



Full length article

# Urinary haloacetic acid concentrations in relation to sex and thyroid hormones among reproductive-aged men

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## ABSTRACT

Sex and thyroid hormones are critical for male reproductive health. However, the associations between haloacetic acid (HAA) exposure – a known endocrine disruptor – and sex and thyroid hormones in humans remains unclear. We thus recruited 502 male participants seeking fertility evaluation from a reproductive center. We measured concentrations of sex and thyroid hormones in a single blood sample and dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) in repeated urine samples. Multivariable linear regression models were constructed to evaluate the associations between HAA concentrations and hormone measurements. After adjusting for potential confounders and urinary creatinine concentrations, urinary concentrations of TCAA were inversely associated with serum levels of sex hormone-binding globulin (SHBG), testosterone (T), T/luteinizing hormone ratio (T/LH), and thyroid stimulating hormone (TSH) (all *P* for trend < 0.10). Compared with participants in the lowest quartile of TCAA concentrations, those in the highest quartile had reduced serum levels of SHBG by 14.2 % (95% CI: –26.7, –3.0 %), T by 11.1 % (95% CI: –21.7, –1.3 %), T/LH by 21.0 % (95% CI: –36.7, –7.1 %), and TSH by 19.1 % (95% CI: –39.7, –1.5 %). Additionally, we observed inverse associations between continuous measurements of urinary HAAs and serum levels of free T, bioactive T, and estradiol. Our findings suggest that male HAA exposure may be associated with disrupted sex and thyroid function.

## 1. Introduction

Sex and thyroid hormones are critical for male reproductive health (Krassas et al., 2010; La Vignera and Vita, 2018; Schulster et al., 2016). Sex hormones, including estradiol (E2), testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone-binding globulin (SHBG), are essential for spermatogenesis and sperm maturation (Schulster et al., 2016; Zhao et al., 2020). Similarly, thyroid dysfunction, such as thyrotoxicosis and hypothyroidism, has also been associated with decreased male testis weight, delayed spermatogenesis,

reduced semen quality, and inhibited Leydig cell proliferation (Krassas et al., 2010; Rijntjes et al., 2008). A growing body of animal and human studies indicate that exposure to chemicals from our built and natural environments, including exposure to disinfection byproducts (DBPs) in drinking water, may disrupt sex and thyroid hormones (Gonsioroski et al., 2020; Jeong et al., 2016; El Alem et al., 2017; Zeng et al., 2013).

Disinfection by-products (DBPs) are unintentionally formed when chlorine or chloride-based chemicals react with organic matter in water (Richardson et al., 2007). Among hundreds of identified DBPs in chlorinated water, haloacetic acids (HAAs) are the leading species of non-

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volatile DBPs (Savitz, 2012; Nieuwenhuijsen et al., 2009). The general population in the United States and across the globe is primarily exposed to HAAs through ingestion of drinking water (Dobaradaran et al., 2020). Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) are two leading species of HAAs; their excretions, particularly TCAA in urine samples, have been strongly associated with ingested trihalomethanes (THMs) and HAAs (Kim et al., 1999; Weisel et al., 1999). Therefore, they are proposed as reliable exposure markers for ingested DBPs in drinking water (Kim et al., 1999; Wang et al., 2014; Chen et al., 2022; Sun et al., 2020).

HAAs have been demonstrated to adversely affect the development of gonads (e.g., testes and ovaries) and the synthesis and secretion of sex hormones, such as E2, T, LH, and FSH (Krassas et al., 2010; Balchak et al., 2000; Goldman and Murr, 2003; Linder et al., 1994). Similarly, HAAs are reported to disrupt thyroid function and thyroid hormone homeostasis in experimental studies (Wang et al., 2022; Revis et al., 1986; Poon et al., 2003; Zhang et al., 2023). Although HAA exposure has been associated with changes in sex hormones among women (Liu et al., 2022; Deng et al., 2022), to our knowledge, no studies have yet examined the associations of HAA exposure with sex or thyroid hormones among reproductive-aged males. To address this gap, the present study aimed to determine DCAA and TCAA concentrations in repeated urine samples to enhance exposure estimation (Wang et al., 2014), and to explore their associations with sex and thyroid hormones in Chinese reproductive-aged males.

