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Exposure to a mixture of arsenic species and growth indicators in 6–12-year-old children from the cycles 2007–2020 NHANES

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ABSTRACT

Background: Exposure to arsenic (As) and its metabolites can affect normal growth in children, but the combine effects at simultaneous low-level exposures, remain uncertain. Hence, this study aims to analyze how the combined effects of As and its metabolites can impact growth indicators in 1,792 US children aged 6–12 years, from the NHANES.

Methods: Levels of arsenic species in urine were measured using HPLC coupled with ICP-DRC-MS during the 2007–2020 NHANES cycles. The sum of iAs ([AsIII + AsV]), MMA, and DMA was used as a biomarker of internal iAs exposure (∑As), and methylation efficiency was assessed using the primary and secondary methylation indices (PMI, SMI). Linear regression and BKMR models were applied to identify adverse effects, nonlinear associations, interactions, and combined effects.

Results: Median concentrations of MMA, DMA, iAs, and ∑As were 0.56 μg/L, 4.07 μg/L, 1.33 μg/L, and 6.40 μg/L, respectively. In the linear regression analyses, higher urinary concentrations of MMA were associated with reductions in several growth indicators. Specifically, each interquartile range (IQR) increase in MMA concentration was linked to decreases of −0.18 (95 % CI: −0.29, −0.06) in Body Mass Index (BMI) Z-score, −0.18 (95 % CI: −0.29, −0.06) in Weight Z-score, and −0.01 (95 % CI: −0.02, −0.01) in Waist circumference/Height ratio. Additionally, higher DMA concentrations were negatively associated with Height Z-score, with a reduction of −0.08 (95 % CI: −0.15, −0.01). In the BKMR analysis, DMA consistently emerged as the dominant contributor across multiple outcomes, showing the highest Posterior Inclusion Probabilities (PIPs) for indicators such as BMI Z-score and Waist circumference/Height ratio. While MMA exhibited notable PIPs in certain models, its influence was generally weaker than that of DMA.

Conclusion: Childhood exposure to a mixture of arsenic species, even at low levels, appears to influence growth indicators and adversely affect physical development in children enrolled in NHANES.

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1. Introduction

Arsenic is widely distributed in the Earth's crust and commonly found in groundwater and food. Exposure to high levels of toxic arsenic forms, such as inorganic arsenic (iAs), can cause long-term health consequences, including heart disease, diabetes, and cancers of the skin and lungs, among others (Nachman et al., 2017; Sanchez et al., 2016; Tsuji et al., 2015). However, it is important to clarify that some arsenic forms, such as arsenobetaine (AsB) found in fish and seafood, are not considered toxic and are excreted in urine unchanged (Nachman et al., 2017; Sanchez et al., 2016; Tsuji et al., 2014). iAs can also have harmful effects on growth and neurodevelopment (Tsuji et al., 2015). Therefore, regulatory efforts have aimed to protect the population, particularly children, who are considered a vulnerable to the effects of arsenic exposure (Alidadi et al., 2019; Spaur et al., 2023).

Determining arsenic speciation in various biological matrices is essential for assessing exposure risks. In drinking water, the main species are iAs, which includes to the sum of arsenite (AsIII) and arsenate (AsV). In contrast, in food and human biological samples, the predominant species are monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and AsB (Villaescusa and Bollinger, 2008). The arsenic profile can vary by biomarker: urine typically contains MMA, DMA, and AsB, while nails accumulate mainly iAs (Cubadda et al., 2017). The process of methylation, which transforms iAs into less toxic metabolites, relies on dietary proteins that provide methionine, a precursor to methyl donor S-adenosylmethionine (SAM). SAM transfers methyl groups to iAs or MMA via the enzyme AS3MT, producing MMA or DMA and S-adenosyl-L-homocysteine (SAH), which is later converted to homocysteine. This cycle requires folate, vitamin B12, or betaine to regenerate methionine and ensure continuous SAM production (Vahter, 2009). Notably, betaine, derived from choline, is upregulated by estrogen (Resseguie et al., 2007). After ingestion, iAs is absorbed through the gastrointestinal tract, transported to the liver, and metabolized into MMA and DMA acid through methylation (Khairul et al., 2017). Biomarkers of arsenic exposure are crucial for understanding its toxicity mechanism and evaluating its health impacts. Blood, urine, hair, and nails are the most commonly used biological substrates in epidemiological studies (Gil and Hernández, 2015). Thus, the sum of urinary iAs ($As^{III} + As^{V}$), MMA, and DMA, defined as ΣAs, is commonly used as a biomarker of iAs internal exposure in environmental epidemiology studies (Cubadda et al., 2017; Navas-Acien et al., 2011; Signes-Pastor et al., 2017b).

