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Blood trihalomethane concentrations in relation to sperm mitochondrial DNA copy number and telomere length among 958 healthy men

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ABSTRACT

Background: In animal and human studies, exposure to trihalomethanes (THMs) has been associated with reduced semen quality. However, the underlying mechanisms remain poorly understood.
Objective: To investigate the associations of blood THM concentrations with sperm mitochondrial DNA copy number (mtDNAcn) and telomere length (TL) among healthy men.
Methods: We recruited 958 men who volunteered as potential sperm donors. A single blood sample was collected from each participant at recruitment and measured for chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM) concentrations. Within a 90-day follow-up, the last semen sample provided by each participant was quantified for sperm mtDNAcn and TL. We used multivariable linear regression models to assess the associations between blood THM concentrations and sperm mtDNAcn and TL. We also performed stratified analyses according to the time intervals between baseline blood THM determinations and semen collection (i.e., 0–9, 10–14, 15–69, or >69 days) to explore potential windows of susceptibility.
Results: After adjusting for potential confounders, we found inverse associations between quartiles (or categories) of blood TBM, brominated THM (Br-THM, the sum of BDCM, DBCM, and TBM), and total THM (TTHM, the sum of all four THMs) concentrations and sperm mtDNAcn (all P for trend$\leq$0.03). Besides, we found inverse associations between quartiles of blood TCM, Br-THM, chlorinated THM (Cl-THM, the sum of TCM, BDCM, and DBCM), and TTHM concentrations and sperm TL (all P for trend$<$0.10). Stratified analyses showed stronger associations between Br-THM concentrations and sperm mtDNAcn determined 15–69 days since baseline exposure determinations, and between blood TCM and TTHM concentrations and sperm TL determined >69 days since baseline exposure determinations.
Conclusion: Exposure to THMs may be associated with sperm mitochondrial and telomeric dysfunction.

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1. Introduction

It is estimated that 48.5 million couples worldwide suffer from infertility and approximately 50% of these cases could be attributed to male factors (Agarwal et al., 2021; Martinez et al., 2012). Semen quality is a strong predictor of male fecundity and has decreased globally over the past several decades (Huang et al., 2017; Levine et al., 2017). While the exact causes of such a decline remain uncertain, growing evidence shows that exposure to environmental pollutants, such as disinfection by-products (DBPs), may play an important role (Jurewicz et al., 2009).

DBPs are unintentionally formed due to the reaction of chlorine with natural organic and inorganic compounds in the water-treating process. Among more than 700 identified DBPs in drinking water, trihalomethanes (THMs) are the most prevalent species (Kali et al., 2021; Srivastav et al., 2020). Humans are exposed to THMs through inhalation, ingestion, and dermal absorption (Genisoglu et al., 2019; Siddique et al., 2015). Animal studies have demonstrated that THMs can induce male reproductive toxicity, such as delayed puberty, decreased testosterone levels, and impaired semen quality parameters (e.g., sperm morphology, motion, and motility) (Klinefelter et al., 1995; Land et al., 1981; Narotsky et al., 2015; Potter et al., 1996). In human studies, THM exposure has been associated with lower semen quality among healthy men (Chen et al., 2020; Fenster et al., 2003), occupational workers (Chang et al., 2001), and male partners of infertile couples (Zeng et al., 2013, 2014a). However, the underlying mechanisms are poorly understood.

Sperm telomeres are repetitive DNA sequences (5′-TTAGGG-3′) that cap the ends of chromosomes to ensure genomic stability and integrity (Berneau et al., 2020; Ling et al., 2016). Sperm mitochondria contain their own genome, which is crucial for cellular energy production, apoptosis regulation, and redox equilibrium (Vertika et al., 2020). Telomeric and mitochondrial dysfunction can disrupt cell metabolism and activate the apoptotic pathway (Boguenet et al., 2021; Rocca et al., 2019), leading to impaired spermatogenesis and fertilization. Growing epidemiological evidence shows that sperm telomere length (TL) and mitochondrial DNA copy number (mtDNAcn), two integrated biomarkers of telomere and mitochondrial function, are associated with male semen quality (Popova et al., 2021; Rocca et al., 2016; Sun et al., 2022). However, no study has explored the associations of THM exposure with sperm mtDNAcn and TL. Blood THM concentrations are sensitive to low levels of environmental exposure (Sun et al., 2020), which are believed to be relatively stable due to the frequent exposure events and slow partitioning out of adipose tissue (Blount et al., 2011). Therefore, we investigated the associations of blood THM concentrations with sperm mtDNAcn and TL among 958 healthy Chinese men.

2. Methods and materials

2.1. Research design

Our study participants were recruited from the Hubei Sperm Bank Cohort, as described in detail in our previous study (Chen et al., 2020). Briefly, healthy men who volunteered as potential sperm donors were considered for inclusion if they met the following criteria: a) had no genetic or sexually transmitted diseases; b) achieved a high school degree or above; c) aged between 22 and 45 years; and d) had no history of occupational or radioactive exposure. Each volunteer provided different types of biospecimen samples (i.e., blood, urine, and semen), completed a questionnaire, and underwent a physical examination at enrollment. Participants were asked to provide an additional number of 2–15 semen samples in the following 3 months. We recruited 1341 men between April and December 2017. After excluding 21 men because of sexually transmitted diseases, 1 because of azoospermia, 120 due to a lack of data on blood THM concentrations, and 241 due to insufficient semen samples for DNA extraction, 958 men were included in the present study. The Ethics Committee of the Center for Reproductive Medicine (Tongji Medical College) approved our study protocol (approval number: [2017] IEC [No.1]), and all participants gave written consent at recruitment.

