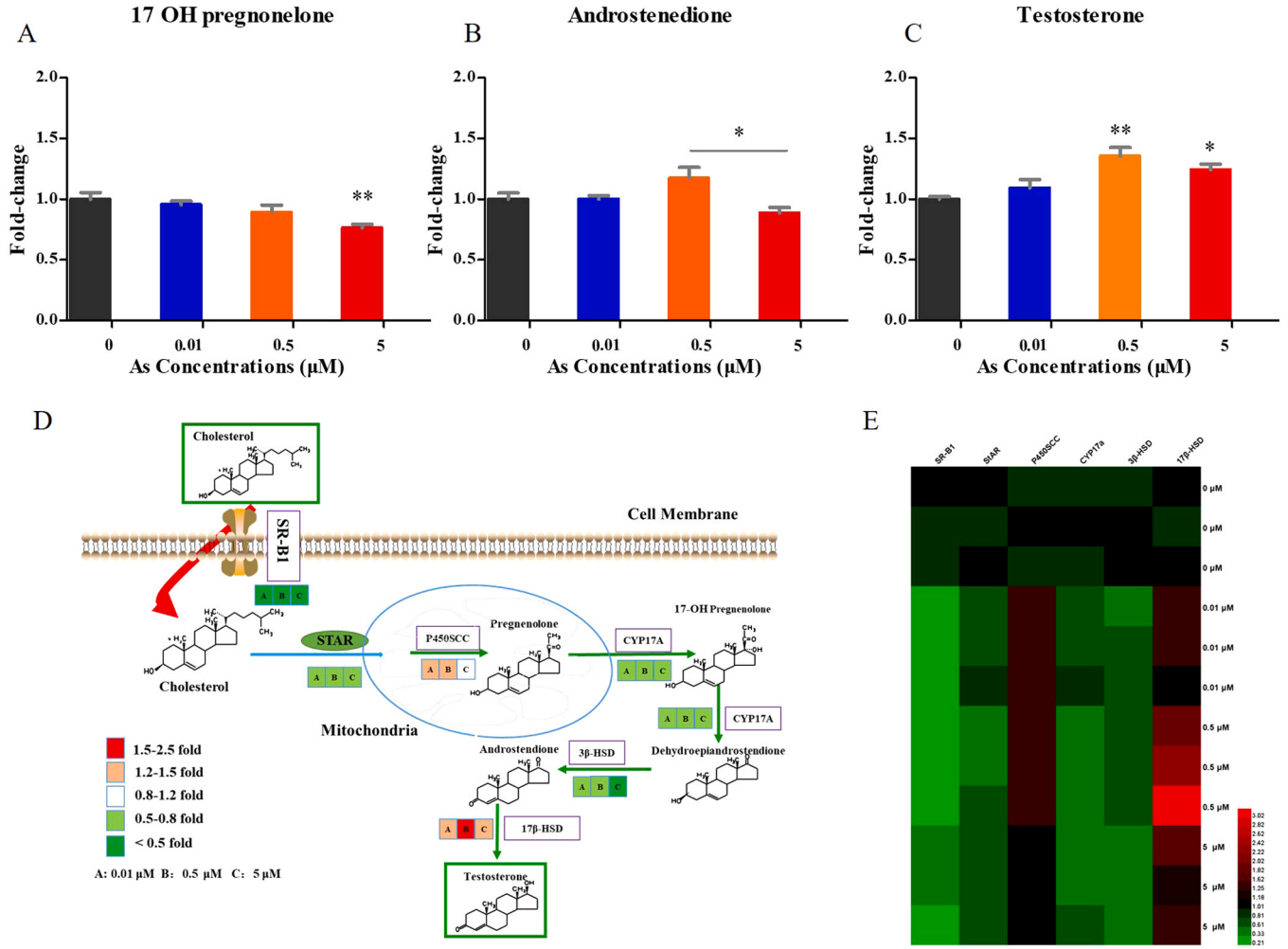

Fig. 3. Arsenic affects steroid hormone biosynthetic pathways in MLTC-1 cells; sex hormone production (A-C), steroidogenesis gene pathway (D) and steroidogenesis gene expression (E). The experiments were repeated four times in duplicate. Data were analyzed by one-way ANOVA with Tukey's multiple comparisons test. *$p < 0.05$, **$p < 0.01$.