## 2. Material and methods

### 2.1. Study population

From March to June 2013, we recruited 1,247 male partners of couples who came to the Reproductive Medicine Center of Tongji Hospital in Wuhan, China for fertility evaluation. The eligibility criteria are described in detail elsewhere (Wang et al., 2018). In the current analysis, we excluded participants who self-reported diseases or illnesses (e.g., thyroid, pituitary gland, or gonad diseases) that may impair sex or thyroid functions ( $n = 121$ ), were azoospermic ( $n = 58$ ), or did not measure for sex or thyroid hormones ( $n = 502$ ). We further excluded 64 participants who did not have sufficient urine samples for the determination of HAAs, leaving 502 participants in the final analysis (Fig. 1).

Each participant underwent a face-to-face interview at enrollment. We collected participants' demographic characteristics (e.g., age, weight, height, ethnicity, education, and income levels), smoking and

drinking status, history of occupational exposure, and history of reproductive or endocrine disease medical history. We did not find any differences in demographic variables between participants included in the present study and the overall population (Table S1). This study has been approved by the Ethics Committee of Tongji Medical College. All participants provided written informed consent before participation.

### 2.2. Urine collection and measurements

Given that urinary HAA concentrations varied greatly within a given day and that the collection of repeated urine samples improved exposure estimation (Wang et al., 2014), two urine samples were collected from each man at different time points (mean duration:  $4.4 \pm 3.7$  hrs; range: 2.0–10.6 hrs) on their visiting day to the clinic. The samples were stored at  $-40^{\circ}\text{C}$  and analyzed for urinary concentrations of DCAA, TCAA, and creatinine using a procedure described in our previous studies (Chen et al., 2022; Wang et al., 2019). The limit of detection (LOD) of the gas chromatograph (GC) for DCAA and TCAA was 1.0 and 0.5  $\mu\text{g/L}$ , respectively. Quality control samples were analyzed alongside each analysis run (30 – 40 samples), comprising 1 blank sample (boiled spring water) and 2 pooled urine samples spiked with HAAs. The spiked recoveries for DCAA and TCAA ranged from 90 % to 115 %.

### 2.3. Blood collection and analyses

Venous blood was drawn from participants between 8:30 and 11:30 am to minimize the diurnal rhythm of serum sex and thyroid hormones (Kerkhof et al., 2015). The detailed methodology for the quantification of sex and thyroid hormones has been described in our studies (Wang et al., 2018; Wang et al., 2016; Wang et al., 2018; Li et al., 2024). Briefly, sex hormones, including E2, T, LH, FSH, and SHBG, were assessed using the direct chemiluminescence assay (Wang et al., 2018). Analysts were blind to any information of the study participants. Quality control samples were analyzed alongside each analysis run, comprising 1 blank sample and 2 serum samples spiked with low and high levels of sex hormones (recovery range: 83 to 108 %). The inter-day variations of E2, T, LH, FSH, and SHBG were all less than 9.0 % (Wang et al., 2018).

Thyroid hormones, including thyroid stimulating hormone (TSH), free triiodothyronine (T3), and free thyroxine (T4), were measured using a Modular E170 automatic analyzer (Wang et al., 2018). We calculated the free androgen index (FAI) by dividing testosterone by SHBG in molarity, as well as free testosterone (Free T) and bioactive testosterone (Bio T) according to Vermeulen's formula (Vermeulen

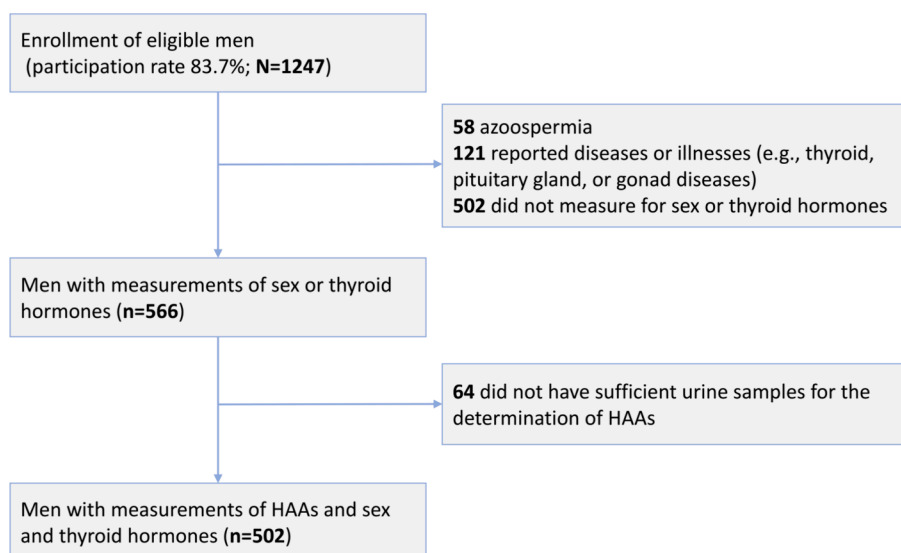


Fig. 1. Flow chart for the study population.

