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Liver-related biomarkers mediate the potential effect of volatile organic compounds on an anti-aging hormone: a cross-sectional study

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Volatile Organic Compounds (VOCs) are a class of air pollutants that have adverse metabolic and endocrine effects. α-Klotho, an anti-aging hormone, is susceptible to environmental pollution. This study aimed to explore the association between urinary VOCs and serum α-Klotho levels and the underlying molecular mechanisms. 1680 individuals from the U.S. were included. The association between VOCs and α-Klotho was explored using multivariate linear regression. Weighted quantile sum (WQS) regressions were used to identify the important distribution of VOCs on α-Klotho, and Bayesian kernel machine regressions (BKMR) were implemented to assess the joint and univariate effects of all VOCs on α-Klotho. Mediation analyses of liver-related biomarkers in the effects of mixed and single VOCs on α-Klotho were also performed. The multivariate linear regression model showed that multiple VOCs were negatively associated with α-Klotho. The WQS models ascertained that *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine (CEMA), *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine (CYMA), and 2-aminothiazoline-4-carboxylic acid (ATCA) play dominant roles. In the BKMR model, we found that the joint effects of mixed VOCs were negatively associated with α-Klotho in the general population (40–79 years) and middle-aged population (40–59 years). Finally, mediation analyses showed that liver-related biomarkers exhibited a substantial mediation effect in the single ATCA on α-Klotho, with a proportion as high as 31.6% in the middle-aged population. Our study demonstrated that liver-related biomarkers mediate the influence of VOCs, especially ATCA, on α-Klotho. Our findings suggest that reducing VOC exposure may be a potential strategy to maintain α-Klotho levels.

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Environmental significance

Volatile organic compounds (VOCs) are a class of air pollutants that have raised increasing concerns about the metabolic and endocrine effects. Here, we discovered that VOCs negatively impact α-Klotho, an anti-aging hormone. In particular, compounds such as *N*-Acetyl-*S*-(2-carboxyethyl)-L-cysteine (CEMA), *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine (CYMA), and 2-aminothiazoline-4-carboxylic acid (ATCA) play dominant roles. VOC exposure is a risk factor for age and aging-related diseases, as it suppresses serum Klotho levels. Noticeably, liver-related markers exhibit substantial mediation effects, driving the impact of VOCs on serum Klotho. More importantly, the adverse effects are more pronounced in middle-aged populations. In conclusion, VOCs may suppress serum Klotho levels and accelerate the aging process *via* liver-related marker mediation.

1. Introduction

Klotho proteins, which are a family of related single-pass transmembrane proteins, are essential components of endocrine fibroblast growth factor (FGF) receptor complexes.[1,2] Currently, Klotho deficiency has been found to play a crucial role in the pathophysiology of aging-related disorders and in general surgical oncology, particularly in tumors such as thyroid, breast, and colorectal cancer.[3–6] Dysregulated Klotho levels are also related to increased all-cause and cause-specific mortality and shortened life expectancy,[7,8] while the restoration of circulating Klotho levels may alleviate age and improve postoperative outcomes.[9–12] Klotho has three homologs, α-Klotho, β-Klotho, and γ-Klotho. Among these, α-Klotho was the first to be discovered, and its role in aging has been extensively discussed.[13]

Volatile Organic Compounds (VOCs) include thousands of chemicals produced by a wide range of household and industrial activities.[14] In medical practice, VOCs are extensively

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applied in the sterilization of medical equipment and pharmaceutical manufacturing and are also emitted during surgical procedures. It has been reported that surgical smoke samples collected from mammary glands using conventional electrosurgical knives exhibited high concentrations of VOCs. Furthermore, the type of electrosurgical unit and the power settings of electrocautery influence the VOC concentrations in the surgical smoke.[15] In addition, it has been established that newborns undergoing cardiac surgery are widely exposed to VOCs from medical devices and from the air within the intensive care unit.[16] VOCs may pose risks because they can be mutagenic, neurotoxic, genotoxic, and carcinogenic.[16]

Prolonged exposure to VOCs has been reported to be linked to biological aging, emerging as a risk factor that can hasten an individual's aging trajectory. For example, Yang and colleagues documented a significant association between VOCs (*e.g.*, benzene, toluene and ethylbenzene) with accelerated aging.[17] Shi *et al.* confirmed that exposure to a range of individual VOCs was linked to biological aging.[18] Van der Laan L *et al.* also revealed a positive correlation between occupational benzene exposure and epigenetic age acceleration.[19] In addition, it has been revealed that biological aging mediated the effects of VOCs on kidney damage.[20] However, the effect of VOCs on Klotho remains unknown. The mechanisms by which these compounds interact and collaborate to affect aging-related biological characteristics are also unclear. Consequently, to better prevent and mitigate the impact of VOCs, it is necessary to elucidate the mechanisms underlying the association between VOCs and Klotho.

Total bilirubin (TB) and liver enzymes including gamma-glutamyl transferase (GGT), alanine transaminase (ALT) and aspartate transaminase (AST) are elevated in various liver diseases, making them crucial indicators of liver function.[21] These liver-related biomarkers have been reported to be linked to aging-related diseases.[22–24] Moreover, extant studies have corroborated the association between VOCs and these biomarkers, providing substantial evidence that exposure to VOCs may affect liver health and function.[25,26] In contrast, alterations in Klotho levels are significantly associated with certain pathological states of the liver, such as non-alcoholic fatty liver disease (NAFLD) and liver fibrosis.[27,28] Based on these reports, we hypothesized that exposure to VOCs might influence Klotho, potentially by damaging liver function. The VOCs assessed in this study were derived from urine and represented metabolites formed through biological processes. Once absorbed, VOCs are metabolized in the body and excreted through various pathways, including urine and blood. Urinary metabolites of VOCs are used as biomarkers because of their stability and ability to reflect recent exposure to VOCs. Moreover, these metabolites are primarily excreted as mercapturic acids, which have longer half-lives than blood-based VOC biomarkers. Therefore, we conducted a cross-sectional study to investigate the effects of 16 urinary VOCs on serum α-Klotho based on 2011–2016 National Health and Nutrition Examination Survey (NHANES) data. We also explored the potential mediating effects of liver-related biomarkers, including TB, GGT, AST and ALT. This study was conducted in accordance with the STROBE guidelines.[29]

2. Methods

2.1 Study population

Data for this study came from the National Health and Nutrition Examination Survey (NHANES), which is an ongoing cross-sectional survey designed to assess the nutrition and health status of a nationally representative sample of U.S. citizens. The study involved 29 902 participants from the NHANES between 2011 and 2016. Fig. S1 outlines the selection process, which involved the exclusion of subjects without serum Klotho data (n = 22 040); subjects without urinary VOC data (n = 5835); 175 subjects with missing BMI, diabetes, cigarette smoking, and alcohol drinking data; and 172 subjects with missing creatinine data. Ultimately, the final study sample consisted of 1680 individuals.

2.2 Urinary VOC measurement

As described previously, quantitative analysis of VOCs in human urine was performed using ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS).[30] A UPLC HSS T3 column (part number 186003540, 1.8 μm × 2.1 mm × 150 mm, Waters Corporation) was used for chromatographic separation with a mobile phase consisting of 15 mM ammonium acetate and acetonitrile. Briefly, the eluent from the chromatographic column was ionized using an electrospray interface to generate negative ions, which were then transferred to a mass spectrometer. The concentrations of the individual analytes were obtained by comparing the relative response factor (the ratio of the analyte of interest to a labeled stable isotope internal standard) with known standard concentrations. A total of 23 VOCs were detected from 2011 to 2016, and their detection limits and rates are summarized in Table S1. VOCs with detection rates <70% were not included in the subsequent analysis.[31] For more detailed information on the laboratory method for detecting urinary VOCs, see https://wwwn.cdc.gov/nchs/data/nhanes/2015-2016/labmethods/UVOC_UVOCS_I_MET.pdf.

2.3 Measurement of serum Klotho

According to the official website instructions, the participants were instructed to fast overnight and provide a blood specimen for examination. Our study focused on NHANES participants aged 40–79 years, incorporating samples from a six-year period. The concentrations of serum Klotho were determined by using ELISA and the analysis was carried out following the manufacturer's protocol.[32] Each specimen was processed twice according to the manufacturer's guidelines. The outcomes were validated based on the preset acceptability criteria in the laboratory, and the results were incorporated into the database only when these criteria were met. Detailed information regarding the Klotho testing process is available on the NHANES website.