2.2. Covariates

Research staff collected data on demographic information, water-use activities, lifestyle factors, medical and reproductive history, and physical examination measurements either at baseline or during follow-up. The research staff also recorded sampling seasons and abstinence duration when semen samples were collected. Body mass index (BMI, kg/m^2) was calculated by dividing the weight in kilograms by the square of height in meters.

2.3. Blood collection and THM measurements

Blood collection, THM measurements, and quality controls have been described in detail in our previous study (Chen et al., 2020). In brief, a single venous blood sample was drawn from each man using an anticoagulant tube and then stored at 4 °C for 2 weeks. Blood chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM) were extracted with headspace solid-phase microextraction and then determined using a gas chromatograph equipped with an electron capture detector (Bonin et al., 2005; Wang et al., 2019). The limits of detection (LODs) of THMs ranged form 0.5–2.0 ng/L. Values lower than the LODs were replaced by the square root of 2 (Sun et al., 2020).

2.4. Semen collection and analyses

Semen samples were collected by masturbation at the Hubei Province Human Sperm Bank both at enrollment and during follow-up. To prospectively investigate the associations of blood THM concentrations with sperm mtDNAcn and TL across the duration of spermatogenesis (e.g., 90 days), we selected the last semen sample with sufficient volume from each participant to extract DNA (Sun et al., 2022). Briefly, DNA was isolated from a 1.5-mL semen sample using the TIANamp Genomic DNA Kit. The extracted DNA concentrations were quantified with an ND-1000 Nanodrop spectrophotometer and then stored at −80 °C until mtDNAcn and TL analyses (Sun et al., 2022).

Sperm mtDNAcn was determined by a real-time quantitative polymerase chain reaction (RT-qPCR) assay and calculated as the ratio of mitochondrial gene copy number [mitochondrially encoded NADH dehydrogenase 1 (MT-ND1)] to the copy number of a nuclear gene taken as a control or housekeeping gene copy number (β-actin) (Sun et al., 2022). TL was determined as the ratio of telomere repeat copy number to single-copy gene (β-actin) copy number ratio (T/S ratio), using our established RT-qPCR method with minor modifications (Hou et al., 2019). Briefly, we constructed a 10-μL reaction mixture containing 1-μL tested DNA sample, 5-μL KAPA SYBR FAST Universal 2 × qPCR master mix, 0.1-μL forward primer (100 nM), 0.9 μL of reverse primer (900 nM), and 3-μL RNase-free water. The forward and reverse telomeric primers can be found in our previous study (Hou et al., 2019). The reaction and quantification were performed using QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems Life Technologies, CA, USA), and the thermal cycling procedure was set as follows: one cycle of 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 63 °C for 30 s, and 72 °C for 30 s. A melting curve analysis was also performed to examine the amplification specificity by using the following conditions: 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. All samples were run in triplicate. The mean cycle threshold (Ct) value was used to calculate the relative mtDNAcn ratio and TL, based on $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The intra- and inter-assay coefficients of variation were both <5%.

2.5. Statistical analysis

We performed descriptive analyses for participants' baseline characteristics, blood THM concentrations, sperm mtDNAcn, and sperm TL. We calculated concentrations of brominated THMs (Br-THMs, the sum of BDCM, DBCM, and TBM), chlorinated THMs (Cl-THMs, the sum of TCM, BDCM, and DBCM), and total THMs (TTHMs, the sum of Br-THMs and TCM). Differences in geometric mean values of mtDNAcn and TL across the categorical covariates were compared using generalized linear models. MtDNAcn and TL were ln-transformed in all subsequent analyses to reduce the skewness of distributions and reduce the influence of extreme values.

The associations between blood THM concentrations and sperm mtDNAcn and TL were estimated using multivariable linear regression models. Blood TCM, BDCM, Br-THMs, Cl-THMs, and TTHMs were categorized into quartiles. Given the low detection rates of DBCM (59.9%) and TBM (42.2%) in blood, we created a three-level ordinal variable (i.e., <60th, 60th–80th, and >80th percentiles) to maintain a sufficient number of participants in each exposure category (Sun et al., 2020). Tests for trends were calculated by modelling the quartiles or categories of blood THM concentrations as ordinal variables (i.e., 0–2 or 0–3). We converted the obtained regression coefficients (βs) into percent changes using the formula [exp (β)–1] × 100%. We also modelled blood measurements of TCM, BDCM, Br-THMs, Cl-THMs, and TTHMs as continuous variables to investigate potential non-linear associations using generalized additive models (GAMs). To identify the potential etiologically relevant window, stratified analyses were conducted according to the time intervals between baseline THM measurements (e.g., 0 days) and semen collection (e.g., 0–9, 10–14, 15–69, and >69 days, corresponding to epididymal storage, development of sperm motility, meiosis, and spermatogenesis, respectively) (Chen et al., 2021).

Potential covariates were selected based on previous studies investigating predictors of blood THM concentrations, sperm TL, or mtDNAcn (Ashley et al., 2020; Aviv, 2018; Fariello et al., 2012; Huffman et al., 2018; Riederer et al., 2014; Rivera-Nunez et al., 2012; Sun et al., 2022; Zeng et al., 2014b). The covariates in the final regression models were then identified using a directed acyclic graph (DAG) that has been recommended to identify potential confounders in population studies (Textor et al., 2016), which included age (continuous), BMI (continuous), household income (<4000, 4000–8000, or >8000 Yuan/month), education background (high school, college, or undergraduate and above), smoking status (current, never, or former), alcohol usage (current, never, or former), and the season of semen collection (spring, summer, autumn, or winter) (Figure S1). A missing category indicator was created for missing data.