et al., 1999). The ratio of T to LH (T/LH) and T3 to T4 (T3/T4) was also calculated. Pathological and normal controls (PeciControl Universal, Roche Diagnostics GmbH, Germany) were tested alongside each analysis run. The inter-day variations of TSH, T3, and T4 were all less than 8.0 % (Wang et al., 2018).

## 2.4. Statistical analyses

Descriptive statistics were conducted for demographic characteristics and the distribution of hormones and HAAs. The missing data on demographic characteristics was less than 1 % and replaced by median values in subsequent analyses. The values of DCAA and TCAA below LOD were replaced with LOD divided by the square root of 2 (Hornung and Reed, 1990). The reproducibility of HAA concentrations in repeated urine samples was assessed by intraclass correlation coefficients (ICCs), which reflect the proportion of between-subject variance to the total variance (Rosner, 2002).

Multivariable linear regression models were used to examine the associations between within-individual average urinary HAA concentrations (ln-transformed) and continuous measurements of hormones. For participants who had a single HAA measurement ( $n = 64$ ), we directly used their single measurement on the ln-transformed scale. In addition, we divided within-individual average HAA concentrations into quartiles and modeled the quartiles as ordinal categorical variables using integer values (i.e., 1–4) to assess potential dose–response relationships. Serum levels of TSH, T3, T4, T3/T4, E2, FSH, LH, SHBG, T, FAI, Free T, Bio T, and T/LH ratio were ln-transformed due to their skewed distribution. The resulting estimates and 95% confidence intervals (CIs) were back-transformed to obtain percent changes using the formula:  $100 \% \times (\exp^{\text{estimate}} - 1)$  (Pitter et al., 2020).

Covariates were selected based on biological plausibility and statistical considerations (Jassat et al., 2021). The confounders associated with HAA exposure and hormones were selected using a Directed Acyclic Graph (DAG) (Figure S1) and retained if their inclusion resulted in changes in estimates for HAA exposure in relation to any hormones by  $> 10 \%$  (Greenland, 1989). The final adjusted models were adjusted for age (continuous), body mass index (BMI; continuous), cigarettes smoked per day (continuous), educational background (less than high school or high school and above), income ( $\leq 3000$ , 3001–6000 or  $\geq 6001$  yuan per month), alcohol consumption (never, former, or current), and smoking status (never, former, or current). The average creatinine concentrations within individuals were also included to account for urinary dilution (Barr et al., 2005). Sensitivity analyses were conducted by using the Markov chain Monte Carlo method of multiple imputations procedure to replace covariates with missing values and by excluding participants who had abnormal semen quality to assess the representativeness of our findings, those who were over 40 years old to evaluate the potential influence of advanced age, or those who had extreme urinary creatinine concentrations (i.e.,  $< 0.3$  or  $> 3$  g/L) that may have led to biased estimations. Statistical Analysis Software (SAS) version 9.2 was used for all data analyses. The Benjamini–Hochberg False Discovery Rate (FDR) method was used to correct for multiple tests where appropriate (Nathan, 2010).

## 3. Results

### 3.1. Characteristics of the study population

The participants in the present study had a mean age of  $32.2 (\pm 5.2)$  years and a BMI of  $23.2 \pm 3.1$  kg/m<sup>2</sup> (Table 1). The majority (97.2 %) were of Han ethnicity, approximately two-thirds (62.0 %) graduated from high school and above, more than half (55.8 %) earned  $\geq 3000$  RMB/month, and one-third (37.6 %) had ever fathered a child. Nearly 60 % of the population ( $n = 297$ ) were current or prior smokers at recruitment and 7.4 % ( $n = 37$ ) drank alcoholic beverages more than once per week.

**Table 1**

Characteristics of the study population ( $n = 502$ )<sup>a</sup>.

