

Influencing Trend and Extent of Combined Exposure Levels of Total Arsenic and Inorganic Arsenic on Arsenic Methylation Capacity Among University Students: Findings from Bayesian Analysis Using Kernel Machine Regression

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Abstract

The arsenic (As) methylation capacity is an important determinant of the susceptibility to arsenic-related diseases. Total As (TAs) or inorganic As (iAs) was reported to associate with As methylation capacity individually, however, influencing trend and extent of their combined exposure levels on methylation capacity remains poorly understood. We measured urinary concentrations of iAs, monomethylarsonic (MMA), and dimethylarsinic (DMA) acids using HPLC-HG-AFS, and calculated the primary (PMI: (MMA+DMA)/TAs) and secondary (SMI: DMA/(MMA+DMA)) methylation capacity indexes in 209 university students in Hefei, China, a non-As endemic area. Subjects were given with a standardized questionnaire to inquire their sociodemographic characteristics. Bayesian kernel machine regression (BKMR) analysis was used to estimate the association of lnTAs and lnIAs levels with methylation indices (ln%MMA, ln%DMA, lnPMI, lnSMI). The median concentration of iAs, MMA and DMA was 1.22, 0.92 and 12.17 µg/L, respectively; the proportions of iAs, MMA and DMA were 8.76%, 6.13% and 84.84%, respectively. Females had higher %DMA and lower %MMA, while males had lower %DMA and higher %MMA. The combined levels of lnTAs and lnIAs showed monotonic decrease in change of ln%DMA and lnSMI other than ln%MMA, additionally, change in ln%PMI was decreased only when levels of lnTAs and lnIAs were larger than their 60th percentiles compared to they were at 50th percentile. With regard to single exposure level, the lnTAs showed positive correlation with ln%DMA, lnPMI, lnSMI when lnIAs was set at a specific level; while lnIAs showed negative correlation with ln%DMA, lnPMI, lnSMI when lnTAs was set at a specific level; and all the dose-response relationships were nonlinear. Our results suggested that combined levels of TAs and iAs played an important role in reducing As methylation capacity, especially iAs; and the reduction only occurred when TAs and iAs were up to a certain combined level.

1. Introduction

Inorganic arsenic (iAs) is a geogenic contaminants widely distributed in the environment and commonly identified in the groundwater, and the WHO stipulated that the guideline value of iAs concentration levels in drinking water is less than 10 µg/L (WHO 2017). However, more than 70 countries have been reported natural As pollution of drinking water, posing a serious health hazard to approximately 150 million people worldwide (Brammer and Ravenscroft 2009). China is one of the most populous countries in the world and faces groundwater iAs pollution of enormous proportions from both industrial and natural sources (Rodríguez-Lado and Sun et al. 2013). IAs exposure could increase the risk of many cancers, such as skin cancer, lung cancer, and bladder cancer (Mochizuki 2019; Palma-Lara and Martínez-Castillo et al. 2020), additionally, iAs exposure is also a possible cause of other non-cancer health problems, such as neurological, cardiovascular, respiratory, urinary, genital and metabolic diseases (Guha Mazumder 2008; Schuhmacher Wolz and Dieter et al. 2009; Mochizuki 2019). Therefore, there are growing concerns about the health problems caused by iAs exposure.

As exists in different chemical forms in nature, the two major iAs species are As^{\square} and As^{\square} . In humans, the primary iAs metabolic pathway is methylation, which could take place in most organs of the body, but mainly in the liver (Drobná and Walton et al. 2010; Rahman and Hassler 2014). At present, the metabolism of iAs in the human body has been suggested through two pathways (Sattar and Xie et al. 2016; Mochizuki 2019). However, whatever the metabolic pathway, most of the As and its metabolites are excreted in urine, and a typical urinary As methylation trait contains 10%-30% iAs, 10%-20% MMA, and 60%-80% DMA (Wei and Yu et al. 2016). Therefore, urinary As metabolite concentrations and their proportions are considered as reliable biomarkers of As exposure and As methylation capacity. Epidemiological studies suggested that high levels of iAs and MMA in urine and low levels of DMA were associated with the incidence of As-related diseases, which included some cancers, heart disease, skin lesion and preschool children's developmental delay and so on (Chen and Wu et al. 2013; Hsu and Tsui et al. 2017; Rasheed and Kay et al.

2018; Lin and Chung et al. 2019). Consequently, growing interest has been focused on the factors that influence As methylation capacity.

As exposure level was one of important determinants for As methylation capacity, so some previous studies have explored effect of TAs and/or iAs level on As methylation capacity among populations in As endemic area by linear regression analysis (Torres-Sánchez and López-Carrillo et al. 2016; Yang and Chai et al. 2017; Olmos and Astolfo et al. 2021), however, in fact, the relationship between them might not be linear, and this relationship among populations in a non-As endemic area is not clear. Additionally, iAs, as a part of TAs in urine, correlates significantly with TAs, they should not be separated when assessing their influence on As methylation capacity. Therefore, a more appropriate analysis method should be performed to explore this relationship. Bayesian kernel machine regression (BKMR) analysis, as a new statistical method, could give a more accurate relationship between one variable and an outcome while controlling an other variable at a specific level which we also wanted to study (Bobb and Valeri et al. 2015). In addition, it could evaluate a nonlinear relationship between exposure and outcome (Bobb and Valeri et al. 2015).

In the present study, we conducted a cross-sectional study to investigate As methylation capacity among some university students in a non-arsenic endemic area, and observe the influencing trend and extent of combined exposure levels of TAs and iAs on As methylation capacity.

2. Method

2.1. Study participants

We randomly recruited volunteers including postgraduates and undergraduates from a medical university in Anhui province, China; in addition, the volunteers must meet the following conditions strictly to ensure that diet and water were major sources of As exposure: eating only in the university canteen for the last two weeks, drinking only municipal water for the last two weeks and not taking medicine or herbs which may contain As for the last two weeks. In total, we enrolled 244 volunteers in the present study and 209 valid participants completed the entire study who completed the questionnaires validly and provided effective urine samples. The study was approved by the ethics committee of Anhui Medical University; in addition, oral and written consent was obtained from all subjects before this study begins.