2.4 Covariates

In this study, we considered several potential factors including age, sex, race/ethnicity, education level, body mass index (BMI), cigarette smoking, diabetes, hypertension, alcohol consumption, and kidney function (creatinine). Data were collected using a self-report questionnaire administered to the participants and their families. Based on previous research, diabetes was defined using the following criteria: (1) a fasting blood glucose level > 6.1 mmol L; (2) in the case of an oral glucose tolerance test (OGTT), a blood glucose level exceeding 11.1 mmol L^{-1} two hours after the participant consumed a solution containing 75 g of glucose; (3) an individual with a glycosylated hemoglobin (HbA1c) level $\geq$ 6.5% or higher; (4) participants who reported being clinically diagnosed with diabetes by healthcare professionals. Additionally, individuals with blood glucose levels close to the threshold were assumed to have diabetes.[32] Hypertension was defined as three consecutive blood pressure readings of 140/90 mmHg or above, with at least a 6-hour interval between measurements. For pregnant individuals, hypertension is defined as two consecutive blood pressure readings of 140/90 mmHg or above, taken at least 6 h apart, and confirmed by a diagnosis from a doctor or other healthcare professional.[33] Furthermore, based on respondents' answers to the question, "have you ever smoked at least 100 cigarettes in your lifetime?", we categorized smoking status into "ever" and "never".[33]

2.5 Statistical analysis

According to NHANES analysis guidelines, we combined the 2011/2012, 2013/2014, and 2015/2016 cycles. To ensure generalizability to the broader U.S. population, we used Mobile Examination Center weights (WTSAU6YR) to estimate representative measures. Population baseline data were expressed as numbers (weighted percentage), weighted mean (standard error), or weighted median (weighted quartile range). Weighted linear regression models were used for continuous data, and weighted chi-square tests were used to calculate *p*-values for categorical data. Additionally, univariate methods were employed to estimate the latent variables. Multivariate linear regression analysis was conducted to compute regression coefficients (β) and 95% confidence intervals (CI) between urinary VOCs and serum Klotho concentrations. Multivariate linear regression allows for simultaneous examination of the effects of multiple independent variables on a dependent variable. This approach provides a more comprehensive understanding of the relationships involved and helps control for confounding variables, thereby reducing bias and improving prediction accuracy. Stepwise adjustments were performed for some regression models (crude model, Model I, and Model II). Model I was adjusted for sex and age, while Model II was further adjusted for race/ethnicity, BMI, education level, hypertension, diabetes, cigarette smoking, alcohol drinking, and creatinine. Subsequently, to examine the relationship between urinary VOCs and serum Klotho concentrations in a specific population, age was used as a stratified variable.

The weighted quantile sum (WQS) allows the assessment of the combined effect of multiple exposures, each weighted by its relative importance. This method is particularly useful when dealing with complex mixtures, as it helps to identify the most influential exposures while accounting for potential interactions and correlations among them. WQS regression was applied to explore the importance distribution of VOCs in Klotho, as it showed good performance in characterizing environmental mixtures. WQS regression is a statistical model for the multivariate regression of high-dimensional datasets commonly encountered in environmental exposure. This model enables the construction of a weighted index using supervised methods to assess the contribution of each component in the mixture to the overall effect. The weights (ranging from 0 to 1) represent the importance level of the individual VOCs. The regression coefficient β of the WQS index was interpreted as the simultaneous effect on Klotho of a one-quantile increase in the mixed VOCs.

However, the linear model mentioned above cannot handle complex multicollinearity and non-additive correlation. The rationale for using Bayesian kernel machine regression (BKMR) is that it provides a flexible and comprehensive approach to modeling the complex, nonlinear effects of multiple exposures on an outcome. BKMR incorporates Bayesian methods to account for uncertainty and allows for the estimation of both the individual and joint effects of exposures, making it suitable for analyzing high-dimensional and potentially correlated exposure data. Using Bayesian kernel machine regression (BKMR), Gaussian kernel could capture high-dimensional features and interactions of VOCs to identify more meaningful factors of VOC exposure. In our work, BKMR was used to assess the joint and univariate effects of all VOCs (iterations = 20 000). Bivariate interactions between VOCs were also assessed. We also explored the dose–response relationship between a single VOC and Klotho when fixing other VOC concentrations to identify which VOC significantly impacts the Klotho level. In order to gain further insight into the mechanisms underlying the influence of VOCs on Klotho and considering the known hepatic metabolism of VOCs, we employed alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT) and total bilirubin (TB) as parallel mediators to analyze the potential mediation effects between VOCs and Klotho. The rationale for using mediation analysis is to understand the mechanisms through which an independent variable affects a dependent variable by examining the role of intermediate variables known as mediators. This method helps to disentangle direct effects from indirect effects, providing insights into causal pathways and improving the interpretation of complex relationships in data. In this study, we conducted mediation analysis on both mixed VOCs (based on WQS weight) and individual VOCs to gain a more comprehensive and rigorous understanding of the various effects of different VOCs on Klotho. All mediation analyses used the bootstrap method with 1500 simulations to estimate the 95% confidence interval of the mediation effect. The total effect (TE) represents the effect of VOC exposure on Klotho without mediators. The indirect effect (IE) represents the

effects of VOC exposure on Klotho through the mediator. The proportion of mediation was calculated using IE divided by TE.

All WQS and BKMR analyses were adjusted for age, sex, race/ethnicity, BMI, education level, hypertension, diabetes, cigarette smoking, alcohol drinking, and serum creatinine level. Considering the correlation between Klotho and age, we further conducted regression analyses stratified by age (40–59 years and 60–79 years). The urinary VOC concentrations were not Ln-transformed during the linear regression, WQS, and BKMR analyses. This decision was made to preserve the original data distribution, which facilitated and promoted a more practical and effective basis for evaluation and implementation in clinical practice. Pearson's correlation analysis was used to assess correlations among the VOCs.

Statistical analysis was conducted using R statistical programming language (version 3.6.1; R Foundation for Statistical Computing) and SPSS software (X&Y Solutions, Inc.). Statistical significance was set at $P < 0.05$.

3. Results

3.1 Baseline traits of included participants

Table 1 presents the demographic characteristics of the participants who underwent both Klotho and VOC measurements. The study included a total of 1680 individuals, with 850 males (50.6%) and 830 females (49.4%). The interquartile range (IQR) for serum Klotho concentrations was 795.100 (657.150–984.750) pg ml^{-1}. The average age of the participants was 57.761 ± 10.643 years. Additionally, 807 participants (48.0%) were smokers, 791 (47.1%) had hypertension, and 322 (19.2%) had diabetes.

In terms of correlations among VOCs, Pearson's coefficients indicated strong correlations between CYMA, 3HPMA, MHBMA3, and HPMMA ($r > 0.8$), whereas BMPA, BMA, 2HPMA, and 2-aminothiazoline-4-carboxylic acid (ATCA) showed relatively weak correlations (Fig. S2).

3.2 Linear association between urinary VOC concentrations and serum Klotho

To investigate the relationship between VOCs and Klotho, a linear regression model was employed to analyze and summarize the data. In univariate analysis, significant associations were observed between serum Klotho concentration and VOCs, including AMCC, CEMA, CYMA, DHBMA, 3HPMA, MA, MHBMA3, PGA, and HPMMA (AMCC: $\beta = -0.055$, $P = 0.007$; CEMA: $\beta = -0.117$, $P = 0.005$; CYMA: $\beta = -0.157$, $P = 0.011$; DHBMA: $\beta = -0.089$, $P = 0.001$; 3HPMA: $\beta = -0.024$, $P = 0.029$; MA: $\beta = -0.082$, $P = 0.009$; MHBMA3: $\beta = -0.828$, $P = 0.022$; PGA: $\beta = -0.095$, $P = 0.003$). However, no statistically significant association was observed between several VOCs and serum Klotho levels, such as ATCA ($\beta = -0.028$, $P = 0.426$). Additionally, strong correlations were found between Klotho levels and certain variables, including sex, age, diabetes, alcohol consumption, and serum creatinine levels (sex: $\beta = -68.465$, $P < 0.001$; age: $\beta = -2.432$, $P < 0.001$; diabetes: $\beta = -42.363$, $P = 0.044$; alcohol consumption: $\beta = -47.024$, $P = 0.009$; and creatinine: $\beta = -112.144$, $P < 0.001$). Considering that the confounders can obscure the true relationship between VOCs and Klotho in the univariate analysis, it was important to perform a multivariable analysis accounting for these