may be attributed to the differences in study design (general population *vs.* subfertility clinical population), sample size, population gender (male *vs.* female), critical exposure time window (adolescents *vs.* adults), population arsenic exposure range and exposure matrices (urine *vs.* blood) selected. More importantly, blood and urine reflected distinct hormone metabolic pathways, many steroid metabolomes were concentrated in urine, and urinary hormone excretion can offer complementary strategies for the assessment of endocrine disrupting effects. In the present population, urinary hormone concentrations were higher than the corresponding serum levels. In particular, there was an order of magnitude difference between the median urinary E_2 concentration (0.31 ng/mL) and serum E_2 concentration (34 pg/mL). Meanwhile, the

lack of an obvious correlation between urinary and serum hormones suggested that different biological matrices have distinct hormone metabolic profiles.

Apart from epidemiological studies, accumulating *in vitro* and animal arsenic exposure reproductive toxicological experiments have been conducted. Zeng et al. observed that high-dose arsenic (5 and 50 ppm in drinking water) is able to decrease serum testosterone and concomitant steroidogenesis gene expression (Zeng et al., 2018). Our previous study also found that exposure to high-dose arsenic (rats exposed through 1, 5 and 25 mg/L drinking water; MLTC-1 cells exposed with 1, 2 and 4 ppm dose) can cause inhibition of sex hormones and steroidogenesis gene expression levels both in rat and mouse Leydig cells (Alamdar et al., 2017, 2019). Due to the selected exposure dose being relatively higher than the general population environmental exposure, the results usually showed that sex hormones and related biosynthesis gene expression were downregulated following arsenic treatment (Sarkar et al., 2003; Renu et al., 2018). To the best of our knowledge, data on reproductive outcomes of environmentally relevant arsenic exposure is still lacking. Hence, we selected 0.01–5 μM (approximately 0.75–375 ng/mL arsenic) to simulate real-world population exposure levels in the present MLTC-1 exposure experiments. In contrast with the results from high-dose exposure, we found that low-dose arsenic exposure significantly induced testosterone synthesis along with *17β-HSD* and *P40SCC* gene expression in this study. A similar phenomenon has been reported for the association of arsenic exposure with birth length and osteoblast growth; arsenic exerted distinct effects on birth length and osteoblast growth based on the exposure dosage (Xu et al., 2014; Shih et al., 2020). Moreover, nonmonotonic dose-response effects of arsenic on glucose metabolism have been published, and the results even suggested that arsenic has opposite glycemic effects at different dose thresholds (Gong et al., 2019). Therefore, various arsenic exposure doses may have different toxic effects.