Characteristic	[n (%) or mean $\pm$ SD]
Age, years	32.2 $\pm$ 5.2
BMI, kg/m <sup>2</sup>	23.2 $\pm$ 3.1
Ethnicity	
Han	488 (97.2)
Other	14 (2.8)
Having ever fathered a pregnancy	
Yes	189 (37.6)
No	313 (62.4)
Abstinence time, days	
< 3	51 (10.2)
3–5	333 (66.3)
> 5	118 (23.5)
Education	
Less than high school	191 (38.0)
High school and above	311 (62.0)
Smoking history	
Never	205 (40.8)
Former	59 (11.8)
Current	238 (47.4)
Daily cigarette consumption	
0	205 (40.8)
1–9	105 (20.9)
$\geq 10$	192 (38.2)
Alcohol consumption	
Never	210 (41.8)
Former	255 (50.8)
Current	37 (7.4)
Income, RMB yuan/month	
$\leq 3000$	222 (44.2)
3001–6000	201 (40.1)
$\geq 6001$	79 (15.7)

<sup>a</sup> A total of 1 participant had missing information on age, 2 on having ever fathered a pregnancy, 5 on education, and 1 on income, which were replaced by median values in subsequent analyses.

### 3.2. Distributions of urinary HAA concentrations and serum hormones

The median creatinine-adjusted DCAA and TCAA concentrations in repeated urine samples were 1.26 and 2.01  $\mu$ g/g creatinine, respectively (Table 2). The reproducibility of HAA measurements in paired urine samples was excellent for TCAA (creatinine-adjusted ICC = 0.80) but poor for DCAA (ICC = 0.20) (Table 2). The median serum levels of E2, FSH, LH, SHBG, T, T/LH, FAI, Free T, and Bio T were 34.08 pg/mL, 4.42 mIU/mL, 3.94 mIU/mL, 31.44 nmol/L, 13.52 nmol/dL, 3.49, 43.91, 7.87 ng/dL, and 188.00 ng/dL, respectively (Table 3). The median serum levels of TSH, T3, T4, and T3/T4 were 2.20 uIU/mL, 3.53 pg/mL, 1.37 ng/dL, and 2.59, respectively (Table 3).

### 3.3. Urinary HAA concentrations and hormones

After adjusting for potential confounders and urinary creatinine concentrations, we found inverse dose–response relationships between urinary TCAA concentrations and serum levels of SHBG, T, and T/LH ratio (all  $P_{\text{FDR}}$  for trend  $< 0.10$ ; Fig. 2). Compared with participants in the lowest urinary quartile of TCAA, those in the highest quartile had a lower serum SHBG, T, and T/LH ratio of  $-14.2 \%$  (95% CI:  $-26.7, -3.0$  %),  $-11.1 \%$  (95% CI:  $-21.7, -1.3$  %), and  $-21.0 \%$  (95% CI:  $-36.7, -7.1$  %), respectively. We also found inverse dose–response relationships between urinary TCAA concentrations and serum levels of TSH ( $P_{\text{FDR}}$  for trend = 0.08; Fig. 3). Compared with participants in the lowest urinary quartile of TCAA, those in the highest quartile had  $-19.1 \%$  (95% CI:  $-39.7, -1.5$  %) lower serum TSH levels. These inverse associations persisted when urinary concentrations of DCAA and TCAA were modeled as continuous variables (Table S2). Additionally, we found inverse associations between continuous measurements of urinary DCAA and serum T, Free T, and Bio T, and between continuous measurements of urinary TCAA and serum E2, Free T, and Bio T (all  $P_{\text{FDR}}$  for

**Table 2**  
Distribution and intraclass correlation coefficients (ICCs) of haloacetic acid concentrations in repeated urine samples (n = 502).

HAA	All urine samples (n = 940)			First urine sample(n = 466)			Second urine sample (n = 474)			ICC (95 % CI) <sup>b</sup>
	%>LOD	Median	Interquartile	%>LOD	Median	Interquartile	%>LOD	Median	Interquartile	
Unadjusted (ug/L)										
DCAA	74.3	1.36	<LOD-2.09	78.8	1.44	1.04–2.13	69.8	1.30	<LOD-2.02	0.10 (0.01, 0.19)
TCAA	95.1	2.19	1.23–4.10	97.2	2.43	1.36–4.41	93.0	1.95	1.04–3.80	0.87 (0.85, 0.89)
Creatinine-adjusted <sup>a</sup> (ug/g creatinine)										
DCAA	–	1.26	0.81–2.41	–	1.24	0.83–2.29	–	1.29	0.81–2.54	0.20 (0.11, 0.29)
TCAA	–	2.01	1.17–3.89	–	2.08	1.27–3.97	–	1.95	1.11–3.87	0.80 (0.77, 0.83)

<sup>a</sup> Adjusted for creatinine concentrations (ug/g creatinine). Urinary quartile levels of creatinine (g/L): 0.8, 1.2, and 1.8 in the first urine sample; and 0.7, 1.2, and 1.8 in the second urine sample, respectively.