Table 1 The demographic characteristics of study participants ($N = 1680$)[a]

Variables	Overall
Participants	$N = 1680$
Continuous variables mean (SD)	
2MHA	62.441 (95.646)
3MHA + 4MHA	432.674 (635.173)
AAMA	71.935 (84.598)
AMCC	266.542 (335.104)
ATCA	175.055 (195.817)
BMA	13.153 (27.569)
BPMA	11.297 (27.417)
CEMA	161.147 (183.864)
CYMA	41.312 (112.589)
DHBMA	365.583 (262.546)
2HPMA	62.895 (132.273)
3HPMA	475.402 (721.487)
MA	188.186 (219.189)
MHBMA3	12.382 (21.472)
PGA	264.243 (251.353)
HPMMA	530.622 (841.268)
Serum Klotho (pg mL^{-1})	852.286 (318.623)
Age (year)	57.761 (10.643)
BMI	29.797 (6.800)
Creatinine (mg dL^{-1})	0.914 (0.323)
Categorical variables, %	
Gender	
Male	850 (50.595%)
Female	830 (49.405%)
Race/Ethnicity	
Mexican American	229 (13.631%)
Non-Hispanic White	211 (12.560%)
Non-Hispanic Black	663 (39.464%)
Other races	577 (34.345%)
Education level	
Below high school	402 (23.929%)
High school	341 (20.298%)
Above high school	937 (55.774%)
Hypertension	791 (47.083%)
Diabetes	322 (19.167%)
Cigarette smoking	807 (48.036%)
Alcohol drinking	1217 (72.440%)

[a] SD, standard deviation; BMI, body mass index; VOCs, volatile organic compounds: 2MHA, urinary 2-methylhippuric acid; 3MHA + 4MHA, urinary 3- and 4-methylhippuric acid; AAMA, urinary *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine; AMCC, urinary *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine; ATCA, urinary 2-aminothiazoline-4-carboxylic acid; BMA, urinary *N*-acetyl-*S*-(benzyl)-L-cysteine; BPMA, urinary *N*-acetyl-*S*-(n-propyl)-L-cysteine; CEMA, urinary *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine; CYMA, urinary *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine; DHBMA, urinary *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine; 2HPMA, urinary *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; 3HPMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine; MA, urinary mandelic acid; MHBMA3, urinary *N*-acetyl-*S*-(4-hydroxy-2-butenyl)-L-cysteine; PGA, urinary phenylglyoxylic acid; HPMMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine.

Table 2 The variable comparisons with univariate analysis to explore the factors affecting serum Klotho concentrations ($N = 1680$)[a]

	Serum Klotho (pg. mL^{-1})	
Variables	N (%)/Mean ± SD	β/OR (95% CI) p value
Gender		
Male	850 (50.595%)	Reference
Female	830 (49.405%)	68.465 (38.081, 98.849) <0.001
Age	57.761 ± 10.643	−2.432 (−3.871, −0.993) <0.001
Education level		
Below high school	402 (23.929%)	Reference
High school	341 (20.298%)	−21.050 (−67.267, 25.166) 0.372
Above high school	937 (55.774%)	0.628 (−40.244, 41.500) 0.976
BMI (kg m^{-2})	29.797 ± 6.800	0.041 (−2.602, 2.684) 0.976
Race/Ethnicity		
Mexican American	229 (13.631%)	Reference
Non-Hispanic White	211 (12.560%)	8.738 (−50.060, 67.536) 0.771
Non-Hispanic Black	663 (39.464%)	−34.482 (−75.589, 6.626) 0.100
Other races	577 (34.345%)	35.409 (−9.196, 80.013) 0.120
Hypertension		
No	889 (52.917%)	Reference
Yes	791 (47.083%)	−18.011 (−48.453, 12.431) 0.246
Diabetes		
No	1358 (80.833%)	Reference
Yes	322 (19.167%)	−42.363 (−83.505, −1.221) 0.044
Cigarette smoking		
No	873 (51.964%)	Reference
Yes	807 (48.036%)	−27.116 (−57.813, 3.581) 0.083
Alcohol drinking		
No	463 (27.560%)	Reference
Yes	1217 (72.440%)	−47.024 (−82.321, −11.727) 0.009
Creatinine (mg dL^{-1})	0.914 ± 0.323	−112.144 (−156.734, −67.554) <0.001
VOCs		
2MHA	62.441 ± 95.646	0.008 (−0.172, 0.187) 0.934
3MHA + 4MHA	432.674 ± 635.173	−0.006 (−0.031, 0.019) 0.638
AAMA	71.935 ± 84.598	−0.120 (−0.281, 0.040) 0.140
AMCC	266.542 ± 335.104	−0.055 (−0.094, −0.015) 0.007
ATCA	175.055 ± 195.817	−0.028 (−0.098, 0.041) 0.426
BMA	13.153 ± 27.569	−0.134 (−0.557, 0.290) 0.535
BPMA	11.297 ± 27.417	−0.145 (−0.539, 0.248) 0.469
CEMA	161.147 ± 183.864	−0.117 (−0.197, −0.036) 0.005
CYMA	41.312 ± 112.589	−0.157 (−0.279, −0.036) 0.011
DHBMA	365.583 ± 262.546	−0.089 (−0.144, −0.034) 0.001
2HPMA	62.895 ± 132.273	−0.082 (−0.174, 0.010) 0.082
3HPMA	475.402 ± 721.487	−0.024 (−0.046, −0.003) 0.029
MA	188.186 ± 219.189	−0.082 (−0.143, −0.020) 0.009
MHBMA3	12.382 ± 21.472	−0.828 (−1.537, −0.120) 0.022
PGA	264.243 ± 251.353	−0.095 (−0.158, −0.032) 0.003
HPMMA	530.622 ± 841.268	−0.020 (−0.039, −0.001) 0.037

[a] SD, Standard deviation; OR, odds ratio; CI, confidence interval; BMI, body mass index. VOCs, volatile organic compounds: 2MHA, urinary 2-methylhippuric acid; 3MHA + 4MHA, urinary 3- and 4-methylhippuric acid; AAMA, urinary *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine; AMCC, urinary *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine; ATCA, urinary 2-aminothiazoline-4-carboxylic acid; BMA, urinary *N*-Acetyl-*S*-(benzyl)-L-cysteine; BPMA, urinary *N*-acetyl-*S*-(n-propyl)-L-cysteine; CEMA, urinary *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine; CYMA, urinary *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine; DHBMA, urinary *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine; 2HPMA, urinary *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; 3HPMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine; MA, urinary mandelic acid; MHBMA3, urinary *N*-acetyl-*S*-(4-hydroxy-2-butenyl)-L-cysteine; PGA, urinary phenylglyoxylic acid; HPMMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine.

confounders to further investigate the association between VOCs and serum Klotho (Table 2).

As shown in Table 3, in the crude model, AMCC ($\beta = -0.055$, 95% CI: −0.100, −0.009), CEMA ($\beta = -0.117$, 95% CI: −0.199, −0.034), CYMA ($\beta = -0.157$, 95% CI: −0.292, −0.022), DHBMA ($\beta = -0.089$, 95% CI: −0.147, −0.031), 3HPMA ($\beta = -0.024$, 95% CI: −0.046, −0.003), MA ($\beta = -0.082$, 95% CI: −0.151, −0.012), MHBMA3 ($\beta = -0.828$, 95% CI: −1.537, −0.11), PGA ($\beta = -0.095$, 95% CI: −0.155, −0.034) and HPMMA ($\beta = -0.020$, 95% CI: −0.038, −0.002) were negatively associated with serum Klotho. All above associations in the crude model were statistically significant ($P < 0.05$). In the fully adjusted model (Model II), the significance of ATCA was observed. Other VOCs, including AMCC ($\beta = -0.051$, 95% CI: −0.099, −0.00), ATCA ($\beta = -0.081$, 95% CI: −0.160, −0.002), CEMA ($\beta = -0.098$, 95% CI: −0.184, −0.012), CYMA ($\beta = -0.186$, 95% CI: −0.330, −0.041), DHBMA ($\beta = -0.067$, 95% CI: −0.126, −0.008), 3HPMA ($\beta = -0.026$, 95% CI: −0.048, −0.004), MA ($\beta = -0.080$, 95% CI: −0.150, −0.010), MHBMA3 ($\beta = -0.894$, 95% CI: −1.644, −0.144), PGA ($\beta = -0.081$, 95% CI: −0.143, −0.019), HPMMA ($\beta = -0.020$, 95% CI: −0.039, −0.001), are all associated Klotho in the fully adjusted model ($P < 0.05$). After adjusting for confounders such as sex, age, and serum creatinine levels, it was found that the presence of these confounding factors obscured the association between ATCA and Klotho, amplified the negative correlation between AMCC, CEMA, DHBMA, MA, PGA and Klotho, and attenuated the negative correlation between CYMA, 3HPMA, MHBMA3 and Klotho. In summary, a negative correlation was confirmed between VOCs and Klotho.