2.2. Urine Samples collection and pretreatment

Morning midcourse urine samples from the participants after getting up and not having breakfast or doing exercise were collected and placed into 10 mL polypropylene plastic tubes respectively. Then the urine samples were sent for determination in time or stored at -80°C when they could not be detected in time. The determination must be completed within one week after sampling to prevent the transformation of As speciations in urine. The fresh urine was filtered directly to remove impurities with microporous membrane ($0.45\mu\text{m}$ i.d.), and the frozen urine was balanced at 4°C for 2 hours before filtration. The urine after filtration could be determined directly.

2.3. Standards and reagents

All the glass needed for the experiment should be soaked in HNO_3 solution (9:1) for 24 hours, then rinsed with water repeatedly, and washed with ultrapure water finally. Up-s (ultrapure) grade HNO_3 was purchased from Suzhou Crystal Clear Chemical Corporation (Suzhou, China). Four standard stock solutions including As^{III} (GBW08666), As^{V} (GBW08667), MMA^{V} (GBW08668) and DMA^{V} (GBW08669) were all purchased from National Institute of Metrology (Beijing, China). The concentration of the above four standard stock solutions was 10.0 mg/L , respectively. The mixed standard working solutions were prepared for each run by serial dilution of the above four standard stock solutions using ultrapure water. Finally, six-point calibration curve including $0.0\text{ }\mu\text{g/L}$, $2.0\text{ }\mu\text{g/L}$, $5.0\text{ }\mu\text{g/L}$, $10.0\text{ }\mu\text{g/L}$, $20.0\text{ }\mu\text{g/L}$ and

50.0 µg/L for each As speciation was used in the present study. The other reagents were HCl (GB/T 622–2006, Sinopharm Chemical Reagent Co. Ltd), KBH_4 (Fuchen (Tianjin) Chemical Reagent Co. Ltd, Tianjin, China), KOH (Tianjin Guang Fu Technology Development Co. Ltd, Tianjin, China) and $(\text{NH}_4)_2\text{HPO}_4$ (Sinopharm Chemical Reagent Co. Ltd), which were all high-grade pure. The experimental water was ultrapure water (18.2 MΩ.cm).

2.4. As speciations determination

The As speciations in urine was determined by high performance liquid chromatography-hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS) according to the method from Guo et al (Guo and Li et al. 2019). The EClassical 3100 High Efficiency Liquid Phase Separation System (Dalian Elite Analytical Instruments Co., Ltd.) with PRP-X100 anion exchange chromatography column (250.0 mm × 4.1 mm × 10.0 µm, Hamilton, Switzerland) was used to separate different As speciations, AFS-8530 atomic fluorescence spectrophotometer (Beijing Haiguang Instrument Co., Ltd.) was used to quantify the four As speciations. The injection volume of the sample was 100 µL. The 7% HCl (GB/T 622–2006, Sinopharm Chemical Reagent Co. Ltd) was used as carrier fluid, 20 g/L KBH_4 (Fuchen (Tianjin) Chemical Reagent Co. Ltd, Tianjin, China) and 3.5 g/L KOH (Tianjin Guang Fu Technology Development Co. Ltd, Tianjin, China) were used as reductants and 15 mmol/L $(\text{NH}_4)_2\text{HPO}_4$ (Sinopharm Chemical Reagent Co. Ltd) was used as the mobile phase. Additionally, 10% HCOOH was used to adjust the pH of the mobile phase. The detailed instrument parameters are shown in Table S1.

2.5. Stability of As speciation

The limit of detection (LOD) of As speciations ranged from 0.218 µg/L to 0.738 µg/L. The recoveries of added standard were ranged from 83.88–96.63% (Table S2). The results of precision test and stability test were shown in Table S3 and Table S4, respectively.

2.6. Determination of TAs in food

In order to explain the source of As in the urine among this group of university students, we purchased a batch of food and drinking water from the canteen and detected the TAs in these samples by hydride generation-atomic fluorescence spectrometry (HG-AFS). Before determination, the weight of the sample was weighed firstly, and then these samples was digested with the prepared digestive solution ($\text{HNO}_3 : \text{H}_2\text{O}_2 = 3:1$) for more than 24 hours. After the sample was completely digested, the acid was heated and driven out with the graphite furnace digestion instrument (LabTech, America). The 5% $\text{CH}_4\text{N}_2\text{O}_4\text{S}$ and 5% $\text{C}_6\text{H}_8\text{O}_6$ were used as pre-reduced catalyst, 5% HCl was used as carrier fluid, 20 g/L KBH_4 and 3.5 g/L KOH were used as reductants.

2.7. Statistical analysis

In our present study, As^{V} in all urine samples could not be detected, so iAs represented merely As^{III} . Concentrations of iAs, MMA, DMA and TAs were used as urine As speciation indicators. The percentages of iAs (%iAs), MMA (%MMA) and DMA (%DMA) were defined as $\text{iAs}/\text{TAs} \times 100\%$, $\text{MMA}/\text{TAs} \times 100\%$ and $\text{DMA}/\text{TAs} \times 100\%$, respectively. Two methylation indices, PMI ($(\text{MMA} + \text{DMA})/\text{TAs}$) and SMI ($\text{DMA}/(\text{MMA} + \text{DMA})$) were also calculated to assess As methylation capacity (Ren and Xu et al. 2019).

The study participants' demographic characteristics were presented as the Mean ± standard deviation (SD) or number (frequency, %), and the information on the daily food matching and weekly exercise was presented as number (frequency, %). The concentration distributions of iAs, MMA, DMA and TAs were described using selected percentiles. The linear regression models were performed to compare the differences of %iAs, %MMA, %DMA, TAs, PMI and SMI between categories of demographic variables after the %iAs, %MMA, %DMA, TAs, PMI and SMI were natural logarithm transformed.