Stratified analyses were conducted according to age (<27 vs. ≥27 years), smoking status (current vs. never or former), and alcohol consumption (current vs. never or former). The multiplicative interaction was estimated by adding a cross-product between blood THMs concentrations and these tested variables in multivariable regression models (Wang et al., 2022). Several sensitivity analyses were also conducted. First, we additionally adjusted for the time interval since the last showering or bathing because recent water-use activities may affect blood THM concentrations (Ashley et al., 2020). Second, we excluded participants whose BMI was >25 kg/m^2 because obesity may affect sperm DNA integrity and mitochondrial activity (Fariello et al., 2012; Ferigolo et al., 2019). Third, we further adjusted for abstinence duration at semen collection because it has been associated with semen quality (Michels et al., 2017). Finally, we re-analyzed the associations of blood THM concentrations with sperm TL by excluding men aged >35 years to avoid age-related changes in TL (Balmori et al., 2021). All data analyses were conducted using SAS (version 9.4, SAS Institute Inc. NC, USA) and R software (version 3.6.0, R Foundation for Statistical Computing, Austria).

3. Results

3.1. Participant characteristics

The arithmetic mean [±standard deviation (SD)] age and BMI of 958 men included in the present study were 28.1 (±5.2) years and 22.7 (±3.2) kg/m^2, respectively. More than half of the men (70.7%) had a normal BMI (18.5–25.0 kg/m^2), possessed a high school degree (65.4%), and used tap water as the main source of water (51.6%) (Table 1). A longer sperm TL was observed among men aged >25 years and those who ever fathered a pregnancy (Table 1). Sperm mtDNAcn was slightly higher among current smokers and those who currently did not drink alcohol or had a shorter abstinence duration (Table 1).

3.2. Distribution of blood THMs, sperm mtDNAcn, and sperm TL

TCM and BDCM concentrations were detectable in 98.2% and 86.5% of blood samples, respectively (Table 2). The detection rates of DBCM and TBM in blood were much lower (i.e., 59.9% and 42.2%, respectively). The median concentrations of blood TCM, BDCM, Br-THMs, Cl-THMs, and TTHMs were 15.3 ng/L, 0.86 ng/L, 4.1 ng/L, 17.6 ng/L, and 27.2 ng/L, respectively. The median values of mtDNAcn and TL were 0.75 and 0.94, respectively.

3.3. Blood THM concentrations and sperm mtDNAcn and TL

We observed inverse dose-response relationships between quartiles (or categories) of blood TBM, Br-THM, and TTHM concentrations and sperm mtDNAcn in crude models (all P for trend≤0.06), which became stronger after adjusting for relevant confounders (all P for trend≤0.03, Table 3). In the fully adjusted modes, percent changes in sperm mtDNAcn comparing highest vs. lowest TBM, Br-THM, and TTHM quartiles (or categories) were −13.3% (95% CI: −23.3%, −1.9%), −13.4% (95% CI: −24.4%, −0.94%), and −18.3% (95% CI: −28.8%, −6.2%), respectively. We also found inverse dose-response relationships between quartiles of blood TCM, Br-THM, Cl-THM, and TTHM concentrations and sperm TL both in crude and fully adjusted models (all P for trend≤0.10, Table 4). Compared with men in the lowest quartiles, those in the highest TCM, Br-THM, Cl-THM, and TTHM quartiles had shorter sperm TL of 5.7% (95% CI: −10.8%, −0.41%), 5.1% (−10.2%, 0.33%), 6.6% (95% CI: −11.6%, −1.3%), and 6.7% (95% CI: −11.8%, −1.2%), respectively. These associations were further confirmed to follow a clear dose-response linear shape in GAMs when blood THM concentrations were modelled as continuous variables (Table S1 and Fig. 1).

3.4. Stratified and sensitivity analyses

The inverse associations between blood Br-THMs and sperm mtDNAcn appeared to be slightly stronger when the exposure was determined 15–69 days before semen examination (i.e., corresponding to meiosis, Fig. 2). We also observed slightly stronger associations between blood TCM and TTHM concentrations and sperm TL determined >69 days since baseline exposure determinations (i.e., corresponding to spermatogenesis). We did not find any evidence of interaction between THM exposure and age or smoking (all P for interaction≥0.09; Table S2). However, the inverse associations of blood Br-THM and TTHM concentrations with mtDNAcn were weaker among current drinkers (P for interaction = 0.006 and 0.04, respectively). The associations between THM exposure and sperm mtDNAcn and TL were substantially unchanged when we further adjusted for the time interval since last showering or bathing (Table S3), when we excluded men aged >35 years (Table S4), when we excluded men whose BMI were >25 kg/m^2 (Table S5), and when we further adjusted for abstinence duration (Table S6).