It is important to note that the observed correlations varied across age groups. After conducting subgroup analyses by age (40–59 years and 60–79 years), differences in the significance of VOC components emerged (Table 4). For instance, ATCA reached only marginal significance ($P = 0.05$) in the general population. However, its significance became highly pronounced in the middle-aged population (40–59 years) ($P < 0.001$), whereas it was not statistically significant in the elderly group (60–79 years).

The relationship between VOCs and Klotho was influenced by age stratification, with varying levels of statistical significance across different age ranges. Specifically, VOCs, such as AMCC, ATCA, CYMA, 3HPMA, MA, MHBMA3, and PGA, were significantly correlated with Klotho in the middle-aged population. In contrast, only AAMA and DHBMA were significantly different in the elderly group. Notably, no single VOC demonstrated significance across either age group, underscoring the importance of considering age stratification when assessing the impact of VOCs on Klotho.

3.3 Weight distribution of urinary VOC concentrations on serum Klotho in the WQS model

The preceding analysis established a negative correlation between certain VOCs and Klotho, thus giving impetus to the notion that different VOCs may have distinct impacts on Klotho. Consequently, our focus was on identifying VOCs that play a dominant role in Klotho.

The WQS model ascertains the importance of VOCs by assigning variable-specific weights to them. In our study, we

Table 3 Multivariable linear regression models for the association between urinary VOC concentrations and serum Klotho concentrations ($N = 1680$)[a]

	β (95% CI), *P* value of serum Klotho concentrations, (pg. mL^{-1})		
Klotho	Crude model	Model I	Model II
2MHA	0.008 (−0.152, 0.167) 0.926	0.018 (−0.141, 0.177) 0.822	0.027 (−0.135, 0.190) 0.744
3MHA + 4MHA	−0.006 (−0.030, 0.018) 0.622	−0.005 (−0.028, 0.019) 0.705	−0.003 (−0.027, 0.022) 0.820
AAMA	−0.120 (−0.301, 0.060) 0.190	−0.118 (−0.298, 0.063) 0.202	−0.126 (−0.312, 0.059) 0.182
AMCC	−0.055 (−0.100, −0.009) 0.018	−0.058 (−0.104, −0.013) 0.011	−0.051 (−0.099, −0.003) 0.036
ATCA	−0.028 (−0.106, 0.050) 0.478	−0.062 (−0.140, 0.017) 0.122	−0.081 (−0.160, −0.002) 0.044
BMA	−0.134 (−0.687, 0.419) 0.635	−0.109 (−0.657, 0.440) 0.697	−0.064 (−0.615, 0.487) 0.820
BPMA	−0.145 (−0.701, 0.411) 0.609	−0.143 (−0.695, 0.409) 0.612	−0.197 (−0.752, 0.358) 0.487
CEMA	−0.117 (−0.199, −0.034) 0.006	−0.099 (−0.181, −0.016) 0.019	−0.098 (−0.184, −0.012) 0.025
CYMA	−0.157 (−0.292, −0.022) 0.023	−0.164 (−0.299, −0.029) 0.017	−0.186 (−0.330, −0.041) 0.012
DHBMA	−0.089 (−0.147, −0.031) 0.003	−0.073 (−0.131, −0.015) 0.013	−0.067 (−0.126, −0.008) 0.027
2HPMA	−0.082 (−0.197, 0.033) 0.163	−0.074 (−0.188, 0.040) 0.204	−0.070 (−0.185, 0.044) 0.228
3HPMA	−0.024 (−0.046, −0.003) 0.023	−0.024 (−0.045, −0.003) 0.025	−0.026 (−0.048, −0.004) 0.021
MA	−0.082 (−0.151, −0.012) 0.021	−0.082 (−0.151, −0.013) 0.020	−0.080 (−0.150, −0.010) 0.026
MHBMA3	−0.828 (−1.537, −0.119) 0.022	−0.860 (−1.566, −0.154) 0.017	−0.894 (−1.644, −0.144) 0.020
PGA	−0.095 (−0.155, −0.034) 0.002	−0.089 (−0.149, −0.028) 0.00	−0.081 (−0.143, −0.019) 0.010
HPMMA	−0.020 (−0.038, −0.002) 0.031	−0.020 (−0.038, −0.002) 0.029	−0.020 (−0.039, −0.001) 0.040

[a] Crude model: unadjusted. Model I = crude model + gender, age. Model II = model I + race/ethnicity, BMI, education level, hypertension, diabetes, cigarette smoking, alcohol consumption, and creatinine level. CI, confidence interval; BMI, body mass index. VOCs, volatile organic compounds: 2MHA, urinary 2-methylhippuric acid; 3MHA + 4MHA, urinary 3- and 4-methylhippuric acid; AAMA, urinary *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine; AMCC, urinary *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine; ATCA, urinary 2-aminothiazoline-4-carboxylic acid; BMA, urinary *N*-acetyl-*S*-(benzyl)-L-cysteine; BPMA, urinary *N*-acetyl-*S*-(n-propyl)-L-cysteine; CEMA, urinary *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine; CYMA, urinary *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine; DHBMA, urinary *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine; 2HPMA, urinary *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; 3HPMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine; MA, urinary mandelic acid; MHBMA3, urinary *N*-acetyl-*S*-(4-hydroxy-2-butenyl)-L-cysteine; PGA, urinary phenylglyoxylic acid; HPMMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine.

Table 4 Age-related stratified analysis for the association between urinary VOC concentrations and serum Klotho concentrations ($N = 1680$)[a]

	β (95% CI) *P* value		
Variables	General population	Middle-aged population (40–59 years)$N = 906$	Elderly population (59–79 years) $N = 774$
2MHA	0.044 (−0.118, 0.206) 0.593	0.038 (−0.260, 0.336) 0.803	0.000 (−0.234, 0.235) 0.999
3MHA + 4MHA	−0.001 (−0.025, 0.024) 0.954	−0.011 (−0.050, 0.029) 0.594	0.002 (−0.033, 0.038) 0.889
AAMA	−0.098 (−0.283, 0.086) 0.296	−0.056 (−0.289, 0.177) 0.638	−0.260 (−0.484, −0.035) 0.023
AMCC	−0.045 (−0.093, 0.003) 0.066	−0.070 (−0.130, −0.010) 0.023	−0.030 (−0.100, 0.040) 0.393
ATCA	−0.079 (−0.158, −0.001) 0.050	−0.195 (−0.301, −0.089) <0.001	0.088 (−0.028, 0.205) 0.136
BMA	−0.068 (−0.620, 0.483) 0.808	−0.155 (−1.076, 0.765) 0.741	0.022 (−0.434, 0.478) 0.926
BPMA	−0.191 (−0.747, 0.364) 0.500	−0.233 (−0.777, 0.310) 0.400	−0.092 (−0.678, 0.495) 0.759
CEMA	−0.095 (−0.181, −0.009) 0.030	−0.107 (−0.234, 0.019) 0.096	−0.098 (−0.214, 0.019) 0.101
CYMA	−0.158 (−0.301, −0.014) 0.031	−0.190 (−0.357, −0.023) 0.025	−0.213 (−0.432, 0.005) 0.055
DHBMA	−0.066 (−0.126, −0.007) 0.028	−0.056 (−0.136, 0.024) 0.172	−0.079 (−0.152, −0.007) 0.038
2HPMA	−0.072 (−0.187, 0.042) 0.2160	−0.132 (−0.265, 0.001) 0.056	−0.007 (−0.148, 0.133) 0.918
3HPMA	−0.022 (−0.044, −0.001) 0.048	−0.033 (−0.061, −0.004) 0.024	−0.016 (−0.057, 0.025) 0.433
MA	−0.071 (−0.141, −0.001) 0.049	−0.090 (−0.166, −0.013) 0.022	−0.052 (−0.171, 0.067) 0.398
MHBMA3	−0.762 (−1.506, −0.018) 0.045	−1.005 (−1.965, −0.044) 0.040	−0.822 (−2.152, 0.507) 0.225
PGA	−0.074 (−0.136, −0.013) 0.019	−0.087 (−0.164, −0.010) 0.026	−0.059 (−0.162, 0.043) 0.256
HPMMA	−0.017 (−0.036, 0.002) 0.078	−0.021 (−0.045, 0.003) 0.091	−0.022 (−0.056, 0.011) 0.193