Bayesian kernel machine regression (BKMR) analysis was used to estimate the association of InTAs and IniAs levels with methylation indices (In%MMA, In%DMA, InPMI, InSMI). BKMR could not only study the linear or nonlinear effects of single factors on methylation indices, but also analyze the possible combined effects among multiple factors (Bobb and Valeri et al. 2015). The exposure-response function does not demand a priori specification and is modeled neatly, which are its main features. The BKMR model is indicated by the equation: $Y_i = h\{TAs_i, iAs_i\} + \beta_q Z_i + e_i$ (Liang and Han et al. 2020). The function $h\{\}$ in the equation is an exposure-response function, which can accommodate nonlinearity and/or interaction between different exposure levels (InTAs, IniAs) in mixture; and $Z = Z_1, Z_2, \dots, Z_q$ was q potential confounders including gender, age, grade, daily food matching, fruit frequency in the last week, time being spent on strenuous physical activity in the last week; and the Gaussian kernel function is applied to simulation researches and real-life scenarios. Models were run up to 10,000 iterations. Firstly, the cumulative effect of combined exposure level to InTAs and IniAs on urinary As methylation indices of university students was evaluated. Secondly, when InTAs or IniAs was fixed at the 25th, 50th, 75th percentile, the effect of another factor level on the As methylation indices were calculated. Finally, we investigated univariate exposure-response function and 95% confidence intervals for TAs and iAs with another one fixed at the median.

All data were statistical analyzed using SPSS for Windows (version 22.0; SPSS UK Ltd., Surrey, UK) and R (version 4.0.5, package “bkmr” and “ggplot2”), and a two-sided p -value < 0.05 was considered statistically significant for all the tests unless otherwise indicated.

3. Results

In our present study, 209 university students completed the questionnaires validly and provided effective urine samples, which included 116 (55.50%) males and 93 (44.50%) females. The mean students' age was 19.94 ± 2.37 years, their mean BMI was 21.42 ± 3.01 kg/m², and most participants (61.24%) were normal weight. There were 118 (56.46%) non-approaching graduation students and 91 (43.54%) approaching graduation students; 96 (45.93%) students came from rural areas and 113 (54.07%) students came from town (Table 1).

Table 1 Characteristics of 209 university students enrolled in the study

Variables	Mean ± SD or n (%)
General demographic characteristics	
Age (y)	19.94 ±2.37
Gender	
Male	116 (55.50)
Female	93 (44.50)
BMI (kg/m²)	
Underweight (< 18.5)	39 (18.66)
Normal (18.5-23.9)	128 (61.24)
Overweight (≥ 24.0)	42 (20.10)
Grade	
Non-Approaching graduation	118 (56.46)
Approaching graduation	91 (43.54)
Residence	
Rural	96 (45.93)
Town	113 (54.07)
Eating habits	
Daily food matching	
More meat and less vegetables	37 (17.70)
Less meat and more vegetable	37 (17.70)
The meat and vegetables are even	132 (63.16)
Fruit frequency in the last week	
1~3 days	144 (68.90)
4~6 days	52 (24.88)
Everyday	13 (6.22)
Daily water consumption in the last week	
< 1000 mL	98 (46.89)
1000~1500 mL	69 (33.01)
≥ 1500 mL	42 (20.10)

The distribution of iAs, MMA, DMA and TAs in urine were shown in Table 2. The detection rates of iAs (As^{III}), MMA and DMA in urine among 209 university students were 100%, 96.65% and 72.73%, respectively. The concentration of DMA was the highest among all the measured As speciations and its median concentration was 12.17 µg/L, the median

concentration of iAs was 1.22 µg/L, and the median concentration of MMA was 1.18 µg/L (Table 2). The proportion distribution of %iAs, %MMA, %DMA in urine was shown in Table 3. The %DMA was the highest, followed by %iAs and %MMA.

Table 2 Urine concentration distributions of As speciations among 209 university students

As speciation	Gender	> LOD (%)	Mean (mg/L)	Min (mg/L)	P_5 (mg/L)	P_{25} (mg/L)	P_{50} (mg/L)	P_{75} (mg/L)	P_{95} (mg/L)	Max (mg/L)
iAs	Total	96.65	1.59	< LOD	0.16	0.73	1.22	2.07	4.28	6.98
	Males	95.69	1.83	< LOD	0.02	0.78	1.44	2.61	4.59	5.3
	Females	97.85	1.3	< LOD	0.17	0.66	1.01	1.66	3.2	6.98
MMA	Total	72.73	1.19	< LOD	< LOD	< LOD	0.92	1.74	3.98	6.47
	Males	80.17	1.45	< LOD	< LOD	0.41	1.18	2.1	4.41	6.47
	Females	63.44	0.87	< LOD	< LOD	< LOD	0.63	1.24	3.36	5.09
DMA	Total	100	14.58	1.09	3.86	7.15	12.17	19.26	33.48	78.36
	Males	100	16.13	1.7	3.51	7.32	14.57	21.14	42.09	78.36
	Females	100	12.65	1.09	4.55	6.91	10.58	16.42	27.44	33.58
TAs	Total	100	17.37	1.09	4.37	8.36	15.02	23.17	39.31	86.94
	Males	100	19.41	1.7	3.79	8.85	17.53	25	48.62	86.94
	Females	100	14.82	1.09	5.11	8.2	12.44	18.91	31.96	40.12

Abbreviations: TAs, total urinary arsenic; iAs, inorganic arsenic (It mainly refers to the As^{III}, since the As^V has not been detected in this population); MMA, monomethylarsonic acid; DMA, dimethylarsinic acid.