The potential effect of arsenic exposure on growth indicators is relevant, as growth patterns during childhood have been linked to some pathologies in adulthood, such as risk of metabolic disease (Rahman et al., 2017). Most research on arsenic exposure and children's growth comes from rural Bangladesh in the MINIMat cohort, an area with high arsenic contamination in groundwater, which is its main source of drinking water (Smith et al., 2000). This study found that maternal urinary arsenic concentrations, which crosses the placenta, was inversely related to birth weight and head and chest circumference, but not birth length (Rahman et al., 2009). Subsequently, at follow-up at 1.5–2 years (Saha et al., 2012) and 5 years of age (Gardner et al., 2013), children's ΣAs concentration was inversely associated with their weight and height. For populations exposed to low levels of arsenic, the number of studies is limited. In the United States (US), infants exposed to higher levels of arsenic in utero had slightly increased length in their first year (i.e., catch-up growth), despite slower linear growth in the first 3.5 months (Muse et al., 2020; Wit and Boersma, 2002). In Spain, the INMA-Asturias cohort conducted one of the few studies that analyzes exposure to a mixture of metals, including iAs, and infant growth in 4–5-year-old children. However, no association was found between arsenic exposure and growth indicators (García-Villarino et al., 2021).

Therefore, the aim of this study was to assess the exposure to arsenic species in urine as a biomarker of exposure in children. We examined the relationship between urinary arsenic speciation and the physical growth and development of children aged 6 to 12 years. Our analysis utilized data from the National Health and Nutrition Examination Survey (NHANES) 2007–2020 cycles. We conducted analyses both on individual compounds and their mixtures, and also evaluated how arsenic methylation could affect children's growth. Previous research has demonstrated that arsenic exposure can lead to sex-specific effects, with differences in susceptibility and health outcomes observed between males and females (Vahter et al., 2007). These distinctions may arise from hormonal differences, variations in methylation capacity, and metabolic factors that influence arsenic biotransformation and toxicity (Naujokas et al., 2013). Additionally, studies have indicated that the association between individual metals, including arsenic, and children's growth indicators may vary by sex (Freire et al., 2019; García-Villarino et al., 2021; Gilbert-Diamond et al., 2016; Signes-Pastor et al., 2019b), the study was also conducted separately for males and females.

2. Methods

2.1. Study design and population

This cross-sectional study used data from the NHANES, a program designed to evaluate the health and nutritional status of non-institutionalized civilian adults and children in the US. This program combines interviews and physical examinations. NHANES is administered through the National Center for Health Statistics (NCHS), which is part of the Centers for Disease Control and Prevention (CDC) and is responsible for developing vital and health statistics for the US.

Participants in NHANES are selected through a stratified, multistage, probability sampling strategy based on the selection of counties, blocks, households, and individuals within households. Study design and recruitment of the participants are described in detail on the NHANES website (NHANES, 2022). The National Center for Health Statistics Research Ethics Review Board approved all the procedures. The participants included in the NHANES cycles 2007–2020 were 10,920, of whom urine spot samples from 2,809 were gathered and analyzed to determine arsenic metabolite concentrations. Of these, 2,714 had complete information on body size and growth parameters. Finally, 1,792 children, evenly distributed among the 2007–2020 cycles, of whom 49.1 % were girls (880) and 50.9 % were boys (912), had complete covariate data and were included in the statistical analyses (**Fig. S1**). **Table S1** in the supplementary material compares the characteristics of the 2714 participants with the 1792 included in the analysis.

2.2. Determination of urine arsenic speciation

Children urine samples were centrifuged, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA for analysis (CDC, 2016). High-performance liquid chromatography (HPLC) was used to determinate the concentration of speciated arsenics and to separate the species when coupled to an ICP-DRC-MS. This analytical technique is based on separation by anion-exchange chromatography (IC), followed by detection using quadrupole ICP-MS technology, and includes DRC™ technology. The lower limit of detection (LOD) was constant for all of the arsenic species in the data set. The LOD was 0.12 μg/L for As^{III}, 0.79 μg/L for As^{V}, 1.16 μg/L for AsB, 1.91 μg/L for DMA, and 0.20 μg/L for MMA for the 2007–2020 study cycles. NHANES employs several techniques to ensure the quality of analyses conducted by contract laboratories. One such method involves conducting blind split sample tests during "dry run" sessions in the Mobile Examination Center (MEC). Additionally, contract laboratories are required to randomly retest 2 % of all collected specimens to maintain accuracy and reliability (Caudill et al., 2008).

2.3. Growth indicators

The body measurements of interest, such as weight (kg), waist circumference (cm), arm circumference (cm), Body Mass Index (BMI [kg/m^2]) and standing height (cm) were evaluated at the MEC by health technicians. Arm circumference was usually measured on the right side of the body, unless participants had a medical condition, amputation, or cast on the right side. Weight was measured using a digital scale in kg. Standing height was measured using a stadiometer with a fixed vertical backboard and an adjustable head piece. Waist Circumference was measured by extending the tape measure around the waist. Detailed protocols have been previously published (NHANES, 2020). The outcomes were standardized by calculating Z-scores using the formula: (Value − Mean)/ Standard Deviation. To ensure comparability across age groups, this standardization was conducted separately for each age between 6 and 12 years. Specifically, values for 6-year-olds were standardized using the mean and standard deviation for 6-year-olds, those for 7-year-olds using the corresponding statistics for 7-year-olds, and so forth.