Similar to the abovementioned inconclusive, nonmonotonic, dose-response effects of arsenic exposure on sex hormone production, early studies have confirmed that certain environmental endocrine disrupting chemicals (EDCs), such as phthalate and BPA, can affect hormone generation in a biphasic dose-response manner in animal studies, *i.e.*, low-dose exposure induces secretion, while high-dose exposure inhibits secretion (Ge et al., 2007; Zhang et al., 2016). Our previous study also indicated that phthalates and perfluorinated compounds have biphasic effects on androgen generation in mouse Leydig cells and low-dose-stimulated testosterone production, whereas these compounds are anti-androgenic at high doses (Tian et al., 2018, 2019). Meanwhile, heavy metals such as cadmium also exert biphasic effects on population hormone generation with different exposure levels. For cadmium exposure and male serum testosterone, a positive association has been observed in the lower dose exposure population, while a negative association has been found in the higher level exposure population [serum cadmium median (IQR): 0.4 (0.2–0.7) μg/L for low exposure *vs* 1.90 (0.60–3.80) μg/L for high exposure] (Kresovich et al., 2015; Chen et al., 2016). Consistent with previous EDCs results, our *in vitro* study found that an environmentally relevant dose of arsenic seems to exert stimulating effects on testosterone production. Interestingly, similar induction effects were also found in the present epidemiological study of urinary hormone excretion but not serum hormone secretion. Given that a series of enzymes are involved in Leydig cell mitochondrial steroidogenesis (Fig. 3), we also investigated the steroidogenic enzyme gene alteration response to arsenic exposure. In human and rodent steroidogenic tissues, SR-B1 and StAR are responsible for transporting cholesteryl esters into cells and mitochondria, which are subsequently converted into pregnenolone by P450SCC in the inner mitochondrial membrane and, further, into the intermediates progesterone and ASD *via* CYP17α and 3β-HSD catalysis, finally producing the end-product testosterone (Azhar and Reaven, 2002). Exposure to high doses of arsenic can attenuate steroidogenic enzyme gene expression, which in turn can inhibit sex hormone production (Renu et al., 2018). In the present study, we observed that arsenic significantly inhibited SR-B1, StAR, CYP17α and 3β-HSD but induced P450SCC and 17β-HSD. It is worth mentioning that the effects of arsenic potency on alterations in 17β-HSD gene expression are in good agreement with the testosterone synthesis results, with a 2.44-fold change in the median-dose (0.5 μM) exposure group and a 1.43-fold change in the high-dose (5 μM) group. Considering that 17β-HSD is responsible for the conversion of ASD to testosterone and its upstream gene expression inhibition, we inferred that arsenic low-dose stimulation corresponds to a negative feedback compensation mechanism that counterbalances the arsenic-induced inhibition of gene expression of steroidogenic enzymes to maintain constant sex hormone homeostasis. We recognize that this hypothesis needs to be confirmed in further studies.

When synthetic testosterone reaches the blood circulatory system, testosterone and other sex hormones are bound to binding proteins. In men, approximately 60% of testosterone is combined with SHBG, approximately 40% is bound to albumin, and only 2% presents as biologically active free testosterone (Rabijewski and Zgliczyński, 2009). SHBG is involved in regulating sex hormone transport, distribution, metabolism, and bioavailability (Goldman et al., 2017). Heavy metals, including arsenic exposure, interact with SHBG, as reported in a previous study (Hsieh et al., 2008; Rotter et al., 2016). In the present population study, we found a positive association between urinary creatinine-adjusted arsenic and serum SHBG but no association between urinary arsenic and serum bioavailable testosterone (data not shown). It is likely that arsenic exposure affects serum hormone binding, transport and elimination homeostasis. Regarding hormone binding in serum and elimination in urine, a related study indicated that physical exercise may have an influence on the elimination of androgenic hormones, mainly relying on changes in their transport protein, SHBG (Maynar et al., 2010). In line with these results, arsenic exposure was associated with increased sex hormone urinary elimination and the ratio of urinary hormones to serum hormones in the present study. Given that MRPs are involved in eliminating conjugated arsenic metabolites and androgen from hepatocytes into urine (Järvinen et al., 2020), we cannot exclude the possibility that the arsenic urinary elimination process directly accelerates hormone urinary excretion. Meanwhile, arsenic was positively associated with urinary androgen hormone excretion, which has been confirmed in general Polish and Chinese population untargeted urinary metabolomics studies (Kozłowska et al., 2019; Zhang et al., 2014). Once again, the arsenic exposure levels in previous Polish (arithmetic mean: 151.71 μg/L for high exposure *vs* 16.91 μg/L low exposure) and Chinese populations (range: 4.79–25.01 μg/g creatinine for first quintile exposure *vs* 46.18–99.42 μg/g creatinine for fifth quintile exposure) were similar to our present study [range: 6.1–21 μg/g creatinine with for lowest quartile exposure (arithmetic mean: 24 μg/g) *vs* 39–194 μg/g creatinine highest quartile exposure (arithmetic mean: 70 μg/g)]. It is worth pointing out that in accordance with entire population results (Wang et al., 2016c), the present subset study concentrations of arsenic and creatinine-adjusted arsenic in repeated urine samples showed excellent reproducibility (ICCs = 0.78 and 0.85, respectively), indicating that urinary As was relatively stable within a given day. However, in another variability study that collected spot samples on 8 days over 3 months, the serial measurements of arsenic showed poor reproducibility over periods of weeks and months (ICC = 0.19). In other words, although the collection of repeated urine samples from each study participant improved the classification, exposure misclassification is still likely due to the availability of only two spot urine samples for each subject and their high within-person variability over periods of weeks or months (Wang et al., 2016a). Based on the serum and urine hormone results, we hypothesized that environmentally relevant arsenic exposure was able to stimulate testicular Leydig cell sex hormone biosynthesis *via* a negative feedback compensation mechanism and further accelerate urinary sex hormone elimination through SHBG regulation to maintain constant sex hormone levels in the blood.