Table 3 Urine As speciations percentages among 209 university students

As speciation percentages	Gender	Mean (%)	Min (%)	P_5 (%)	P_{25} (%)	P_{50} (%)	P_{75} (%)	P_{95} (%)	Max (%)
%iAs	Total	9.25	0	2.16	6.26	8.76	11.77	17.32	46.12
	Males	9.54	0	0.46	6.2	8.98	12.04	18.93	24.49
	Females	8.88	0	2.23	6.28	8.3	10.64	16.66	46.12
%MMA	Total	6.23	0	0	0	6.13	10.1	15.08	21.09
	Males	6.23	0	0	0	6.13	10.1	15.08	21.09
	Females	5.15	0	0	0	4.58	8.94	14.32	20.34
%DMA	Total	84.52	43.01	71.21	79.45	84.84	90.33	96.6	100
	Males	83.36	64.53	70.69	78.07	82.91	89	99.54	100
	Females	85.97	43.01	72.92	81.86	87.54	91.91	95.28	100

The association of demographic features, eating habits with As speciations in urine among 209 university students were shown in Table 4. The InTAs concentration in urine of students over 20 years old was 0.31 $\mu\text{g/L}$ lower than that of students under 20 years old ($p < 0.01$). Similar to the age, the urine InTAs concentration of approaching graduation students was 0.34 $\mu\text{g/L}$ lower than that of non-approaching graduation students ($p < 0.01$). Compared with the males, In%MMA in urine of females was lower while In%DMA and InSMI was higher, and the difference was statistically significant. Dietary habits were only correlated with the urine InTAs. The InTAs level of students with balanced diet was higher than that of students with unbalanced diet, but the difference was only statistically significant when compared to those with less meat and more vegetables ($p < 0.01$). There was also a significant correlation between fruit consumption and urinary InTAs level, so were water consumption. The more frequent fruit consumption, the lower InTAs concentration, and the more water consumption, the lower InSMI.

Table 4

The analyses about influencing factors of As speciations in urine and TAs by linear regression models

	ln%iAs	ln%MMA	ln%DMA	lnTAs	lnPMI	lnSMI
Age						
< 20 y	Ref	Ref	Ref	Ref	Ref	Ref
≥ 20 y	-0.11 (-0.25, 0.03)	0.16 (-0.07, 0.39)	0.01 (-0.02, 0.03)	-0.31 (-0.50, -0.13)**	0.01 (-0.01, 0.03)	-0.01 (-0.02, 0.01)
Gender						
Male	Ref	Ref	Ref	Ref	Ref	Ref
Female	0.10 (-0.24, 0.04)	-0.38 (-0.60, -0.15)**	0.03 (0.003, 0.06)*	-0.19 (-0.38, 0.004)	0.01 (-0.01, 0.03)	0.02 (0.01, 0.04)*
BMI						
Underweight	0.07 (-0.12, 0.27)	-0.08 (-0.38, 0.22)	0.004 (-0.03, 0.04)	0.03 (-0.23, 0.29)	0.004 (-0.02, 0.03)	0.001 (-0.02, 0.02)
Normal	Ref	Ref	Ref	Ref	Ref	Ref
Overweight	0.02 (-0.07, 0.12)	-0.01 (-0.16, 0.13)	-0.003 (-0.02, 0.02)	-0.02 (-0.15, 0.10)	0.00001 (-0.01, 0.01)	-0.003 (-0.01, 0.01)
Residence						
Urban	Ref	Ref	Ref	Ref	Ref	Ref
Rural	0.07 (-0.07, 0.21)	0.07 (-0.16, 0.30)	-0.02 (-0.05, 0.004)	0.10 (-0.09, 0.29)	-0.01 (-0.03, 0.004)	-0.01 (-0.03, 0.01)
Grade						
Non-Approaching graduation	Ref	Ref	Ref	Ref	Ref	Ref
Approaching graduation	-0.11 (-0.25, 0.03)	0.17 (-0.06, 0.40)	0.003 (-0.03, 0.03)	-0.34 (-0.53, -0.16)**	0.01 (-0.01, 0.03)	-0.01 (-0.02, 0.01)
Eating habits						
Daily food matching						
More meat and less vegetables	-0.07 (-0.26, 0.11)	-0.03 (-0.34, 0.28)	0.01 (-0.03, 0.04)	-0.04 (-0.29, 0.20)	0.01 (-0.02, 0.03)	-0.002 (-0.02, 0.02)

The univariate linear regression models analysis was conducted.

*, P value < 0.05; **, P value < 0.01; Ref, reference group; TAs, total urinary arsenic; iAs, inorganic arsenic (It mainly refers to the As^{III}, since the As^V has not been detected in this population); MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; PMI, the Primary Methylation Index; SMI, the Secondary Methylation Index; ln, the natural logarithm transformed.

	ln%iAs	ln%MMA	ln%DMA	lnTAs	lnPMI	lnSMI
Less meat and more vegetable	-0.02 (-0.11, 0.08)	0.05 (-0.11, 0.20)	0.01 (-0.01, 0.03)	-0.26 (-0.40, -0.13)**	0.01 (-0.01, 0.02)	-0.002 (-0.01, 0.01)
The meat and vegetables are even	Ref	Ref	Ref	Ref	Ref	Ref
Fruit frequency in the last week						
1 ~ 3 days	0.004 (-0.31, 0.32)	-0.48 (-0.96, 0.01)	0.01 (-0.05, 0.07)	0.43 (0.03, 0.84)*	-0.01 (-0.05, 0.03)	0.03 (-0.01, 0.06)
4 ~ 6 days	0.02 (-0.12, 0.16)	-0.15 (-0.38, 0.08)	-0.003 (-0.03, 0.02)	0.20 (-0.01, 0.41)	-0.01 (-0.03, 0.02)	0.01 (-0.01, 0.02)
Everyday	Ref	Ref	Ref	Ref	Ref	Ref
Daily water consumption in the last week						
< 1000 mL	Ref	Ref	Ref	Ref	Ref	Ref
1000 ~ 1500 mL	-0.10 (-0.26, 0.05)	0.42 (0.16, 0.69)	-0.03 (-0.05, 0.003)	-0.002 (-0.22, 0.22)	-0.001 (-0.02, 0.02)	-0.02 (-0.04, -0.01)*
≥ 1500 mL	-0.44 (-0.13, 0.04)	0.13 (-0.03, 0.29)	-0.01 (-0.03, 0.10)	-0.04 (-0.16, 0.09)	-0.004 (-0.02, 0.01)	-0.01 (-0.02, 0.01)
The univariate linear regression models analysis was conducted.						
*, <i>P</i> value < 0.05; **, <i>P</i> value < 0.01; Ref, reference group; TAs, total urinary arsenic; iAs, inorganic arsenic (It mainly refers to the As ^{III} , since the As ^V has not been detected in this population); MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; PMI, the Primary Methylation Index; SMI, the Secondary Methylation Index; ln, the natural logarithm transformed.						