[a] Adjusted for sex, race/ethnicity, BMI, education level, hypertension, diabetes, cigarette smoking, alcohol drinking, and creatinine level. CI, confidence interval; BMI, body mass index. VOCs, volatile organic compounds: 2MHA, urinary 2-methylhippuric acid; 3MHA + 4MHA, urinary 3- and 4-methylhippuric acid; AAMA, urinary *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine; AMCC, urinary *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine; ATCA, urinary 2-aminothiazoline-4-carboxylic acid; BMA, urinary *N*-acetyl-*S*-(benzyl)-L-cysteine; BPMA, urinary *N*-acetyl-*S*-(n-propyl)-L-cysteine; CEMA, urinary *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine; CYMA, urinary *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine; DHBMA, urinary *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine; 2HPMA, urinary *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; 3HPMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine; MA, urinary mandelic acid; MHBMA3, urinary *N*-acetyl-*S*-(4-hydroxy-2-butenyl)-L-cysteine; PGA, urinary phenylglyoxylic acid; HPMMA, urinary *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine.

conducted both negative and positive WQS experiments. In general population, the WQS index of mixed VOCs was negatively associated with serum Klotho levels ($\beta = -29.56$, $P = 0.026$), and the highest weighted VOCs were CEMA (25.8%), CYMA (22.3%), and ATCA (15.1%) (Fig. 1A). We also used a positive WQS model while assuming that an increase in mixed VOC concentration would increase serum Klotho ($\beta = -16.1$, $P = 0.200$) (weights shown in Fig. 1B), which was not statistically significant.

In the age-stratified analysis, it was observed that none of the WQS models for the elderly population (60–79 years) displayed any statistically significant impact (weights are shown in Fig. S3), implying that the joint effects of VOCs on Klotho may not be evident in the middle population (40–59 years). In contrast, the WQS model showed a significant statistical effect for the middle-aged population. The highest weights of VOCs were CYMA (47.3%), ATCA (29.1%), and CEMA (7.72%) in the negative WQS model ($\beta = -48.80$, 95% CI: −83.12, −14.08). In summary, the weight distribution in the middle-aged population was relatively concentrated in the WQS model. Therefore, it is reasonable to suggest that the impact of VOCs on Klotho in the middle-aged population may be primarily due to one or a few specific VOCs. The details of all the WQS models are shown in Table S2.

3.4 Dose–response and joint effects of VOCs on Klotho in the BKMR model

To perform a more in-depth assessment of the association between VOCs and Klotho, we utilized the BKMR method to study the joint effects and univariable dose–response relationship of VOCs on Klotho.

In the evaluation of the joint effects of VOCs, it was observed that all stratified groups exhibited a declining tendency in Klotho levels as the level of VOCs increased (Fig. 2). In the general population (Fig. 2A), when all exposures were fixed at their minimum values and then increased to the 90th percentile, the serum Klotho decreased by 45.06 pg ml^{-1} (95% CI: −1.92, −92.03). Similar results were observed in the age-stratified BKMR analysis. In the middle-aged population (40–59 years), a robust negative correlation was found in joint effects of VOCs on Klotho (Fig. 2B), as a statistically significant reduction in Klotho ($\beta = -14.03$, 95% CI: −27.49, −0.57) was observed when VOCs exceeded the 20th percentile. However, in the elderly population, the effect of VOCs on Klotho was weak (Fig. 2C). As VOCs increased, the changes in Klotho ($\beta = -24.99$, 95% CI: −82.75, 32.76 at the 90th percentile of VOCs) did not reach statistical significance. Moreover, we explored the potential interactions between VOCs in the middle-aged population (Fig. S4), and no obvious interaction was observed. In the single-exposure effect, we explored the univariate exposure-response functions of VOCs and Klotho in the general population (Fig. S5). A negative exposure–response relationship was observed between AMCC, ATCA, and CEMA. CYMA, 3HPMA, PGA, and MHBMA3. Furthermore, we found a nonlinear relationship between VOCs and Klotho in the elderly population (Fig. S6). However, this relationship could not be confirmed because of the large variance. In the middle-aged population, only ATCA showed a significant negative relationship (Fig. S7).

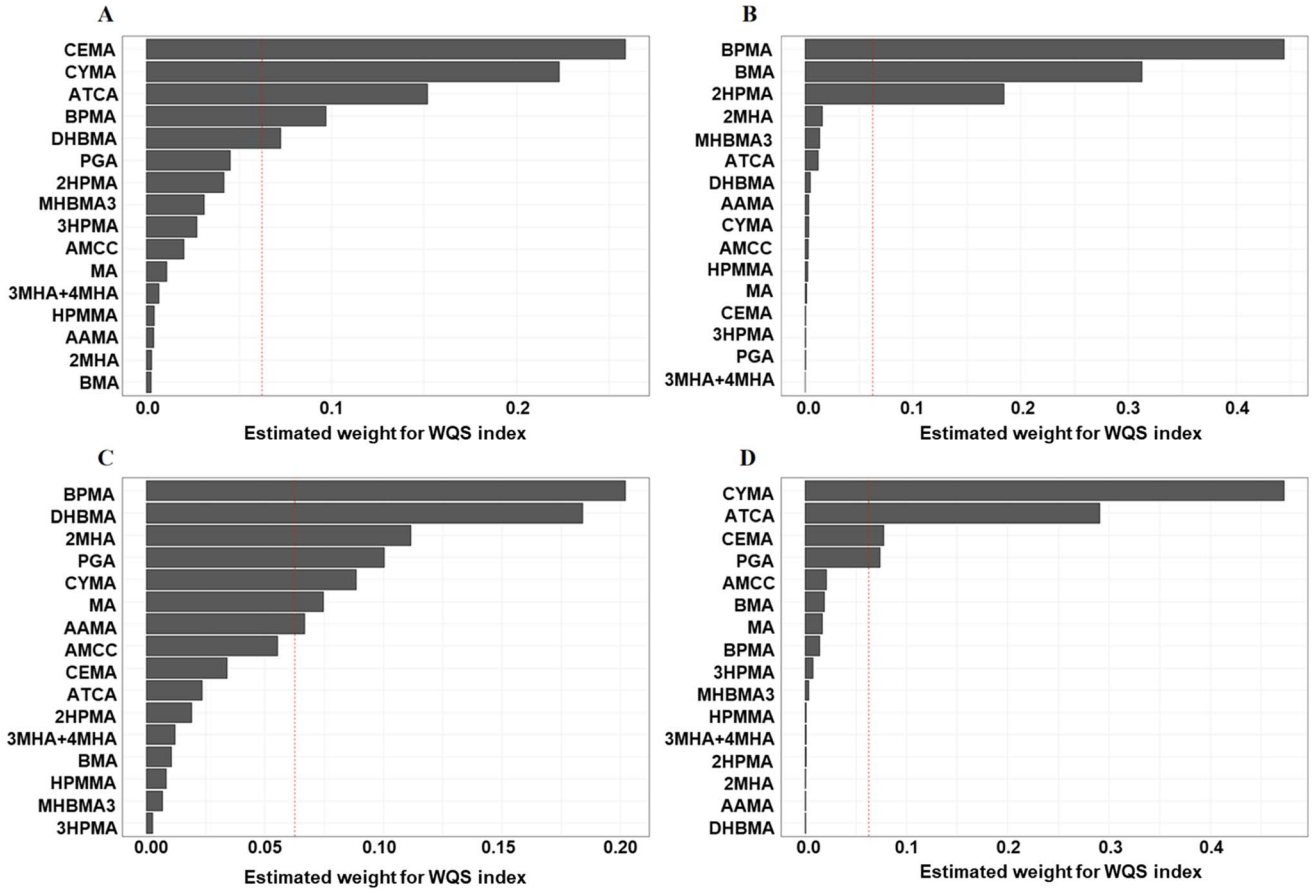

Fig. 1 WQS model regression index weights in different populations. (A) Negative WQS model regression index weights in the general population, *P* value of wqs < 0.05. (B) Positive WQS model regression index weights in the general population, *P* value of wqs > 0.05. (C) Negative WQS model regression index weights in the elderly population, *P* value of wqs > 0.05. (D) Negative WQS model regression index weights in the middle-aged population, *P* value of wqs < 0.05.