<sup>b</sup> Intraclass correlation coefficients.

**Table 3**  
Distribution of serum sex hormone and thyroid hormone concentrations (n = 502).

Hormones	Geometric mean (95 % CI)	Median (25th, 75th)	Range
Sex hormones			
E2 (pg/mL)	33.89 (33.05, 34.75)	34.08 (28.66, 40.57)	12.25–89.82
FSH (mIU/mL)	4.41 (4.22, 4.61)	4.42 (3.22, 6.15)	0.78–24.08
LH (mIU/mL)	3.87 (3.72, 4.01)	3.94 (2.95, 5.02)	0.80–15.97
SHBG (nmol/L)	30.63 (29.41, 31.90)	31.44 (23.24, 42.07)	6.25–140.08
T (nmol/L)	13.36 (12.93, 13.81)	13.52 (10.42, 17.38)	2.69–43.56
T/LH	3.46 (3.31, 3.60)	3.49 (2.56, 4.78)	0.69–12.55
FAI	43.62 (42.25, 45.04)	43.91 (34.18, 55.75)	10.29–120.34
Free T (ng/dL)	7.88 (7.67, 8.11)	7.87 (6.6, 9.84)	1.62–16.90
Bio T (ng/dL)	188.16 (182.93, 193.54)	188.00 (156.00, 235.00)	38.70–403.00
Thyroid hormones			
TSH (uIU/mL)	2.14 (2.03, 2.26)	2.20 (1.51, 2.95)	0.02–21.18
T3 (pg/mL)	3.54 (3.49, 3.58)	3.53 (3.28, 3.86)	1.81–5.77
T4 (ng/dL)	1.37 (1.36, 1.39)	1.37 (1.25, 1.49)	0.82–2.22
T3/T4	2.58 (2.55, 2.61)	2.59 (2.36, 2.84)	1.80–4.62

Abbreviations: E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; T, testosterone, T/LH, testosterone/luteinizing hormone; FAI, free androgen index; Free T, free testosterone; Bio T, bioactive testosterone; TSH, thyroid stimulating hormone; T3, free triiodothyronine; T4, free thyroxine; and T3/T4, free triiodothyronine/free thyroxine;

trend ≤ 0.15). Our results were largely unchanged when we used multiple imputations procedures to replace missing data and when we excluded participants who had abnormal semen quality, those who were over 40 years old, or those who had extreme urinary creatinine concentrations (Tables S3–S6).

4. Discussion

Among 502 reproductive-aged Chinese male adults who provided repeated urine samples, urinary TCAA concentrations were inversely associated with serum levels of SHBG, T, T/LH ratio, and TSH. These associations were consistent, regardless of whether TCAA concentrations were modeled as quartile or continuous variables. Meanwhile, we found inverse associations between urinary DCAA concentrations and serum T, Free T, and Bio T, and between urinary TCAA concentrations and serum E2, Free T, and Bio T when HAA concentrations were modeled as continuous variables.