The estimated joint effect of lnTAs and lnIAs on As methylation indices (ln%MMA, ln%DMA, lnPMI, lnSMI) are shown in Fig. 1–4. We firstly displayed some numerical summaries of their overall effect, which was identified as the change in As methylation indices (ln%MMA, ln%DMA, lnPMI, lnSMI) associated with a simultaneous change in lnTAs and lnIAs from a particular percentile as compared to when lnTAs and lnIAs were at their median values (50th percentile) (Fig. 1A, Fig. 2A, Fig. 3A, Fig. 4A). As can be seen from Fig. 1A, combined exposure was not associated with ln%MMA. When both lnTAs and lnIAs levels are above the 60th percentile, the lnTAs and lnIAs were significantly negatively correlated with ln%DMA; on the contrary, when both lnTAs and lnIAs levels are below the 55th percentile, a significantly positive correlation was observed when compared to the 50th percentile, and the similar result was also found about lnSMI. While compared with the 50th percentile, the lnTAs and lnIAs were positively correlated with lnPMI only at 35th, 40th and 55th percentile. The variation in the association of lnTAs or lnIAs when it increased from 25th to 75th percentile accompanied by another factor was set at 25th, 50th or 75th percentile with As methylation indices (ln%MMA, ln%DMA, lnPMI, lnSMI), respectively was shown in Fig. 1B, Fig. 2B, Fig. 3B and Fig. 4B. lnTAs displayed a significantly positive association with ln%DMA and lnPMI levels when lnIAs was set at the 25th, 50th, and 75th percentiles. And lnIAs displayed a significantly negative association with ln%DMA, lnPMI, and lnSMI levels when lnTAs is set at the 25th, 50th, and 75th percentiles, and displayed a significantly positive association with ln%MMA. Finally, we also examined the

potential nonlinear exposure-response relationship when another factor was held at the corresponding median concentration by BKMR analysis. We found that InTAs was positively correlated with In%DMA, InPMI and InSMI. However, InIAs was negatively correlated with these three indices (Fig. 1C, Fig. 2C, Fig. 3C and Fig. 4C).

Considering the study participants are university students and environment As exposure level is not very serious in Anhui province (Zhong and Zhang et al. 2019), we thought that their urinary As are mainly source from food and drinking water. Therefore, we measured the TAs content in the food and drinking water from the canteen. The results showed that the TAs content in cereals was the highest among all the our purchased foods, with an average of 90.340 µg/kg, while TAs in fruits, vegetables and meats were similar to be lower. The mean concentration of TAs in drinking water was 0.284 µg/L (Table 5).

Table 5
Average levels of total arsenic in some foods.

Food group	Levels of total arsenic (mean ± SD, µg/kg or µg/L)
Cereals (polished rice, flour)	90.340 ± 41.834
Fruits (banana, cantaloupe, dragon fruit)	17.507 ± 8.540
Vegetables (potato, tomato, cabbage, bean sprout, carrot)	7.338 ± 1.844
Meats (pork, chicken, beef, fish, shrimp)	7.369 ± 11.086
Drinking water	0.284 ± 0.324

4. Discussion

4.1. Key findings

In summary, these students exposed to As generally and widely; the levels of iAs, MMA, DMA and TAs had gender difference. With respect to As methylation capacity, gender and exposure level of TAs and iAs were important determinants. Females seem to have stronger As methylation capacity than males; combined levels of TAs and iAs played an important role in reducing As methylation capacity, especially iAs; and the reduction only occurred when TAs and iAs were up to a certain combined level.

4.2. As speciations distribution patterns

Total urinary As for our samples (17.37 µg/L) was similar with that reported for Belgium (Hoet and Jacquerye et al. 2013) (14.1 µg/L) and the U.S. (Pace and Smith-Gagen et al. 2018) (15.67 µg/L). But is slightly lower compared with some other regions, such as Hainan island and Northern Vietnam (Inoue and Umezaki et al. 2014; Pham and Nguyen et al. 2017), this difference may be due to the differences in eating habits and exposure levels in different regions. Furthermore, urinary proportions of As speciations in the present study were coincident with reports that most person had 10%-30% iAs, 10%-20% MMA, and 60%-80% DMA, regardless of their As exposure levels (Hsueh and Ko et al. 2003; Steinmaus and Yuan et al. 2010). The %iAs, %MMA and %DMA in our subjects' urine were 8.76%, 6.13% and 84.84%,

respectively. The toxicity of As extremely differs among their chemical speciations, and previous studies verified that monomethylarsonous acid (MMA^{III}) could result in the induction of cytotoxic and genotoxic and more MMA in urine meant more MMA^{III}, and more cytotoxic and genotoxic (Tseng 2009; Wnek and Medeiros et al. 2009; Ren and McHale et al. 2011). Both the percentages of MMA and iAs were lower in our study showed that university students in our study had stronger As methylation capacity.

4.3. Demographic characteristics and eating habits

The percentage of As metabolites in urine is commonly used to reflect the As methylation capacity. Previous studies suggested that variability in As methylation capacity was due, in part to variations in gene polymorphisms, as well as gender, age, BMI, smoking, drinking alcohol and eating habits (Tseng 2009; Wnek and Medeiros et al. 2009; Ren and McHale et al. 2011).