Table 1
Geometric means (standard deviation) of sperm mtDNAcn and telomere length by demographic characteristics and water-use activities among 958 men screened as potential sperm donors.[a]

Characteristics	N (%) or mean ± SD	MtDNAcn	Telomere length
Age (years)	28.1 ± 5.2		
<25 (ref)	304 (31.7)	0.77 (2.0)	0.88 (1.3)
25–30	391 (40.8)	0.76 (2.0)	0.93 (1.3)*
>30	263 (27.5)	0.77 (2.2)	1.02 (1.4)*
BMI (kg/m^2)	22.7 ± 3.2		
<18.5	73 (7.6)	0.82 (1.9)	0.94 (1.3)
18.5–25 (ref)	677 (70.7)	0.77 (2.1)	0.94 (1.4)
>25	208 (21.7)	0.73 (2.0)	0.95 (1.4)
Education			
High school (ref)	626 (65.4)	0.79 (2.1)	0.94 (1.4)
College	265 (27.7)	0.72 (2.0)	0.93 (1.3)
Undergraduate and above	66 (6.9)	0.68 (2.2)	0.91 (1.4)
Income (Yuan/month)			
<4000 (ref)	300 (31.4)	0.72 (2.0)	0.93 (1.3)
4000–8000	366 (38.2)	0.77 (2.1)	0.96 (1.4)
>8000	291 (30.4)	0.81 (2.2)	0.93 (1.4)
Abstinence time (days)			
≤2	16 (1.7)	1.17 (2.5)*	0.84 (1.4)
2.1–7 (ref)	798 (83.3)	0.78 (2.0)	0.94 (1.4)
>7	144 (15.0)	0.65 (2.2)*	0.95 (1.4)
Smoking status			
Current (ref)	361 (37.7)	0.83 (2.1)*	0.94 (1.4)
Never or former	597 (62.3)	0.73 (2.0)	0.94 (1.4)
Alcohol usage			
Current (ref)	125 (13.1)	0.66 (2.1)	0.94 (1.4)
Never or former	833 (86.9)	0.78 (2.1)*	0.94 (1.3)
Having ever fathered a pregnancy			
No (ref)	682 (71.4)	0.78 (2.0)	0.93 (1.3)
Yes	273 (28.6)	0.75 (2.2)	0.98 (1.4)*
Source of drinking water			
Tap water	494 (51.6)	0.77 (2.1)	0.94 (1.3)
Others	464 (48.4)	0.76 (2.0)	0.94 (1.4)
Season of semen collection			
Spring (ref)	108 (11.3)	0.76 (2.1)	0.98 (1.4)
Summer	352 (36.7)	0.76 (2.0)	0.96 (1.3)
Autumn	353 (36.9)	0.80 (2.1)	0.91 (1.4)*
Winter	145 (15.1)	0.71 (2.1)	0.95 (1.3)
Tap water consumption (mL/day)			
0 (ref)	467 (48.8)	0.75 (2.0)	0.94 (1.4)
1–1000	139 (14.5)	0.79 (2.3)	0.96 (1.4)
>1000	352 (36.7)	0.78 (2.1)	0.93 (1.3)
Time interval since last bathing/showering (hours)			
≤12 (ref)	190 (19.9)	0.77 (2.2)	0.93 (1.3)
12.1–24	623 (65.0)	0.78 (2.0)	0.94 (1.4)
>24	145 (15.1)	0.71 (2.1)	0.97 (1.4)

* *P*-value <0.05 for the differences in biomarker concentrations compared to the reference category, estimated by generalized linear models.
Abbreviations: SD, standard deviation; BMI, body mass index; mtDNAcn, mitochondrial DNA copy number; ref, reference.
[a] A total of 3 men had missing information on the history of having ever fathered a pregnancy, 1 on education level, and 1 on income.

4. Discussion

Among 958 healthy men screened as potential sperm donors, we found inverse dose-response relationships between quartiles (or categories) of blood TBM, Br-THM, and TTHM concentrations and sperm mtDNAcn, and between blood TCM, Br-THM, Cl-THM, and TTHM concentrations and sperm TL. These inverse dose-response relationships were demonstrated to be linear when blood THM concentrations were modelled as continuous variables.

Toxicological evidence has shown that THMs can induce mitochondrial dysfunction. For instance, Faustino-Rocha and colleagues reported that brominated THMs induced decreased mitochondrial bioenergetic activity and inhibited adenosine triphosphate (ATP) synthase in hepatic tissues of male ICR mice (Faustino-Rocha et al., 2016). Yang and colleagues observed that drinking water extracts disrupted mitochondrial signalling pathways and induced mitochondria-mediated apoptosis in rat liver (Yang et al., 2015a). Similarly, extensive toxicological literature has reported that THM exposure can induce DNA damage in different types of cells (de Castro et al., 2019), which may be triggered by shortened TL or altered telomere structures in chromosomes (Aguado et al., 2020). For instance, THMs have been demonstrated to induce DNA strand breaks in primary human lung epithelial cells (Landi et al., 2003), as well as DNA damage and impaired DNA repair capacity in human lymphoblastic leukemia cells (Geter et al., 2004). Meanwhile, telomere damage can cause mitochondrial compromise (Sahin et al., 2011), and mitochondrial dysfunction, in turn, can lead to changes in telomere biology (Lin and Epel, 2022; Van Der Stukken et al., 2022). Nevertheless, while sperm mitochondria and TL are critical for spermatogenesis, hyperactivation, capacitation, acrosome reaction, and fertilization (Durairajanayagam et al., 2021), and have been associated with semen quality, male fecundity, embryo quality, and *in vitro* fertilization success (Rocca et al., 2016; Rosati et al., 2020; Wu et al., 2019; Yang et al., 2015b), no toxicological or population study to date has investigated the potential influence of THM exposure on sperm mtDNAcn and TL.