This study had several strengths. First, to the best of our knowledge, this was the first study integrating epidemiological and *in vitro* studies to evaluate the association of arsenic exposure and sex hormone levels. *In vitro* results might elucidate the potential causal associations. In addition, repeated measures of urinary arsenic more accurately reflect exposure levels. Moreover, urinary hormone excretion provides complementary strategies for arsenic endocrine disruption assessment. Finally, the relatively large study size reduced the probability of falsely significant results.

However, some limitations of our study should be considered. First, only *in vitro* MLTC-1 Leydig cell exposure experiments were conducted in this study, which lacked hypothalamic-pituitary-testis axis interactions, limiting a comprehensive assessment of hormone responses to arsenic exposure. Animal arsenic exposure studies will help elucidate the underlying mechanism. In addition, we only detected the total arsenic concentration for exposure assessment, whereas inorganic and organic species vary in their toxicity. Therefore, due to the lack of speciation analysis for arsenic, we were unable to distinguish the predominantly toxic arsenic species in our study population. Moreover, although the overall intra-individual variability in sex hormone levels appears to be primarily attributable to individual specific environmental factors, sex hormones such as testosterone also vary in accordance with their diurnal rhythm (Diver et al., 2003). Repeated sampling measurement of free testosterone over the course of a day may be necessary to establish reliable and robust overall hormone levels (Bird et al., 2016). In the present study, repeated urine samples were pooled equally for hormone determination to decrease variability to some extent. However, we cannot exclude other factors contributing to urinary variability over long periods. Finally, our study participants were recruited from an infertility clinic. Such a study design may have resulted in the inclusion of more subfertile men; thus, it is unclear whether extrapolation of our findings to the general population can be made.

5. Conclusion

In conclusion, the present study showed that urinary arsenic concentrations were significantly associated with increased urinary sex hormone excretion in reproductive-aged men. *In vitro* Leydig cell exposure experiments confirmed that environmental low-dose arsenic exerted a stimulatory effect on steroidogenesis. These findings extend the previous knowledge of arsenic exposure inversely associated with sex hormones when these hormones are assumed to be the cause of antiandrogens. Moreover, compared with blood sex hormones, urinary hormone excretion is more sensitive to the response to environmentally relevant arsenic exposure, and urine matrices could be an ideal tool for noninvasive endocrine disruption assessment.