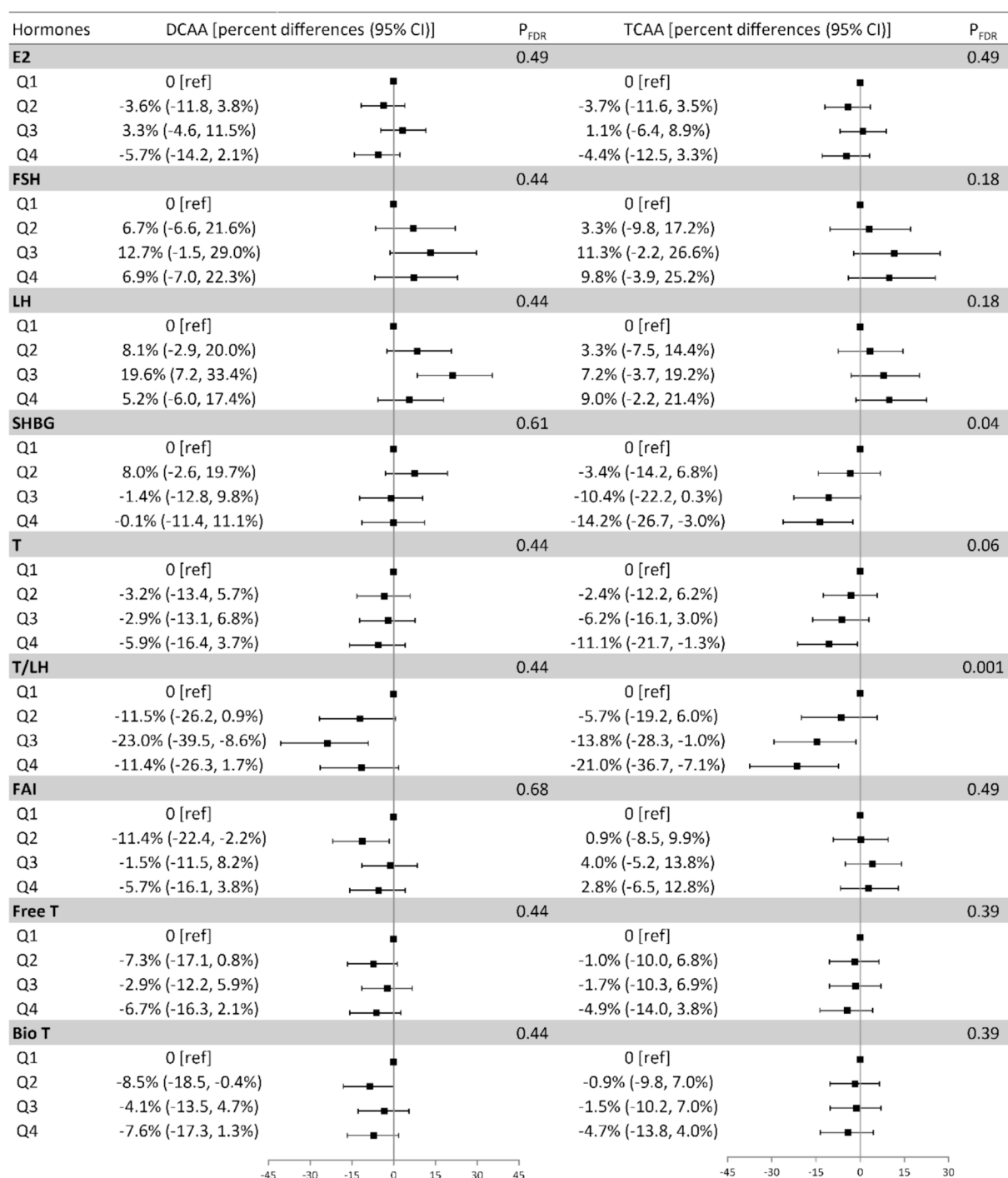
TCAA concentrations in urine have been strongly correlated with ingested HAAs and thus are proposed as candidate biomarkers for ingested DBPs in drinking water (Bader et al., 2004; Froese et al., 2002; Zhang et al., 2009). In our present study, the estimated creatinine-

adjusted ICCs of urinary TCAA concentrations in repeated urine samples were 0.80, indicating excellent reproducibility. In contrast, the reproducibility of DCAA concentrations in repeated urine samples was poor (creatinine-adjusted ICC = 0.20), indicating a high degree of exposure misclassification when limited samples were collected to estimate individuals' HAA exposure. The reproducibility of DCAA and TCAA concentrations in urine samples is influenced by the kinetics of absorption, distribution, metabolism, and elimination, as well as the intervals between exposure events and the elimination half-life of HAAs (Lesá et al., 2014). For instance, DCAA has a half-life of approximately 1 day in humans; TCAA has a slightly longer half-life, ranging from 2 to 6 days (Bader et al., 2004; Schultz and Shangraw, 2006), which may partly explain the higher ICCs of TCAA observed in the present study. The estimated ICC of DCAA in the present study was comparable to that reported for 11 men providing 529 spot urine samples on 8 days over 3 months (ICC = 0.08–0.37) (Wang et al., 2014). However, an apparently higher ICC was found for TCAA measurements in our present study (0.80 versus 0.09–0.23), which could be largely explained by the differences in study design and sampling strategy. In our present study, we only collected two spot urine samples from each man on a single day, which may have ignored the within-day and between-day variability of urinary TCAA concentrations captured in our previous study (Wang et al., 2014).