In the present study, inverse dose-response relationships were exhibited between blood THM concentrations and sperm mtDNAcn and TL. Stratified analyses further showed slightly stronger associations between Br-THM concentrations and sperm mtDNAcn determined 15–69 days since baseline exposure determinations (i.e., corresponding to meiosis), and between blood TCM and TTHM concentrations and sperm TL determined >69 days since baseline exposure determinations (i.e., corresponding to spermatogenesis), suggesting potential etiologic windows. These window-specific findings are biologically plausible. Sperm mtDNA is mainly synthesized during meiosis and early spermiogenesis (Hecht and Liem, 1984). Experimental studies also have suggested that meiosis is the most susceptible stage for the loss of mitochondrial fusion (Meyer et al., 2017; Varuzhanyan and Chan, 2020). The sperm TL primarily elongates during the early stage of spermatogenesis and thus is more likely to be influenced by exogenous insults (Fice and Robaire, 2019). Interestingly, we found evidence of interaction between THM exposure and alcohol consumption; the inverse associations of blood Br-THM and TTHM concentrations with mtDNAcn were only observed among participants who currently did not drink alcohol. Alcohol consumption can induce the activity of CYP2E1, which may have neutralized THM biotransformation and toxicity (Burch et al., 2015).

The involvement of mechanisms in the inverse associations between THM exposure and sperm mtDNAcn and TL remains unclear. However, growing evidence indicates that oxidative stress may play a vital role. On the one hand, sperm mitochondrial and telomeric DNA has an abundant amount of guanine bases but has a lack of DNA repair mechanisms and protective histones and, thus, are highly vulnerable to oxidative stress (Durairajanayagam et al., 2021; Fice and Robaire, 2019). Excessive generation of free radicals can cause single-strand DNA break, telomere binding protein depletion, lipid peroxidation, and defective mitochondrial membranes (Chai et al., 2017; Mishra et al., 2016), eventually leading to telomeric and mitochondrial dysfunction. On the other hand, *in vivo* and *in vitro* studies have consistently demonstrated that THMs can not only induce oxidative stress damage but also impair the anti-oxidant capacity (Abbassi et al., 2010; Beddowes et al., 2003; Faustino-Rocha et al., 2016). Among 1760 pregnant women, we observed positive associations between blood THM concentrations and urinary oxidative stress biomarkers, such as 8-hydroxy-2-deoxyguanosine (8-OHdG) (Liu et al., 2020). Furthermore, THMs can alter DNA methylation levels in rodent species (Coffin et al., 2000), which may eventually lead to telomeric and mitochondrial damage

Table 2
Distribution of blood trihalomethane concentrations and sperm mtDNAcn and telomere length among 958 men screened as potential sperm donors.

Biomarkers	%>LOD	Arithmetic mean	Geometric mean	Percentile				
				10%	25%	50%	75%	90%
Blood THMs (ng/L)								
TCM	98.2	21.7	14.6	6.7	10.4	15.3	21.5	29.2
BDCM	86.5	1.0	0.87	<LOD	0.63	0.86	1.2	1.6
DBCM	59.9	0.96	0.84	<LOD	<LOD	0.85	1.2	1.7
TBM	42.2	42.2	6.9	<LOD	<LOD	<LOD	55.3	135.7
Br-THMs	–	44.2	11.2	2.5	2.9	4.1	57.6	137.8
Cl-THMs	–	23.7	16.9	8.3	12.0	17.6	23.5	32.0
TTHMs	–	65.9	35.2	11.1	16.3	27.2	79.9	157.5
MtDNAcn	–	1.0	0.77	0.31	0.47	0.75	1.2	1.9
Telomere length	–	0.99	0.94	0.64	0.77	0.94	1.2	1.4

Abbreviations: mtDNAcn, mitochondrial DNA copy number; THMs, trihalomethanes; TCM, chloroform; BDCM, bromodichloromethane; DBCM, dibromochloromethane; TBM, bromoform; Br-THMs, brominated THMs; Cl-THMs, chlorinated THMs; TTHMs, total THMs; LOD, the limit of detection.

Table 3
Percent changes (95% CI) in sperm mtDNAcn in relation to blood THM concentrations among 958 men screened as potential sperm donors.[a]