Credit authorship contribution statement

Meiping Tian and Yi-Xin Wang: conceived the idea and conducted the majority of the experiments, wrote the paper. **Xiaofei Wang**: participated MLTC-1 cell experiments. **Heng Wang and Bingru Nan**: conducted urinary sex hormones analysis. **Liangpo Liu and Jie Zhang**: statistical analysis of data. **Heqing Shen**: writing- reviewing and editing. **Qingyu Huang**: supervision and editing. All authors discussed the results and assisted in the preparation of the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124904.

References

Alamdar, A., Xi, G., Huang, Q., Tian, M., Eqani, S.A.M.A.S., Shen, H., 2017. Arsenic activates the expression of 3β-HSD in mouse Leydig cells through repression of histone H3K9 methylation. Toxicol. Appl. Pharmacol. 326, 7–14.

Alamdar, A., Tian, M., Huang, Q., Du, X., Zhang, J., Liu, L., Shah, S.T.A., Shen, H., 2019. Enhanced histone H3K9 tri-methylation suppresses steroidogenesis in rat testis chronically exposed to arsenic. Ecotoxicol. Environ. Saf. 170, 513–520.

Azhar, S., Reaven, E., 2002. Scavenger receptor class BI and selective cholesteryl ester uptake: partners in the regulation of steroidogenesis. Mol. Cell. Endocrinol. 195, 1–26.

Banerjee, M., Carew, M.W., Roggenbeck, B.A., Whitlock, B.D., Naranmandura, H., Le, X. C., Leslie, E.M., 2014. A novel pathway for arsenic elimination: human multidrug resistance protein 4 (MRP4/ABCC4) mediates cellular export of dimethylarsinic acid (DMAV) and the diglutathione conjugate of monomethylarsonous acid (MMAIII). Mol. Pharmacol. 86 (2), 168–179.

Banerjee, M., Marensi, V., Conseil, G., Le, X.C., Cole, S.P., Leslie, E.M., 2016. Polymorphic variants of MRP4/ABCC4 differentially modulate the transport of methylated arsenic metabolites and physiological organic anions. Biochem. Pharmacol. 120, 72–82.

Bélanger, A., Pelletier, G., Labrie, F., Barbier, O., Chouinard, S., 2003. Inactivation of androgens by UDP-glucuronosyltransferase enzymes in humans. Trends Endocrinol. Metab. 14 (10), 473–479.

Bird, B.M., Cid Jofré, V.S., Geniole, S.N., Welker, K.M., Zilioli, S., Maestripieri, D., Arnocky, S., Carré, J.M., 2016. Does the facial width-to-height ratio map onto variability in men's testosterone concentrations? Evol. Hum. Behav. 37, 392–398.

Bochud, M., Jenny-Burri, J., Pruijm, M., Ponte, B., Guessous, I., Ehret, G., Petrovic, D., Dudler, V., Haldimann, M., Escher, G., Dick, B., Mohaupt, M., Paccaud, F., Burnier, M., Péchère-Bertschi, A., Martin, P.Y., Vogt, B., Ackermann, D., 2018. Urinary cadmium excretion is associated with increased synthesis of cortico- and sex steroids in a population study. J. Clin. Endocrinol. Metab. 103 (2), 748–758.

Carmean, C.M., Yokoi, N., Takahashi, H., Oduori, O.S., Kang, C., Kanagawa, A., Kirkley, A.G., Han, G., Landeche, M., Hidaka, S., Katoh, M., Sargis, R.M., Seino, S., 2019. Arsenic modifies serotonin metabolism through glucuronidation in pancreatic β-cells. Am. J. Physiol. Endocrinol. Metab. 316 (3), 464–474.

Castiello, F., Olmedo, P., Gil, F., Molina, M., Mundo, A., Romero, R.R., Ruíz, C., Gómez-Vida, J., Vela-Soria, F., Freire, C., 2020. Association of urinary metal concentrations with blood pressure and serum hormones in Spanish male adolescents. Environ. Res. 182, 108958.