In the present study, TAs and %MMA in the urine of male was higher than that of female, while the ln%DMA and lnSMI was lower than that of female. Our findings were consistent with previous study researched by Huang et al. (Huang and Hsueh et al. 2009) and Steinmaus et al (Steinmaus and Carrigan et al. 2005). However, differences in As metabolic profile between genders were not observed in Argentine children (Olmos and Astolfo et al. 2021). Some researchers speculated the gender difference of As methylation capacity possibly linked with sex hormones, and the peak in sex hormone secretion occurs in the 20 years old in both males and females, which could explain why gender effects are less prominent in children (Lindberg and Kumar et al. 2007; Lindberg and Ekström et al. 2008; Gomez-Rubio and Roberge et al. 2011). Our study also displayed a significantly negative correlation between age and TAs level in urine. Interestingly, previous studies have also shown that the level of TAs increased significantly with age in humans (Lindberg and Kumar et al. 2007; Lindberg and Ekström et al. 2008), which was not consistent with our results. The study participants in our study was a group of Chinese university students and their age span was small, which may be one of the important reason why the conclusions were inconsistent. Epidemiological studies evaluating the relationship between BMI and the distribution of As metabolites in urine presented that higher BMIs were associated with a lower %MMA, suggesting that BMI might be related with As methylation capacity (Lindberg and Kumar et al. 2007; Gomez-Rubio and Roberge et al. 2011; Gribble and Crainiceanu et al. 2013), but the other studies did not show any association between them (Li and Ekström et al. 2008; Bocca and Pino et al. 2020). Our results were in accordance with the latter.

Although several studies have suggested that eating habits could affect the As methylation capacity (Xu and Wang et al. 2008; Bozack and Hall et al. 2019; Garcia-Rico and Valenzuela-Rodriguez et al. 2020; Xue and Zhao et al. 2020), this is not the case in the present study, and we only found that diet (food matching daily) was associated with TAs. However, our study didn't assess the real nutritional status of the participants since a food frequency questionnaire was not used to find out all information of those substances capable to modify both metabolism and toxicity of As.

4.4. TAs and iAs levels

We explored the correlation between urinary TAs and iAs levels with the indexes of As methylation capacity by BKMR analyses. The results showed that there were positive correlations between TAs with the three As methylation indices (%DMA, PMI, SMI), and negative correlation with %MMA; however, iAs presented an opposite trend. It should be noted that both PMI and SMI were correlated negatively with combined levels of TAs and iAs. These results reminded us that iAs may played a more important role in reducing As methylation. Our findings differed from a study from Xu et al. (Xu and Wang et al. 2008; Bozack and Hall et al. 2019; Garcia-Rico and Valenzuela-Rodriguez et al. 2020; Xue and Zhao et al. 2020), who found that with the increasing of TAs level, the %iAs and %MMA increasing while the %DMA decreasing in people from Inner Mongolia. In addition, Kong et al. (Kong and Yang et al. 2020) also discovered that As methylation decreased with the increasing of TAs level. However, there are some other studies suggested that PMI and SMI rised as exposure levels rised (Kong and Yang et al. 2020). The differences may be due to that these previous studies mainly

obtained the independent relationship between TAs or iAs with As methylation indices by linear regression analysis, while we not only obtained the nonlinear relationship between TAs and iAs with those indices and analyzed the possible combined effects by BKMR analyses. Besides, this method allowed for a more accurate correlation between one variable and an outcome while controlling an other variable that we also wanted to study (Li and Xu et al. 2019; Wang and Gao et al. 2020). Specifically, when we analyzed the relationship between TAs and methylation capacity, iAs was set at a specific level; otherwise, TAs was set at a specific level.

4.5. Strengths and limitations

This study explored influencing trend and extent of combined exposure levels of total arsenic and inorganic arsenic on arsenic methylation capacity among university students using BKMR analysis for the first time. Nevertheless, our study still has several limitations. Firstly, spot urine samples could reflect only short-term exposure, and the time of day at sample collection may influence the observation of %iAs, %MMA and %DMA. In order to minimize this influencing effect, morning urine samples were collected at the same time every day during the execution stage of study. Secondly, the dilution degree of urine was not corrected by the specific gravity of urine creatinine, although it doesn't have any impact on the As methylation capability. Thirdly, we didn't have data about complete dietary in university students, only determined a few common TAs contents of some food, it's an important factor for As methylation capacity. Finally, this was a cross-sectional study, which could only explain the correlation between the As methylation capacity and the influencing factors investigated, and the causal conclusion could not be obtained.

5. Conclusions

In the present study, we found that these students exposed to As generally and widely; the levels of iAs, MMA, DMA and TAs had gender difference, and females seem to have stronger As methylation capacity than males. The combined exposure levels of TAs and iAs played an important role in As methylation, especially iAs, and the reduction only occurred when TAs and iAs were up to a certain combined level. The impact of TAs and iAs on As methylation capacity should be paid more attention in the future.

Declarations

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Authors' Contributions Fangbiao Tao, Yuyou Yao, and Chunmei Liang figured out the conception of this study; Rui Jiang, Tingting Jiang, Yuan Hu, Shitao He, Long Tao tested the urine samples; Juan Shen, Wei Zhang, Yuxiang Song, Yicheng Ma collected the urine samples and questionnaires; Rui Jiang, Qing Zhang, Dongmei Ji and Chunmei Liang analyzed the data and wrote the manuscript; Fangbiao Tao, Yuyou Yao, Chunmei Liang, Qing Zhang and Dongmei Ji offered proposals and provided financial support.

Availability of Data and Material The data sets supporting the results of this article are included within the article and its additional files.

Conflicts Interests No conflict of interest exists in the submission of this manuscript, and the submission is approved by all the authors listed.

Ethics Approval The study was approved by the ethics committee of Anhui Medical University.

Consent to Participate Oral and written consent was obtained from all subjects.

Consent for Publication Not applicable.

Code Availability Not applicable.