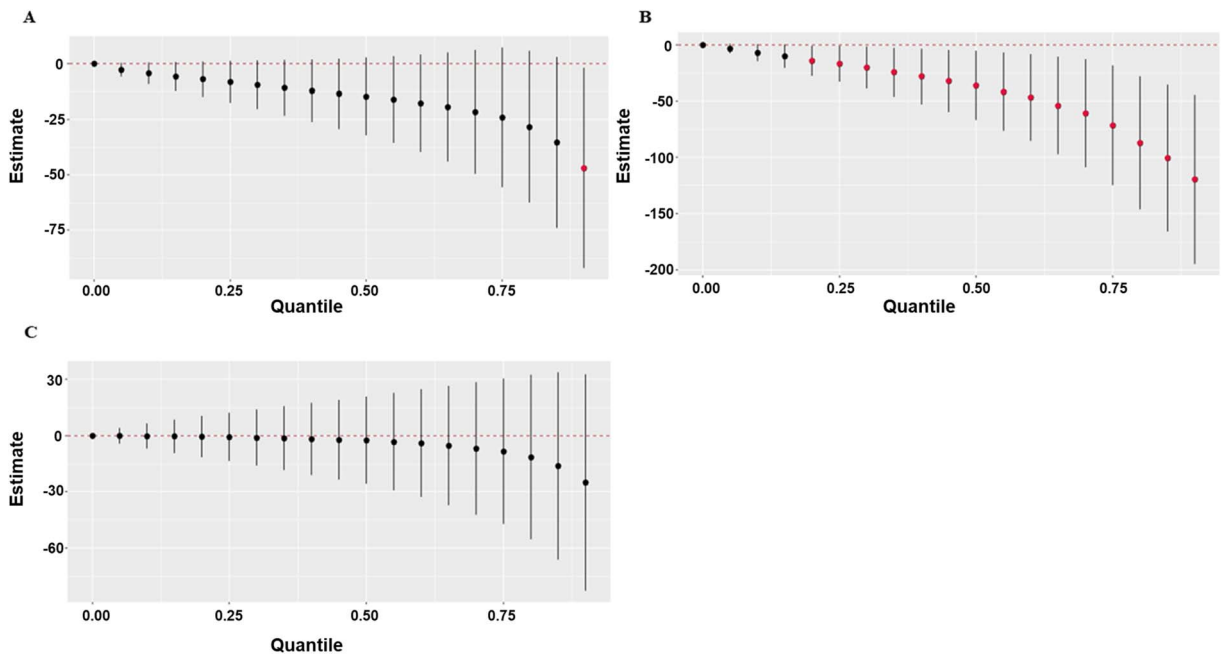

Fig. 2 Joint effects (95% confidence interval) of urinary VOCs on serum Klotho were estimated by Bayesian Kernel Machine Regression (BKMR) models. All the chemicals at particular percentiles were compared to all the VOCs at their 0th percentile. Abbreviation: BMKR: Bayesian kernel machine regression. (A) General population; (B) Elderly population; (C) Middle-aged population. Red dots indicate that the estimate value of Klotho is significant (*P* < 0.05).

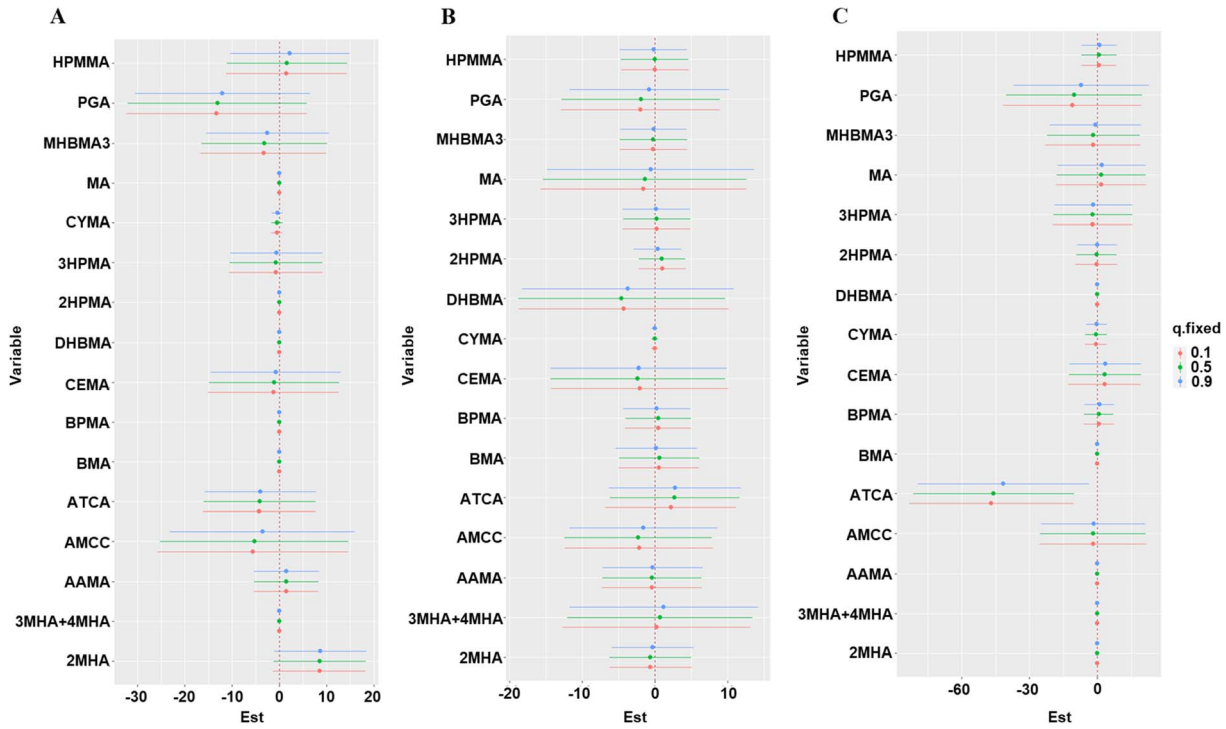

Fig. 3 Associations between single urinary VOCs and serum Klotho were estimated by Bayesian Kernel Machine Regression (BKMR) in the total population and different subgroups, when other all metals were held at their corresponding 10th (red), 50th (green) or 90th (blue) percentiles, respectively. (A) General population; (B) Elderly population; (C) Middle-aged population.

We also estimated the contribution of individual VOC exposure to the joint effect. Fig. 3 compares the risks when a single VOC is at the 75th percentile with those when it is at its 25th percentile, where all of the remaining VOCs are fixed at a particular quantile. Only ATCA in the middle-aged population (40–59 years) showed statistically significant dose–response differences (q. Fixed = 0.5 $\beta = -45.88$, 95% CI: -85.45, -10.30) (Fig. 3C). Based on our BKMR analysis, ATCA may emerge as the primary VOC responsible for the decline in Klotho levels, and it is mainly detrimental to the middle-aged population. All BKMR models' posterior inclusion probabilities (PIPs) are shown in Table S3.

3.5 Mediation analysis of liver-related biomarkers in the effect of VOCs on Klotho

We further investigated potential molecular factors mediating the influence of VOCs on serum Klotho levels. Through parallel mediation analyses, we assessed the mediation effects of liver-related biomarkers on the association between VOCs and serum Klotho levels. In the general population, direct effects were the main mechanism by which mixed VOCs affected Klotho, with indirect effects accounting for a small portion of the total effect (TB: 5.7%) (Fig. 4A). Similar findings were observed in the middle-aged population (Fig. 4B). We also performed a mediation analysis for individual VOCs in the general population, discovering significant total effects for certain VOCs, including AMCC, CYMA, CEMA, 3HPMA, MA, MHBMA3, PGA, and HPMMA. Most of these VOCs demonstrated a direct effect, and a TB-mediated effect was present in many cases (Table S4 and 5).