Chen, C., Wang, N., Nie, X., Han, B., Li, Q., Chen, Y., Zhai, H., Zhu, C., Chen, Y., Xia, F., Lu, M., Lin, D., Lu, Y., 2016. Blood cadmium level associates with lower testosterone and sex hormone-binding globulin in Chinese men: from SPECT-China Study, 2014. Biol. Trace Elem. Res. 171, 71–78.

Diver, M.J., Imtiaz, K.E., Ahmad, A.M., Vora, J.P., Fraser, W.D., 2003. Diurnal rhythms of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those in young men. Clin. Endocrinol. 58 (6), 710–717.

Drobná, Z., Walton, F.S., Harmon, A.W., Thomas, D.J., Stýblo, M., 2010. Interspecies differences in metabolism of arsenic by cultured primary hepatocytes. Toxicol. Appl. Pharmacol. 245 (1), 47–56.

Ge, R.S., Chen, G.R., Dong, Q., Akingbemi, B., Sottas, C.M., Santos, M., Sealfon, S.C., Bernard, D.J., Hardy, M.P., 2007. Biphasic effects of postnatal exposure to diethylhexylphthalate on the timing of puberty in male rats. J. Androl. 28 (4), 513–520.

Goldman, A.L., Bhasin, S., Wu, F.C.W., Krishna, M., Matsumoto, A.M., Jasuja, R., 2017. A reappraisal of testosterone's binding in circulation: physiological and clinical implications. Endocr. Rev. 38 (4), 302–324.

Gong, Y., Liu, J., Xue, Y., Zhuang, Z., Qian, S., Zhou, W., Li, X., Qian, J., Ding, G., Sun, Z., 2019. Non-monotonic dose-response effects of arsenic on glucose metabolism. Toxicol. Appl. Pharmacol. 377, 114605.

Hsieh, F.I., Hwang, T.S., Hsieh, Y.C., Lo, H.C., Su, C.T., Hsu, H.S., Chiou, H.Y., Chen, C.J., 2008. Risk of erectile dysfunction induced by arsenic exposure through well water consumption in Taiwan. Environ. Health Perspect. 116 (4), 532–536.

Huang, Q., Zhang, J., Peng, S., Tian, M., Chen, J., Shen, H., 2014. Effects of water soluble PM2.5 extracts exposure on human lung epithelial cells (A549): a proteomic study. J. Appl. Toxicol. 34 (6), 675–687.

Hubaux, R., Becker-Santos, D.D., Enfield, K.S., Rowbotham, D., Lam, S., Lam, W.L., Martinez, V.D., 2013. Molecular features in arsenic-induced lung tumors. Mol. Cancer 12, 20.

Hughes, M.F., Kenyon, E.M., Kitchin, K.T., 2007. Research approaches to address uncertainties in the risk assessment of arsenic in drinking water. Toxicol. Appl. Pharmacol. 222 (3), 399–404.