Several human studies have assessed the association of DBP exposure with sex hormones (Zeng et al., 2013; Liu et al., 2022; Deng et al., 2022; Sun et al., 2021; Nickmilder and Bernard, 2011). For instance, Nickmilder and colleagues reported that swimming in indoor chlorinated pools during childhood was associated with lower serum levels of inhibin B and T among 361 male adolescents (Nickmilder and Bernard, 2011). Zeng and colleagues reported an inverse association between blood THM concentrations and serum levels of T among 401 men recruited from a reproductive center (Zeng et al., 2013). In more recent studies among Chinese populations, Deng and colleagues reported inverse associations between urinary TCAA concentrations and anti-Müllerian hormone (Deng et al., 2022), and a U-shaped association between urinary DCAA concentrations and serum levels of progesterone and prolactin among women receiving medically assisted reproduction (Liu et al., 2022). Very few studies to date have explored the association of DBP exposure with thyroid hormones. Based on a nationally representative survey of 2233 U.S. adults, our previous study found that blood THM concentrations were associated with altered serum levels of T4 and T3 (Sun et al., 2021).

Our results are biologically plausible. Both *in vitro* and *in vivo* studies have indicated that moderate (1/4 the lethal dose 50 test, LD50) doses of HAAs induce testicular toxicity, manifested as atrophy of testes, degeneration of seminiferous tubules, and depletion of germ cells (El Arem et al., 2017; Bhat et al., 1991), which, in turn, resulted in the dysfunction of Leydig cells and insufficient secretion of testosterone (Zirkin and Papadopoulos, 2018; Carney et al., 2014). HAAs also have demonstrated weak estrogenic or androgenic activities (Kim et al., 2020). Previous studies have shown that some HAAs can either mimic