Given the increased susceptibility of the middle-aged population to VOCs, analyzing the mediation effects in this group could yield more insightful results. WQS analyses identified ATCA and CYMA as the primary VOCs affecting the Klotho levels in this age group. Therefore, we conducted a more in-depth investigation of the mediating effects of ATCA and CYMA (Fig. 4C and D). The results indicated that CYMA did not have a significant mediating effect. However, our analysis found a substantial mediation effect for ATCA, with a proportion as high as 31.6%, primarily mediated by TB (5.3%) and AST (26.3%). This suggests that inhibiting the mediation pathway of ATCA could have significant preventive benefits, potentially reducing ATCA-induced damage to Klotho by nearly 30% through liver protection.

4. Discussion

VOCs, which are air pollutants, have a significant impact on human health. When the VOCs in the room reach a certain concentration, people will experience headaches, nausea, vomiting, fatigue, *etc.*, resulting in impairment of the human liver, kidney, brain, and nervous system, causing memory loss and other serious consequences. During surgery, VOCs are directly derived from medical devices and inhaled from the air in the intensive care unit. During the submucosal dissection phase of endoscopic submucosal dissection, various carcinogenic and non-carcinogenic VOCs were detected at levels

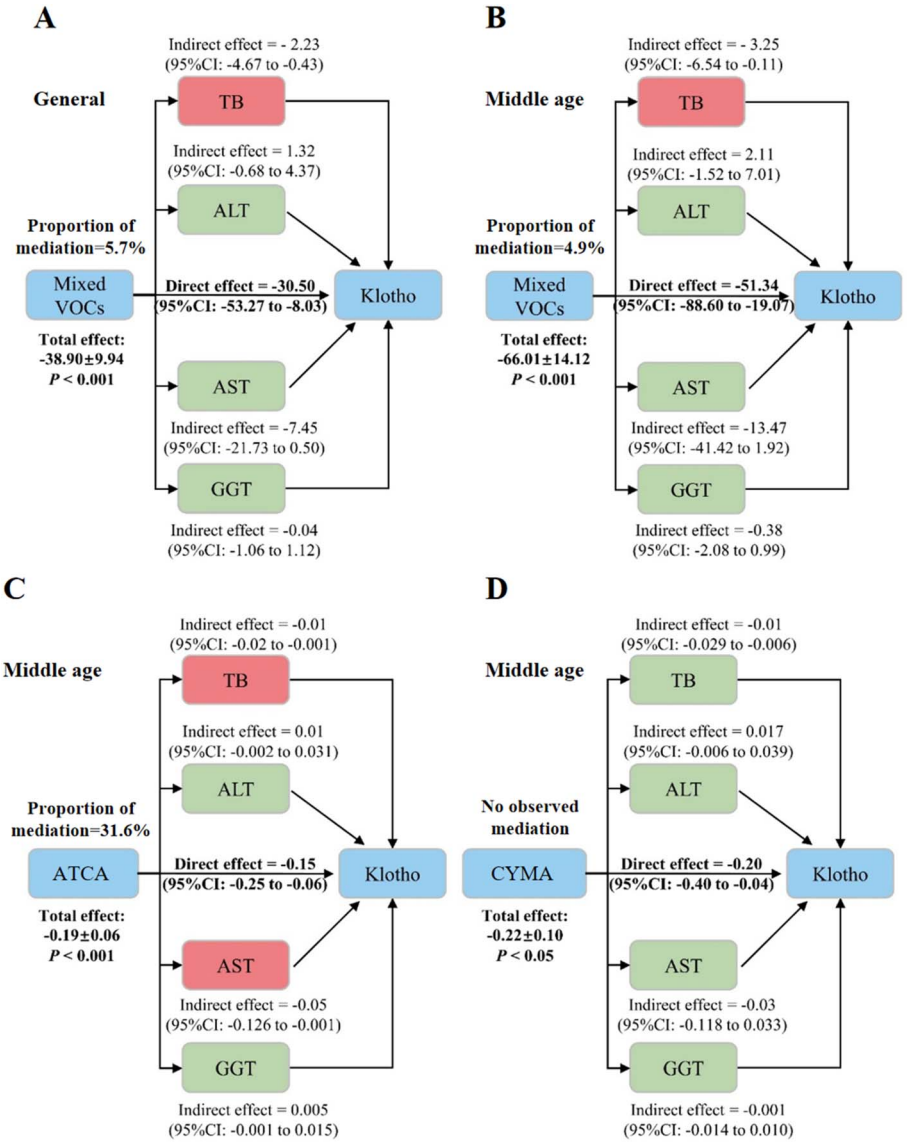

Fig. 4 Estimated proportion of mediation in VOCs and Klotho. (A) Mixed VOCs in the general population; (B) mixed VOCs in the middle-aged population; (C) mediation of ATC in the middle-aged population; (D) mediation of CYM in the middle-aged population. Mediation models were adjusted for sex, age, race/ethnicity, education, body mass index, education level, hypertension, diabetes, cigarette smoking, alcohol drinking and serum creatinine. IE, the estimate of the indirect effect; TE, the estimate of the total effect; proportion of mediation = IE/TE. Red boxes indicate statistical significance.

exceeding the reference "safe" values.[34] Therefore, more scientific research is required to study the correlation between VOCs and human health. Klotho is a pivotal protein in health assessment, as it plays a vital role in the anti-aging process and cancers. The FGF-Klotho endocrine system plays a crucial role in the pathophysiology of aging-related disorders and cancers, including colorectal cancer, diabetes, and cardiovascular disease.[35] This work investigated the joint effects of VOC exposure (measured urinary VOCs) on serum Klotho in the United States.

In this study, 16 VOCs were selected for further analysis. Through single factor analysis, we found that nine compounds, AMCC, CEMA, CYMA, DHBMA, 3HPMA, MA, MHBMA3, PGA, and HPMMA, may be correlated with serum Klotho concentration. After further adjusting for other covariables and correcting for serum creatinine (an indicator that may affect the human excretion of VOCs), we found that ATCA also had a significant impact on serum Klotho. If the results of the single factor analysis and multi-factor analysis of this compound were inconsistent, we suspected that the effect of ATCA was masked by other variables. After adjusting for other possible confounding factors, an effect of ATCA was observed.

In summary, after adjusting for potential confounding variables such as serum creatinine and alcohol consumption, we identified 10 potential VOCs (AMCC, ATCA, CEMA, CYMA, DHBMA, 3HPMA, MA, MHBMA3, PGA, and HPMMA) that show a significant negative correlation with Klotho levels. This suggests that an increase in VOC exposure can significantly

reduce the Klotho levels. However, owing to collinearity among some of these VOCs, the effects of a few VOCs may be inaccurately estimated. Despite this, it is important to note that collinearity does not generate spurious correlations. Therefore, the evidence supporting a negative correlation between VOCs and Klotho levels remains valid.

To identify the specific VOCs that play a significant role and to elucidate their mechanisms of action, we used WQS analysis to rank their importance. Our findings indicated that only a limited number of models in the negative direction, specifically the total population and the 40–59 years age group, exhibited statistical significance. Within the 40–59 age group, the WQS weights were highly concentrated, primarily highlighting the importance of CYMA and ATCA. However, when the elderly population was included, CEMA became more prominent. It is crucial to note that this does not imply that CEMA is the most influential VOC among the elderly population. Upon analyzing the elderly population in isolation, the WQS model effect was not significant, suggesting a lack of reliable evidence linking VOC exposure to Klotho levels in this age group. In the general population, the concentration of importance notably decreased compared with that in the middle-aged population. This discrepancy may be attributable to the inclusion of data from older individuals. When the model was fitted to the additional but non-significant data, the weights of each VOC were recalculated. The presence of confounding data may have led to an underestimation of the importance of CYMA and ATCA while overestimating the importance of other, less relevant VOCs, such as CEMA. Therefore, based on the results of the WQS analysis, we propose that the impact of VOCs on Klotho levels is primarily evident in the middle-aged population, with CYMA and ATCA being the predominant contributors.

Consistent findings were also observed in the BKMR analysis results. As illustrated in Fig. 2, the sensitivity to VOCs was most pronounced in the middle-aged population. However, this strong correlation was less apparent in the overall population analysis, further supporting the notion that the impact of VOCs on Klotho levels was primarily evident in the middle-aged population. Importantly, there was no evidence of a ceiling effect on the impact of VOCs on Klotho levels. However, the negative influence of VOCs continued to intensify, even at higher concentrations. Notably, at higher quantiles of VOC exposure, an increase in the same percentile range led to a more substantial decrease in Klotho levels than at lower quantiles. This finding suggests that timely interventions to reduce VOC exposure in individuals with high levels of exposure could yield significant health benefits.