Järvinen, E., Kidron, H., Finel, M., 2020. Human efflux transport of testosterone, epitestosterone and other androgen glucuronides. J. Steroid Biochem. Mol. Biol. 197, 105518.
Kozłowska, L., Janasik, B., Nowicka, K., Wąsowicz, W., 2019. A urinary metabolomics study of a Polish subpopulation environmentally exposed to arsenic. J. Trace Elem. Med. Biol. 54, 44–54.
Kresovich, J.K., Argos, M., Turyk, M.E., 2015. Associations of lead and cadmium with sex hormones in adult males. Environ. Res. 142, 25–33.
Luo, Q., Zhao, H., Jiang, Y., Guo, J., Lv, N., Tang, J., Li, S., Zhang, D., Bai, R., Chen, G., 2020. Association of blood metal exposure with testosterone and hemoglobin: a cross-sectional study in Hangzhou Birth Cohort Study. Environ. Int. 136, 105451.
Maynar, M., Timon, R., González, A., Olcina, G., Toribio, F., Maynar, J.I., Caballero, M.J., 2010. SHBG, plasma, and urinary androgens in weight lifters after a strength training. J. Physiol. Biochem. 66 (2), 137–142.
Molin, M., Ulven, S.M., Dahl, L., Lundebye, A.K., Holck, M., Alexander, J., Meltzer, H.M., Ydersbond, T.A., 2017. Arsenic in seafood is associated with increased thyroid-stimulating hormone (TSH) in healthy volunteers - A randomized controlled trial. J. Trace Elem. Med. Biol. 44, 1–7.
Ommati, M.M., Heidari, R., Zamiri, M.J., Sabouri, S., Zaker, L., Farshad, O., Jamshidzadeh, A., Mousapour, S., 2020. The footprints of oxidative stress and mitochondrial impairment in arsenic trioxide-induced testosterone release suppression in pubertal and mature F1-male balb/c mice via the downregulation of 3β-HSD, 17β-HSD, and CYP11a expression. Biol. Trace Elem. Res. 195 (1), 125–134.
Rabijewski, M., Zgliczyński, W., 2009. Pathogenesis, evaluation and treatment of hypogonadism in men. Endokrynol. Pol. 60 (3), 222–233.
Reddy, P.S., Rani, G.P., Sainath, S.B., Meena, R., Supriya, Ch, 2011. Protective effects of N-acetylcysteine against arsenic-induced oxidative stress and reprotoxicity in male mice. J. Trace Elem. Med. Biol. 25 (4), 247–253.
Renu, K., Madhyastha, H., Madhyastha, R., Maruyama, M., Vinayagam, S., Valsala Gopalakrishnan, A., 2018. Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. Life Sci. 212, 37–58.
Rodríguez-Lado, L., Sun, G., Berg, M., Zhang, Q., Xue, H., Zheng, Q., Johnson, C.A., 2013. Groundwater arsenic contamination throughout China. Science 341 (6148), 866–868.
Rotter, I., Kosik-Bogacka, D.I., Dołęgowska, B., Safranow, K., Kuczyńska, M., Laszczyńska, M., 2016. Analysis of the relationship between the blood concentration of several metals, macro- and micronutrients and endocrine disorders associated with male aging. Environ. Geochem. Health 38 (3), 749–761.
Sanderson, J.T., 2006. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. Toxicol. Sci. 94 (1), 3–21.
Sarkar, M., Chaudhuri, G.R., Chattopadhyay, A., Biswas, N.M., 2003. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J. Androl. 5 (1), 27–31.
Schiffer, L., Barnard, L., Baranowski, E.S., Gilligan, L.C., Taylor, A.E., Arlt, W., Shackleton, C.H.L., Storbeck, K.H., 2019. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: a comprehensive review. J. Steroid Biochem. Mol. Biol. 194, 105439.
Shih, Y.H., Scannell, Bryan, M., Argos, M., 2020. Association between prenatal arsenic exposure, birth outcomes, and pregnancy complications: an observational study within the National Children's Study cohort. Environ. Res. 183, 109182.