THM (ng/L)	N	Crude models	Adjusted models[b]
TCM			
Q1 (<10.4)	239	Ref	Ref
Q2 (10.4–15.3)	240	11.3 (−2.3, 26.7)	11.4 (−2.3, 26.9)
Q3 (15.3–21.5)	240	1.8 (−10.6, 15.9)	0.65 (−11.7, 14.8)
Q4 (>21.5)	239	3.9 (−8.8, 18.3)	3.4 (−9.6, 18.2)
P for trend		0.67	0.98
BDCM			
Q1 (<0.63)	239	Ref	Ref
Q2 (0.63–0.86)	240	−6.7 (−18.1, 6.3)	−7.2 (−18.5, 5.7)
Q3 (0.86–1.2)	240	−1.2 (−13.3, 12.5)	−2.5 (−14.4, 10.9)
Q4 (>1.2)	239	1.1 (−11.2, 15.2)	0.92 (−11.6, 15.2)
P for trend		0.67	0.73
DBCM			
<60th (<0.99)	575	Ref	Ref
60th–80th (0.99–1.35)	192	−1.6 (−12.6, 10.9)	−3.2 (−14.2, 9.1)
>80th (>1.35)	191	6.4 (−5.5, 19.8)	4.3 (−7.4, 17.5)
P for trend		0.39	0.62
TBM			
<60th (<6.3)	575	Ref	Ref
60th–80th (6.3–75.9)	192	−5.4 (−16.0, 6.5)	−5.6 (−16.3, 6.4)
>80th (>75.9)	191	**−11.7 (–21.6, –0.51)**	**−13.3 (–23.3, –1.9)**
P for trend		**0.03**	**0.02**
Br-THMs			
Q1 (<2.9)	239	Ref	Ref
Q2 (2.9–4.1)	240	−1.5 (−13.5, 12.2)	−3.5 (−15.3, 10.0)
Q3 (4.1–57.6)	240	−5.7 (−17.2, 7.4)	−7.1 (−18.7, 6.0)
Q4 (>57.6)	239	−10.8 (−21.7, 1.7)	**−13.4 (–24.4, –0.94)**
P for trend		0.06	**0.03**
Cl-THMs			
Q1 (<12.0)	239	Ref	Ref
Q2 (12.0–17.6)	240	11.9 (−1.8, 27.4)	12.1 (−1.7, 27.8)
Q3 (17.6–23.5)	240	−1.1 (−13.1, 12.7)	−1.3 (−13.5, 12.6)
Q4 (>23.5)	239	2.5 (−10.0, 16.8)	2.3 (−10.6, 17.0)
P for trend		0.82	0.75
TTHMs			
Q1 (<16.3)	239	Ref	Ref
Q2 (16.3–27.2)	240	−9.9 (−20.9, 2.6)	−11.8 (−22.7, 0.71)
Q3 (27.2–79.9)	240	−5.6 (−17.1, 7.5)	−7.3 (−19.0, 6.2)
Q4 (>79.9)	239	**−15.3 (–25.6, –3.5)**	**−18.3 (–28.8, –6.2)**
P for trend		**0.03**	**0.01**

AbrAbbreviations: mtDNAcn, mitochondrial DNA copy number; BMI, body mass index; THM, trihalomethane; TCM, chloroform; BDCM, bromodichloromethane; Br-THMs, brominated THMs; Cl-THMs, chlorinated THMs; TTHMs, total THMs.

[a] The mtDNAcn was ln-transformed. The regression coefficients were back-transformed using 100 × [exp(β)–1] to obtain the percent changes.

[b] The models were adjusted for age, BMI, income, education background, smoking status, alcohol usage, and sampling season.

Table 4
Percent changes (95% CI) in sperm telomere length in relation to blood THM concentrations among 958 men screened as potential sperm donors.[a]

THM (ng/L)	N	Crude models	Adjusted models[b]
TCM			
Q1 (<10.4)	239	ref	Ref
Q2 (10.4–15.3)	240	**−7.7 (–12.6, –2.5)**	**−7.7 (–12.5, –2.6)**
Q3 (15.3–21.5)	240	**−6.4 (–11.4, –1.2)**	**−7.1 (–11.9, –1.9)**
Q4 (>21.5)	239	−5.0 (−10.0, 0.33)	**−5.7 (–10.8, –0.41)**
P for trend		0.11	0.06
BDCM			
Q1 (<0.63)	239	Ref	Ref
Q2 (0.63–0.86)	240	2.9 (−2.5, 8.7)	2.0 (−3.4, 7.6)
Q3 (0.86–1.2)	240	−1.7 (−6.9, 3.8)	−1.7 (−6.8, 3.7)
Q4 (>1.2)	239	0.58 (−4.8, 6.2)	−1.0 (−6.3, 4.6)
P for trend		0.74	0.44
DBCM			
<60th (<0.99)	575	ref	Ref
60th–80th (0.99–1.35)	192	−2.3 (−7.1, 2.7)	−1.8 (−6.6, 3.1)
>80th (>1.35)	191	−2.6 (−7.4, 2.3)	−3.1 (−7.7, 1.8)
P for trend		0.23	0.19
TBM			
<60th (<6.3)	575	ref	Ref
60th–80th (6.3–75.9)	192	2.0 (−3.0, 7.2)	1.5 (−3.4, 6.7)
>80th (>75.9)	191	−3.4 (−8.1, 1.6)	−3.1 (−7.9, 1.9)
P for trend		0.31	0.34
Br-THMs			
Q1 (<2.9)	239	ref	Ref
Q2 (2.9–4.1)	240	**−5.7 (–10.7, –0.41)**	−4.6 (−9.6, 0.70)
Q3 (4.1–57.6)	240	−3.4 (−8.6, 2.0)	−4.1 (−9.2, 1.3)
Q4 (>57.6)	239	**−5.4 (–10.5, –0.15)**	−5.1 (−10.2, 0.33)
P for trend		0.10	0.09
Cl-THMs			
Q1 (<12.0)	239	ref	Ref
Q2 (12.0–17.6)	240	**−8.1 (–12.9, –2.9)**	**−8.4 (–13.2, –3.3)**
Q3 (17.6–23.5)	240	**−7.6 (–12.4, –2.4)**	**−8.5 (–13.3, –3.4)**
Q4 (>23.5)	239	**−5.4 (–10.4, –0.11)**	**−6.6 (–11.6, –1.3)**
P for trend		0.06	**0.02**
TTHMs			
Q1 (<16.3)	239	ref	Ref
Q2 (16.3–27.2)	240	**−6.3 (–11.3, –1.1)**	**−6.4 (–11.4, –1.2)**
Q3 (27.2–79.9)	240	−3.0 (−8.1, 2.4)	−4.6 (−9.8, 0.91)
Q4 (>79.9)	239	**−6.1 (–11.1, –0.87)**	**−6.7 (–11.8, –1.2)**
P for trend		0.08	**0.04**

Abbreviations: BMI, body mass index; THM, trihalomethane; TCM, chloroform; BDCM, bromodichloromethane; Br-THMs, brominated THMs; Cl-THMs, chlorinated THMs; TTHMs, total THMs.