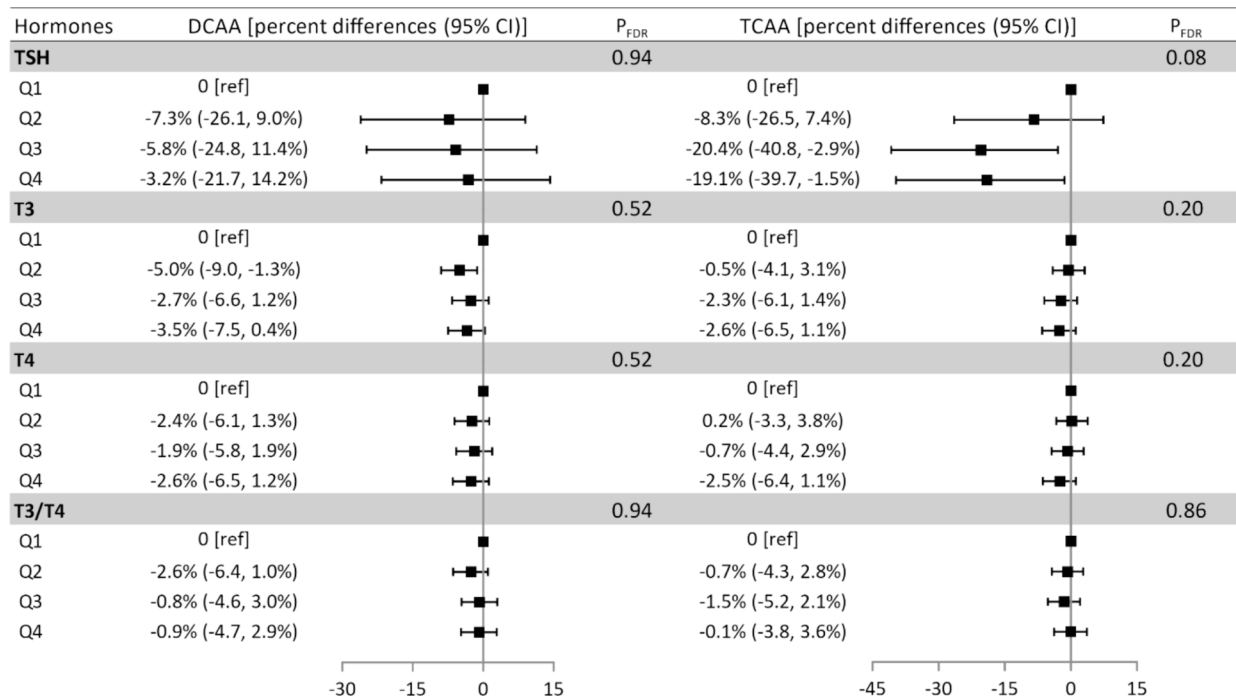




**Fig. 2.** Percent differences (95 % CI) in sex hormone concentrations in relation to haloacetic acid concentrations (n = 502). Models were adjusted for age, BMI, smoking status, daily cigarette consumption, alcohol consumption, education, income, and urinary creatinine concentrations. We divided within-individual HAA concentrations into quartiles; the number of participants for Q1, Q2, Q3, and Q4 is 125, 126, 126, and 125, respectively. Abbreviations: DCAA, dichloroacetic acid; TCAA, trichloroacetic acid; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; T, testosterone; T/LH, testosterone/luteinizing hormone; FAI, free androgen index; Free T, free testosterone; Bio T, bioactive testosterone; BMI, body mass index; and P<sub>FDR</sub>, FDR-adjusted p values for trends.

the effect of estrogen or androgen or disrupt the interactions between hormones and their receptors, resulting in androgen secretion disorders (Long et al., 2021; Gonzalez et al., 2021). Thyroid toxicity induced by THMs has been reported in animal studies. For instance, Narotsky and colleagues reported that exposure to environmentally relevant levels of DBP mixtures caused a significantly increased incidence of thyroid follicular cell hypertrophy in rats (Narotsky et al., 2013). Further investigation is needed to elucidate underlying mechanisms.

Our present study is the first to explore the association between HAA exposure and sex and thyroid hormones among reproductive-aged males attending a fertility center. The strength of our study is the measurements of internal HAA exposure markers in repeated urine samples, which have been demonstrated to improve exposure estimation (Wang et al., 2014). However, this study has several limitations. First, the participants were recruited from a reproductive medical center. Although such a design improved the participation rate and the quality



**Fig. 3.** Percent differences (95 % CI) in thyroid hormone levels (ln-transformed) in relation to haloacetic acid concentrations (ln-transformed) (n = 502). Models were adjusted for age, BMI, smoking status, daily cigarette consumption, alcohol consumption, education, income, and urinary creatinine concentrations. We divided within-individual HAA concentrations into quartiles; the number of participants for Q1, Q2, Q3, and Q4 is 125, 126, 126, and 125, respectively. Abbreviations: DCAA, dichloroacetic acid; TCAA, trichloroacetic acid; TSH, thyroid stimulating hormone; T3, free triiodothyronine; T4, free thyroxine; T3/T4, free triiodothyronine/free thyroxine; BMI, body mass index; and P<sub>FDR</sub>, FDR-adjusted p values for trends.

of hormone measurements, our findings may not be generalized to the overall population. Second, we did not measure other species of DBPs, particularly THMs that are the most abundant species of DBPs. Since previous studies have shown that exposure to THMs is associated with altered sex and thyroid hormones (Zeng et al., 2013; Potter et al., 1996). the inability to account for THMs may have underestimated effects in the current study. Third, although we measured HAA concentrations in repeated urine samples, exposure misclassification cannot be fully excluded. Fourth, we only measured sex and thyroid hormones at a single time point, which may have measurement error due to daily variations, although we strictly collected blood samples from 8:30 to 11:30 a.m. to reduce the diurnal variability of hormones. Finally, the cross-sectional design prevented us from establishing any causal relationships.

5. Conclusion

Among 502 reproductive-aged male adults, we found that urinary TCAA concentrations were inversely associated with serum levels of SHBG, T, T/LH ratio, and TSH. Additionally, we found inverse associations between between urinary DCAA concentrations and serum T, Free T, and Bio T, and between urinary TCAA concentrations and serum E2, Free T, and Bio T when HAA concentrations were modeled as continuous variables. Our findings suggest that exposure to drinking water HAAs may affect male reproductive health by disrupting sex and thyroid hormones.

CRedit authorship contribution statement

**Ying-Jun Chen:** Writing – original draft, Methodology, Data curation, Conceptualization. **Carmen Messerlian:** Writing – review & editing, Conceptualization. **Qi Lu:** Writing – review & editing. **Vicente Mustieles:** Writing – review & editing. **Yu Zhang:** Writing – review & editing. **Yang Sun:** Writing – review & editing. **Liang Wang:** Writing –

review & editing. **Wen-Qing Lu:** Writing – review & editing. **Chong Liu:** Writing – review & editing, Visualization, Validation. **Yi-Xin Wang:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

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Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2024.108785>.

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