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Figures

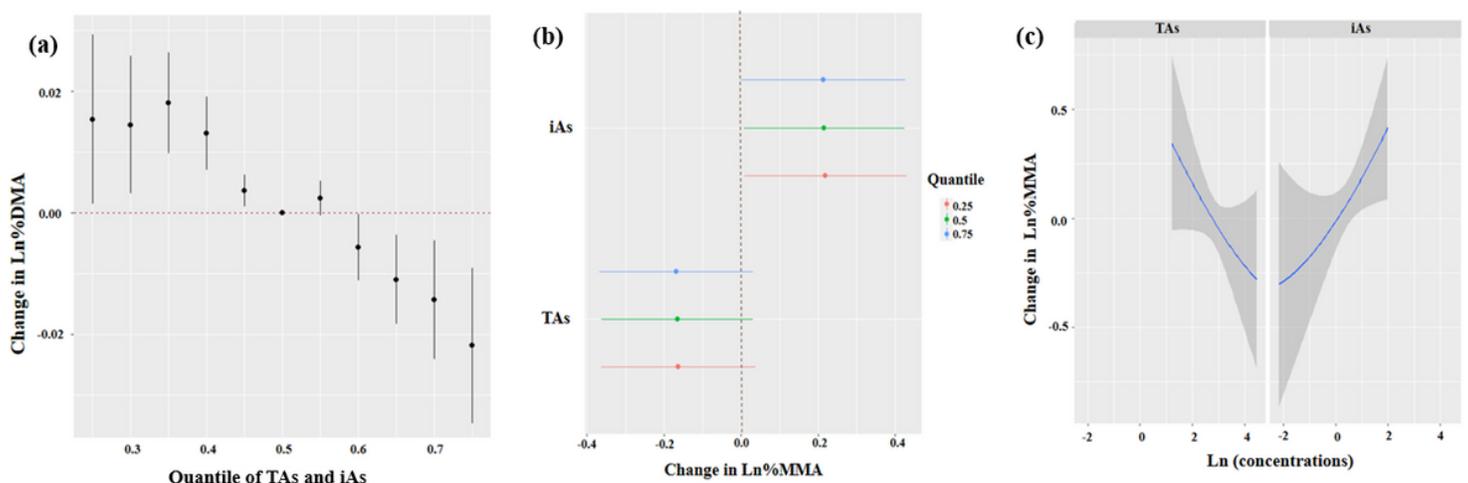


Figure 1

Joint effect of the total arsenic and inorganic arsenic in urine on Ln%MMA by Bayesian Kernel Machine Regression (BKMR) Model was adjusted for gender, age, grade, daily food matching, fruit frequency in the last week and time being spent on strenuous physical activity in the last week. Fig. 1a: overall effect of the LnTAs and IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the Ln%MMA level when LnTAs and IniAs were at a particular quantile to when they were at the 50th percentile, respectively. Fig. 1b: independent association of LnTAs or IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the Ln%MMA level when LnTAs or IniAs was at the 75th vs. 25th percentile, when another factor was fixed at either the 25th, 50th or 75th percentile. Fig. 1c: univariate exposure-response function and 95% confidence bands for LnTAs or IniAs with another factor being fixed at the median.

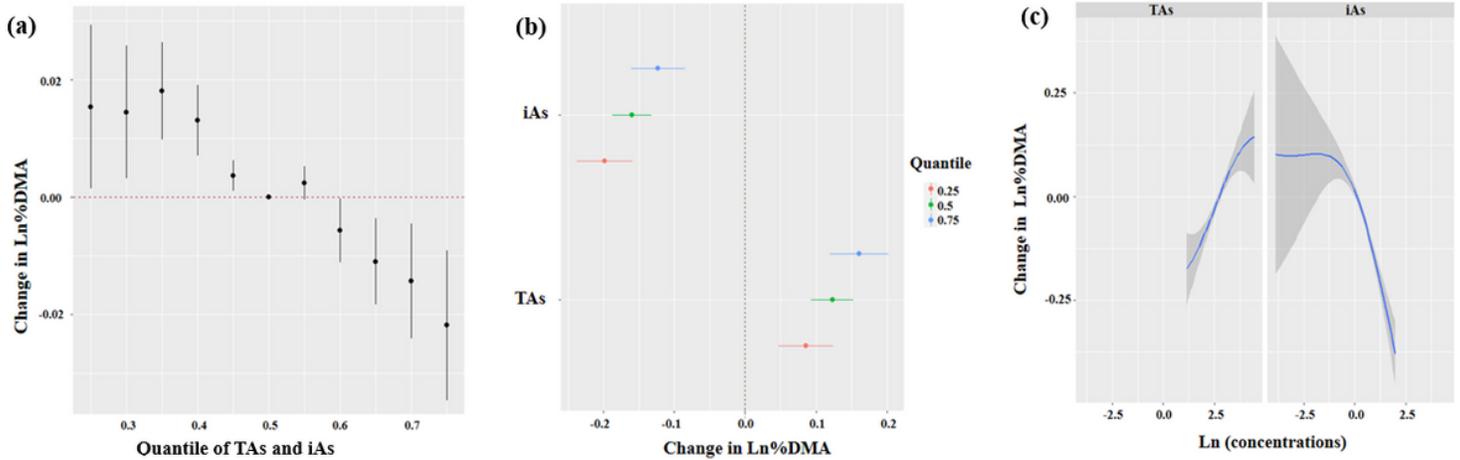


Figure 2

Joint effect of the total arsenic and inorganic arsenic in urine on Ln%DMA by Bayesian Kernel Machine Regression (BKMR) Model was adjusted for gender, age, grade, daily food matching, fruit frequency in the last week and time being spent on strenuous physical activity in the last week. Fig. 2a: overall effect of the LnTAs and IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the Ln%DMA level when LnTAs and IniAs were at a particular quantile to when they were at the 50th percentile, respectively. Fig. 2b: independent association of LnTAs or IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the Ln%DMA level when LnTAs or IniAs was at the 75th vs. 25th percentile, when another factor was fixed at either the 25th, 50th or 75th percentile. Fig. 2c: univariate exposure-response function and 95% confidence bands for LnTAs or IniAs with another factor being fixed at the median.