[a] The telomere length was ln-transformed. The regression coefficients were back-transformed using 100 × [exp(β)–1] to obtain the percent changes.

[b] The models were adjusted for age, BMI, income, education background, smoking status, alcohol usage, and sampling season.

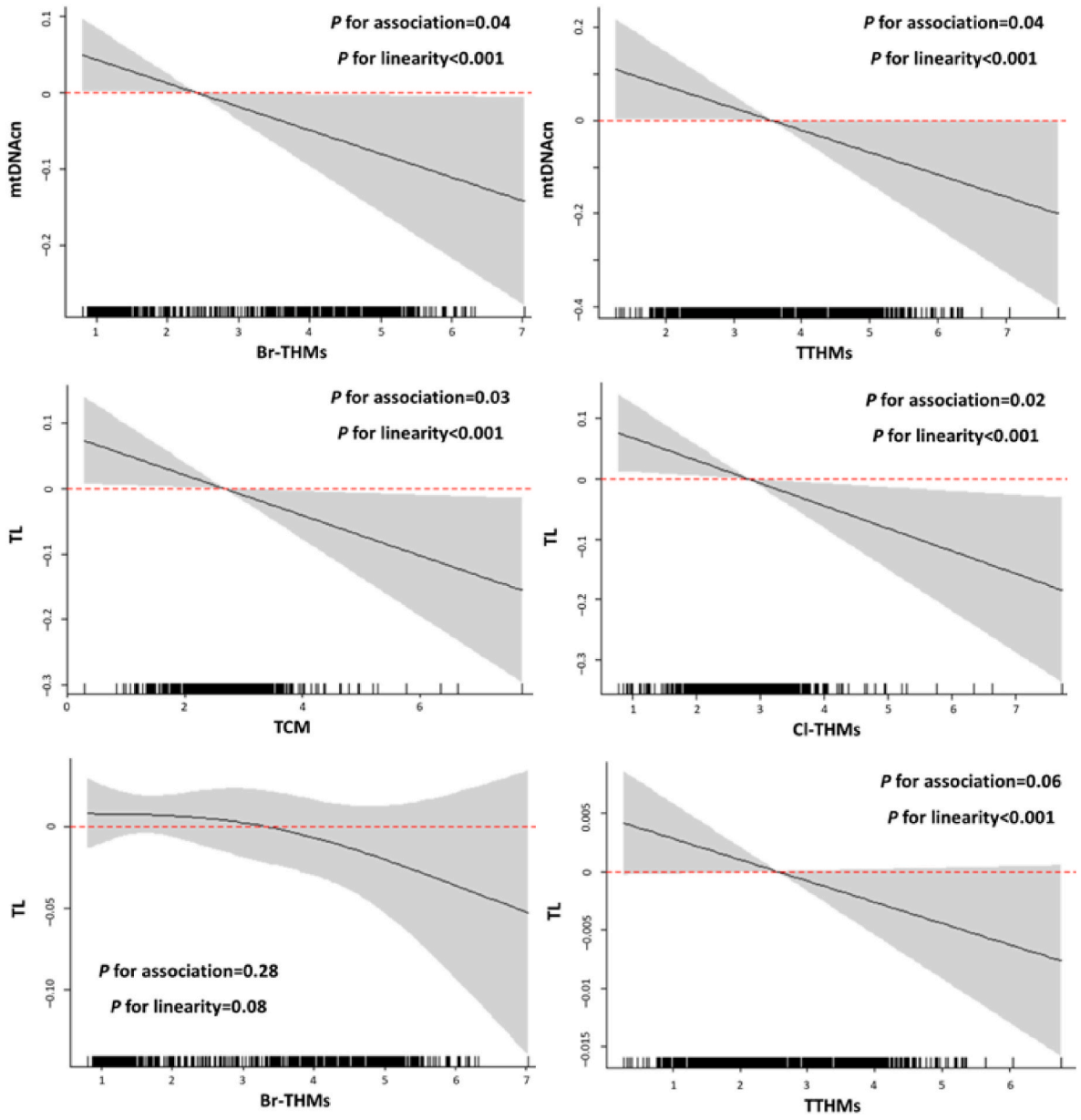

Fig. 1. Dose-response relationships between blood THM concentrations and sperm mtDNAcn and TL based on generalized additive models, with adjustment for age, BMI, household income, education background, smoking status, alcohol usage, and sampling season. Blood THM concentrations, sperm mtDNAcn, and TL were ln-transformed. Abbreviations: mtDNAcn, mitochondrial DNA copy number; TL, telomere length; BMI, body mass index; THMs, trihalomethanes; TCM, chloroform; Br-THMs, brominated THMs; Cl-THMs, chlorinated THMs; TTHMs, total THMs.

(Sang et al., 2021; Yehezkel et al., 2008).