Similarly, BKMR analysis was employed to examine the effects of various VOC components while attempting to avoid any potential collinearity. Consistent with the findings of the WQS analysis, we observed that only the concentration difference effect of ATC was found in the middle-aged population. This finding suggests that with fixed concentrations of other pollutants, a significant change in Klotho only occurred when ATC changed from the 25th to 75th percentile, whereas such an effect was not observed for other VOCs. To further elucidate the crucial role of ATC in VOCs, we compared the overall risk (75th–25th) of all VOCs with the risk of single ATC (75th–25th), resulting in a value of 85.40%, suggesting that ATC contributes significantly to the impact of VOCs on Klotho.

Contrary to our expectations, the effect of CYMA was not significant in any age group, which may be partially due to the collinearity problem with many variables. This finding does not contradict the WQS analysis results, which showed the high importance of ATCA. As mentioned earlier, the false high value of CEMA and collinearity of variables could influence the estimation of variable importance in linear models such as WQS. Therefore, the WQS model acts as a reference for considering collinearity issues when interpreting the variable importance. It should be emphasized that we do not necessarily reject the possibility of CYMA's effect on Klotho, but our current analyses do not strongly support this conclusion. Finally, the comparison between ATCA and CYMA showed that ATCA was the most significant component of VOCs for Klotho regulation, as it remained more stable across the analyses conducted in this study, a finding that enhances confidence in the results for ATCA.

Previous studies[36] have reported the role of ATCA in cardiovascular diseases. A previous study[37] reported that ATCA increases DNA oxidative stress damage. Xu *et al.*[38] reported that Klotho can inhibit oxidative stress. Our findings reveal that ATCA reduces Klotho levels, consistent with the literature. To elucidate how VOCs affect Klotho, we performed mediation analysis. Our findings revealed that, regarding the mixed VOC effect on Klotho, TB played a robust mediating role, albeit with a proportion of only approximately 5%. Notably, the mediated proportion of ATCA was as high as 31%, suggesting a more substantial contribution of ATCA to Klotho regulation. The proportion of TB remains stable at approximately 5%, with most of it mediated by AST.

Bilirubin has been recognized as an important antioxidant and anti-inflammatory factor.[39,40] Bilirubin exerts a protective effect on oxidant and inflammatory insults through several processes.[41,42] It has been reported that the bilirubin system in aging and age-related diseases is dysregulation.[42,43] In addition, it also revealed negative relationship between TB levels and frailty among middle-aged and elderly individuals.[44] Therefore, TB may be an anti-aging factor. The VOC metabolites have been proven to independently cause oxidative damage both *in vivo* and *in vitro*.[45] The VC metabolites also lead to a significant increase in neutrophil infiltration and a significant rise in the expression of pro-inflammatory cytokines.[46] We speculate that excess ROS generated by VOC metabolism may disturb bilirubin homeostasis and further deteriorate oxidative stress *via* activation of redox–sensitive pathways, finally suppressing Klotho transcription and expression. These results indicate that the use of hepatoprotective agents may mitigate the damage caused by ATCA in Klotho to some extent. Given prior literature indicating ATCA's importance as the most significant VOC, we recommend targeting the exposure index of ATCA and implementing liver protection measures for individuals with high exposure levels to reduce the harm caused by ATCA. Despite an incomplete understanding of the underlying mechanisms, our study elucidated the toxicological consequences of VOCs on Klotho and their potential modes of action.

Due to the age group (40–79 years) from NHANES, younger populations or those outside the U.S. were not included in this study. This study included patients aged 40–79 years from the NHANES database, as individuals in this age group are more likely to undergo surgical procedures and, therefore, better represent the surgical patient population.[47,48] Investigating the relationship between VOCs and Klotho in this cohort is important. Although the NHANES database is limited to the U.S. population, its large sample size and diverse racial and ethnic representation confer a high degree of reliability and generalizability to the findings. Nevertheless, future studies should be conducted across different age groups, countries, and ethnicities to further validate these results.

To investigate the relationship between VOCs and Klotho in a relatively large population, we utilized four statistical methods that have progressively delved into the topic. Our analyses successfully identified ATCA as the most hazardous VOC and explored its influence on Klotho *via* mediation by liver-related biomarkers. However, this study had several limitations. First, urinary VOC measurements may not accurately represent an individual's typical exposure level. Additionally, the VOCs detected in the NHANES dataset may not be directly attributable to specific sources, such as surgical smoke, which limits the ability to draw definitive conclusions about exposure in clinical contexts. Second, despite controlling for various potential confounding covariates, residual and unmeasured confounders as well as measurement errors could still introduce bias into our analysis. The self-reported nature of certain covariates, including smoking and alcohol consumption, may also be subject to recall or reporting bias. Third, our findings suggest that a decline in renal function may affect Klotho levels and that VOC excretion could be directly influenced by renal function. Although we adjusted for kidney function using blood creatinine levels, future studies may benefit from incorporating more specific indicators, such as estimated glomerular filtration rate (eGFR) or urinary albumin levels, to further refine these analyses. Finally, regarding occupational exposure, higher VOC levels in surgical smoke give surgeons more opportunity for occupational exposure than physicians, but such data are not available in the database to compare the VOC levels in surgeons and physicians, which need to be further validated. While our study utilized the NHANES database to examine the relationship between urinary VOCs and serum Klotho levels in the general population, it highlights broader implications for public health, considering both medical and industrial sources of VOC exposure. In the future, we suggest designing longitudinal cohorts with repeated measurements of VOC metabolites, α-Klotho, and aging-related endpoints (*e.g.*, frailty and cardiovascular events) to clarify temporal associations. In addition, we also propose combining animal models with molecular assays to validate the biological plausibility of VOC-induced α-Klotho downregulation, strengthening the causal inference framework.

5. Conclusion

In summary, this study aimed to demonstrate an inverse relationship between VOC exposure and serum α-Klotho levels in American adults, specifically in the age group of 40–59 years. These results suggest a potential role for VOC exposure in aging and carcinogenesis. Furthermore, this study identified the significant impact of ATCA among VOCs and its mediation through liver-related biomarkers.

Statement of ethical compliance

All the data of live subjects (human urine and serum) in our study are the from National Health and Nutrition Examination Survey (NHANES) database. The National Health and Nutrition Examination Survey (NHANES) is a population-based cross-sectional survey designed to collect information on the health and nutrition of the U.S. household population. Data were collected through structured interviews at home, physical examination at a mobile center, and laboratory tests, and the survey was designed with multistage probability sampling. The protocol of NHANES was approved by the National Centre for Health Statistics (NCHS) ethics review board, and all participants provided written informed consent.

Author contributions

Conceptualization: Ding Zeng and Shuaifei Ji; data curation: Bochen An and Yiming Bi; formal analysis: Yiming Bi and Jingcheng Zhou; methodology: Yiming Bi and Bochen An; software: Yiming Bi and Lixin Yu; supervision: Yiming Bi and Ding Zeng; validation: Yiming Bi and Jingcheng Zhou; visualization: Yiming Bi and Shuaifei Ji; writing – original draft: Shuaifei Ji, Bochen An and Yiming Bi; writing – review & editing: Ding Zeng and Shuaifei Ji.

Conflicts of interest

The authors declare no actual or potential conflicts of interest.

Abbreviations

SD	Standard deviation
OR	Odds ratio
CI	Confidence interval
BMI	Body mass index
VOCs	Volatile organic compounds
2MHA	Urinary 2-methylhippuric acid
3MHA + 4MHA	Urinary 3- and 4-methylhippuric acid
AAMA	Urinary *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine
AMCC	Urinary *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine
ATCA	Urinary 2-aminothiazoline-4-carboxylic acid
BMA	Urinary *N*-acetyl-*S*-(benzyl)-L-cysteine
BPMA	Urinary *N*-acetyl-*S*-(n-propyl)-L-cysteine
CEMA	Urinary *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine
CYMA	Urinary *N*-acetyl-*S*-(2-cyanoethyl)-L-cysteine
DHBMA	Urinary *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine
2HPMA	Urinary *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine

3HPMA	Urinary *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine
MA	Urinary mandelic acid
MHBMA3	Urinary *N*-acetyl-*S*-(4-hydroxy-2-butenyl)-L-cysteine
PGA	Urinary phenylglyoxylic acid
HPMMA	Urinary *N*-acetyl-*S*-(3-hydroxypropyl-1-methyl)-L-cysteine

Data availability

Data will be made available on request.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5va00270b.

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