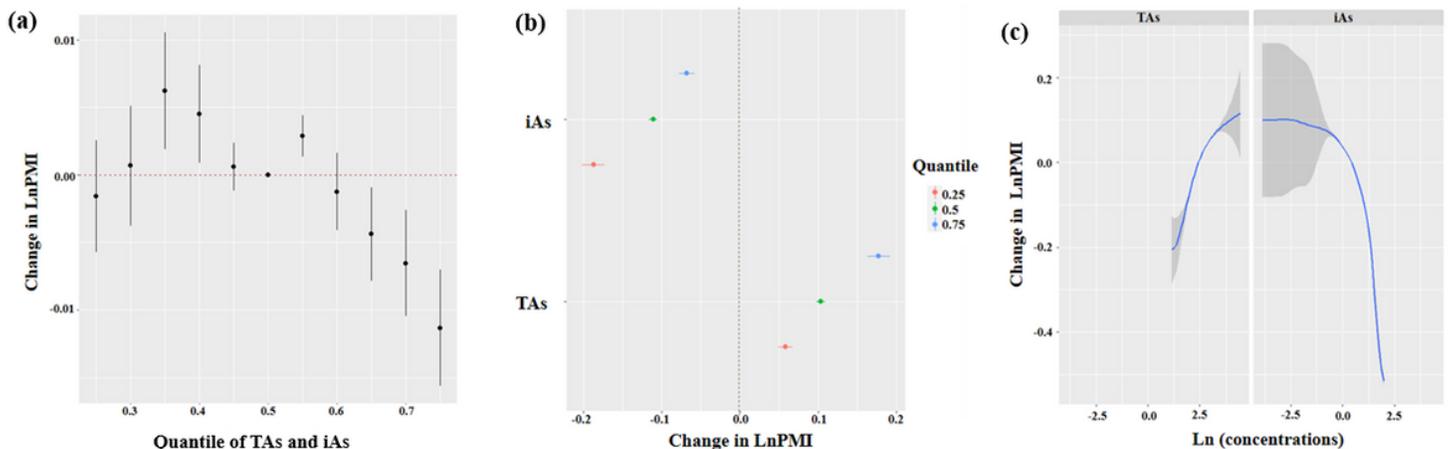


Figure 3

Joint effect of the total arsenic and inorganic arsenic in urine on InPMI by Bayesian Kernel Machine Regression (BKMR) Model was adjusted for gender, age, grade, daily food matching, fruit frequency in the last week and time being spent on strenuous physical activity in the last week. Fig. 3a: overall effect of the InTAs and IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the InPMI level when InTAs and IniAs were at a particular quantile to when they were at the 50th percentile, respectively. Fig. 3b: independent association of InTAs or IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the InPMI level when InTAs or IniAs was at the 75th vs. 25th percentile, when another factor was fixed at either the 25th, 50th or 75th percentile. Fig. 3c: univariate exposure-response function and 95% confidence bands for InTAs or IniAs with another factor being fixed at the median.

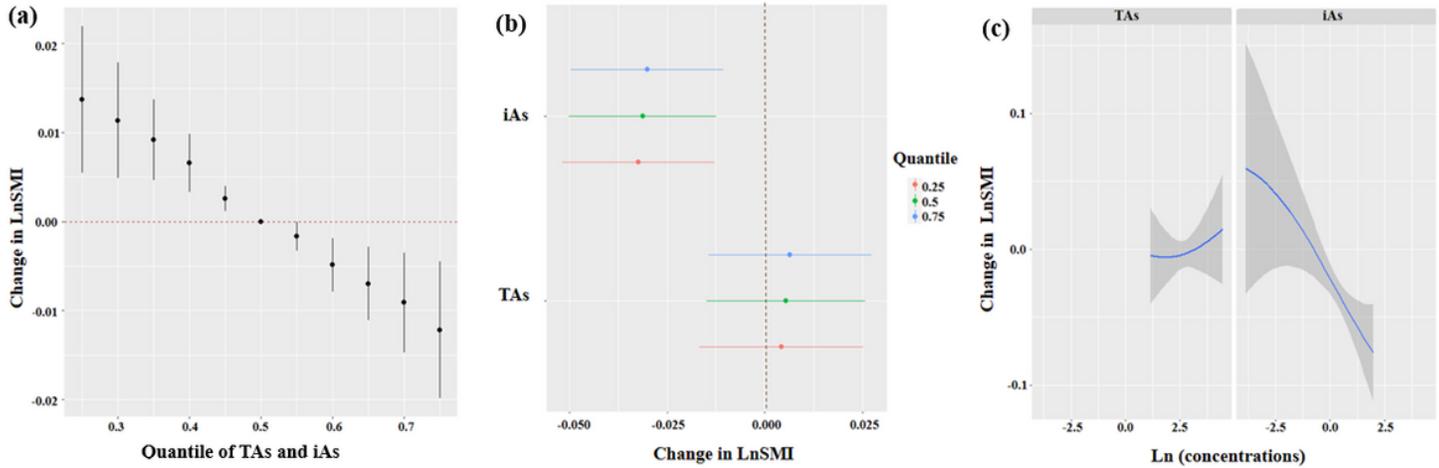


Figure 4

Joint effect of the total arsenic and inorganic arsenic in urine on InSMI by Bayesian Kernel Machine Regression (BKMR) Model was adjusted for gender, age, grade, daily food matching, fruit frequency in the last week and time being spent on strenuous physical activity in the last week. Fig. 4a: overall effect of the InTAs and IniAs (estimates and 95%CI,gray dashed line at the null). This plot compared the InSMI level when InTAs and IniAs were at a particular quantile to when they were at the 50th percentile, respectively. Fig. 4b: independent association of InTAs or IniAs (estimates and 95%CI, gray dashed line at the null). This plot compared the InSMI level when InTAs or IniAs was at the 75th vs. 25th percentile, when another factor was fixed at either the 25th, 50th or 75th percentile. Fig. 4c: univariate exposure-response function and 95% confidence bands for InTAs or IniAs with another factor being fixed at the median.

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