In the present study, we have adjusted for various covariates related to sperm mtDNAcn and TL, such as age, smoking, alcohol consumption, and abstinence duration. We observed longer sperm TL among men aged >25 years and those who ever fathered a pregnancy, which supports the previous evidence showing a positive association between telomere elongates and age (Aston et al., 2012; Balmori et al., 2021). Both smoking and alcohol consumption may affect mitochondrial function (Manzo-Avalos and Saavedra-Molina, 2010; Mori et al., 2022). In the present study, we found that sperm mtDNAcn was slightly higher among current smokers and those who currently did not drink alcohol. We also found that sperm mtDNAcn was lower among men who had a longer abstinence duration, which could be partly related to decreased antioxidant capacity in spermatozoa over time (Shen et al., 2019). However, our results may still be confounded by unmeasured factors such as parental age (Ferlin et al., 2013), genetic factors (e.g., expression of mitochondrial transcription factor A and single nucleotide polymorphisms in telomerase-associated protein 1 that may affect sperm telomeric and mitochondrial function) (Faja et al., 2019; Yan et al., 2014), and co-exposure to other pollutants (e.g., air pollution, phthalate, and polycyclic aromatic hydrocarbons) (Huffman et al., 2018; Ling et al., 2016; Zhang et al., 2020; Zhou et al., 2021). Moreover, we determined blood THM concentrations at a single time point at recruitment, which may have led to exposure misclassification due to the considerable within-person variation of blood THM concentrations over time (Wang et al., 2019). We also cannot ascertain any causal relationships due to the observational nature of the study design. Finally, mtDNAcn and TL were measured in sperm samples that may contain a small proportion of white blood cells, which may have influenced the preciseness of our findings. The major strength of this study is the recruitment of healthy men screened as potential sperm donors, which improves the representativeness of the study population. Other

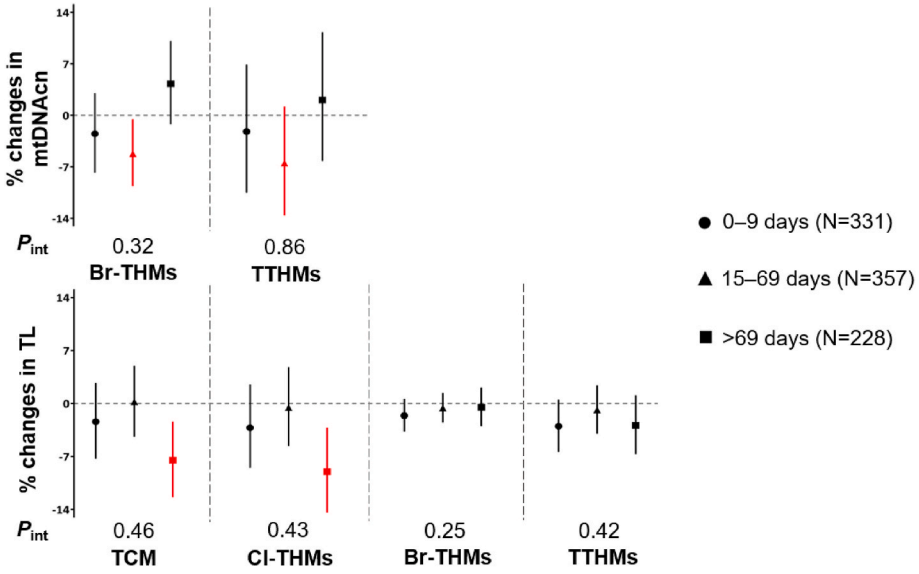

Fig. 2. Percent changes in sperm mtDNAcn and telomere length associated with blood THM concentrations stratified by the time intervals between baseline THM measurements (e.g., 0 days) and semen collection (i.e., 0–9, 15–69, and >69 days since baseline). The stage of sperm motility development (10–14 days since baseline, N = 42) was excluded due to the limited sample size. Models were adjusted for age, BMI, education background, household income, smoking status, alcohol usage, and sampling season. Blood THM concentrations were ln-transformed. The mtDNAcn and telomere length were ln-transformed and then back-transformed using 100 × [exp(β)–1] to obtain the percent changes. Abbreviations: mtDNAcn, mitochondrial DNA copy number; BMI, body mass index; THMs, trihalomethanes; TCM, chloroform; Br-THMs, brominated THMs; Cl-THMs, chlorinated THMs; TTHMs, total THMs.

strengths include the application of internal exposure biomarkers (i.e., blood THM concentrations), a large sample size, and prospective measurements of mtDNAcn and TL in semen samples collected during follow-up.

5. Conclusions

Among 958 healthy men screened as potential sperm donors, we found inverse dose-response relationships between blood TBM, Br-THM, and TTHM concentrations and sperm mtDNAcn, and between blood TCM, Br-THM, Cl-THM, and TTHM concentrations and sperm TL. Part of these associations differed across the duration of spermatogenesis, indicating potential etiologic windows. Our findings suggest that sperm mtDNAcn and TL are potential relevant mechanisms underlying the adverse effect of THM exposure on male reproductive health, although more studies are needed to verify these novel results.

Author contribution statement

Conceptualization: C.L. J.H. and Y.X.W.; data curation: C.L. B.S. and H.G.C.; investigation: C.L. Y.J.C. B.S. H.G.C. T.Q.M, and X.C.L.; methodology: V.M. C.M. and Y.X.W.; formal analysis: C.L. and Y.J.C.; validation: B.S. H.G.C, and Y.S.; visualization: C.L. and X.F.P.; resources: W.Q.L. C.L.X, and J.H.; project administration: W.Q.L. C.L.X. and Y.X.W.; funding acquisition: W.Q.L. and Y.X.W.; supervision: V.M. and Y.X.W; writing – original draft: C.L. and Y.X.W; writing – review & editing: all authors. All the authors had full access to the data in this research and accept responsibility to submit for publication.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envres.2022.114737.

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