Singh, G.K., Balzer, B.W., Desai, R., Jimenez, M., Steinbeck, K.S., Handelsman, D.J., 2015. Requirement for specific gravity and creatinine adjustments for urinary steroids and luteinizing hormone concentrations in adolescents. Ann. Clin. Biochem. 52 (Pt 6), 665–671.
Tian, M., Liu, L., Wang, H., Wang, X., Martin, F., Zhang, J., Huang, Q., Shen, Q., 2018. Phthalates induce androgenic effects at exposure levels that can be environmentally relevant in humans. Environ. Sci. Technol. Lett. 5, 232–236.
Tian, M., Zhang, X., Liu, L., Martin, F.L., Wang, H., Zhang, J., Huang, Q., Wang, X., Shen, H., 2019. Phthalate side-chain structures and hydrolysis metabolism associated with steroidogenic effects in MLTC-1 Leydig cells. Toxicol. Lett. 308, 56–64.
Wang, Y.X., Feng, W., Zeng, Q., Sun, Y., Wang, P., You, L., Yang, P., Huang, Z., Yu, S.L., Lu, W.Q., 2016a. Variability of metal levels in spot, first morning, and 24-hour urine samples over a 3-month period in healthy adult Chinese men. Environ. Health Perspect. 124 (4), 468–476.
Wang, Y.X., Sun, Y., Feng, W., Wang, P., Yang, P., Li, J., Huang, Z., Chen, Y.J., Liu, C., Sun, L., Yue, J., Gu, L.J., Zeng, Q., Lu, W.Q., 2016b. Association of urinary metal levels with human semen quality: a cross-sectional study in China. Environ. Int 91, 51–59.
Wang, Y.X., Sun, Y., Huang, Z., Wang, P., Feng, W., Li, J., Yang, P., Wang, M., Sun, L., Chen, Y.J., Liu, C., Yue, J., Gu, L.J., Zeng, Q., Lu, W.Q., 2016c. Associations of urinary metal levels with serum hormones, spermatozoa apoptosis and sperm DNA damage in a Chinese population. Environ. Int. 94, 177–188.
Wang, Y.X., Zeng, Q., Sun, Y., You, L., Wang, P., Li, M., Yang, P., Li, J., Huang, Z., Wang, C., Li, S., Dan, Y., Li, Y.F., Lu, W.Q., 2016d. Phthalate exposure in association with serum hormone levels, sperm DNA damage and spermatozoa apoptosis: a cross-sectional study in China. Environ. Res. 150, 557–565.
Wang, H., Liu, L., Wang, J., Tong, Z., Yan, J., Zhang, T., Qin, Y., Jiang, T., She, J., Shen, H., 2017. Urinary sexual steroids associated with bisphenol A (BPA) exposure in the early infant stage: preliminary results from a Daishan birth cohort. Sci. Total Environ. 601–602, 1733–1742.
Xu, W.X., Liu, Y., Liu, S.Z., Zhang, Y., Qiao, G.F., Yan, J.L., 2014. Arsenic trioxide exerts adouble effect on osteoblast growth in vitro. Environ. Toxicol. Pharmacol. 38, 412–419.
Zeng, Q., Zhou, B., Feng, W., Wang, Y.X., Liu, A.L., Yue, J., Li, Y.F., Lu, W.Q., 2013. Associations of urinary metal concentrations and circulating testosterone in Chinese men. Reprod. Toxicol. 41, 109–114.
Zeng, Q., Yi, H., Huang, L., An, Q., Wang, H., 2018. Reduced testosterone and Ddx3y expression caused by long-term exposure to arsenic and its effect on spermatogenesis in mice. Environ. Toxicol. Pharmacol. 63, 84–91.
Zhang, J., Shen, H., Xu, W., Xia, Y., Barr, D.B., Mu, X., Wang, X., Liu, L., Huang, Q., Tian, M., 2014. Urinary metabolomics revealed arsenic internal dose-related metabolic alterations: a proof-of-concept study in a Chinese male cohort. Environ. Sci. Technol. 48 (20), 12265–12274.
Zhang, Y., Tao, S., Yuan, C., Liu, Y., Wang, Z., 2016. Non-monotonic dose-response effect of bisphenol A on rare minnow Gobiocypris rarus ovarian development. Chemosphere 144, 304–311.
Zorimar, R., 2009. Application of Urinary Arsenic Metabolites to Assess Arsenic Exposure in Southeastern Michigan: Advancing Exposure Assessment for Epidemiology Research. Dissertations & Theses Gradworks.
Zubair, M., Ahmad, M., Qureshi, Z.I., 2017. Review on arsenic-induced toxicity in male reproductive system and its amelioration. Andrologia 49 (9), e12791.