2.4. Covariates

The socio-demographic and biological variables for the children included in the study such as age, sex, and race were collected through a structured in-person interview (parents-children), with the details of the information collection procedures described in the NHANES study (NHANES, 2020). Total calorie intake (kcal/per day) was calculated using a two 24-hour dietary recall interviews. In addition, using the same dietary questionnaire, the frequency of fish and seafood consumption in the last 30 days was collected. Socioeconomic status was measured by the poverty-to-income ratio (PIR) and categorized using the median as the cutoff point. A questionnaire administered by an interviewer asked about the number of people in the household who smoked tobacco. Household smokers (i.e., household members who smoked $\geq$ 1) were used as an indirect indicator of passive smoke exposure. As this information was not available for all collected cycles, ln-transformed urine cotinine (ng/ml) was used as a reference for tobacco exposure. Additionally, the NHANES cycle to which each individual in the study belonged was included in the analysis, but no weights were used.

2.5. Statistical analysis

Descriptive statistics were calculated to summarize the variables: median (first − third quartile), values for continuous variables, and absolute and relative frequency for categorical variables. Differences in the study variables between included and excluded participants were assessed using t-tests, Fisher's exact test, or chi-square tests, as appropriate based on the statistical assumptions and conditions required for each test. Arsenic concentrations were standardized to creatinine content (μg/g creatinine). Values below the LOD were entered as the LOD divided by the square root of two and included in statistical analyses (Helsel, 1990; Hornung and Reed, 1990). To address the concern regarding statistical method requirements, we transformed the arsenic species concentration variables by applying natural logarithm transformations (ln). These transformed values were subsequently centered and scaled by dividing by their interquartile range (IQR). These transformed variables were used in all statistical analyses described below. In addition, iAs = arsenite (As^{III}) + arsenate (As^{V}), ΣAs = iAs + MMA + DMA were calculated to provide a comprehensive assessment of total arsenic internal exposure and speciation. These calculations allow for the differentiation between arsenic species and their metabolites, which is critical for understanding the overall arsenic burden and its potential health effects (Gao et al., 2019; Hata et al., 2012). Primary methylation index (PMI: MMA/iAs) and the secondary methylation index (SMI: DMA/MMA), were calculated to measure arsenic methylation efficiency (Kuo et al., 2017). PMI and SMI were transformed using the ln to correct for skewness and enhance normality. The coefficients from the PMI and SMI models were then converted using the formula (e^{β}-1) x 100 to calculate the expected percent differences in the mean of each anthropometric measure for each IQR-unit change of PMI or SMI (Smith et al., 2023). Spearman correlation coefficients were also calculated for each pair of arsenic species and anthropometric measurements. Arm circumference, waist circumference, BMI, standing height, and weight had approximately normal distributions.

A linear regression analysis adjusted for covariates was conducted to quantify the effects of arsenic speciation exposure on growth indicators. Potential confounding variables were selected based on previous studies and their relationship with growth indicators, using a directed acyclic graph (DAG) to identify the minimum sufficient set (**Fig. S2**) (DAGitty) (Textor et al., 2016). The linear regression models included the transformed concentrations of different arsenic metabolites as independent variables. The dependent variables were the infant growth indicators, adjusted for the child's age and sex, NHANES cycle, total calorie intake, seafood and shellfish consumption, race, poverty index, and cotinine levels measured in urine, as identified through the DAG. These variables were selected based on prior evidence of their influence on arsenic exposure and growth outcomes in children. Age and sex adjust for natural growth variation (Castiello et al., 2020), while total calorie intake and seafood consumption account for dietary arsenic exposure (Notario-Barandiaran et al., 2023). Race and socioeconomic factors, such as the poverty index (Desai et al., 2021; Signes-Pastor et al., 2020), address disparities in exposure and health outcomes, and cotinine levels control for the confounding effect of secondhand smoke, which is linked to growth and development (Robinson et al., 2016). As a sensitivity analysis, BMI was categorized into four groups (Underweight, Healthy weight, Overweight, and Obesity) based on BMI growth curves percentiles from the CDC (Hales et al., 2022). Logistic regression models were employed to evaluate the associations between arsenic species as independent variables and categorical BMI as dependent variable. Healthy weight category was used as the reference category in logistic regression models. Additionally, we conducted a sensitivity analysis using single-value median imputation to handle missing data. The results obtained through this approach were consistent with those derived from the complete-case analysis (see Supplementary Material: **Figs. S8-S13**), further supporting the robustness of our findings.

The nonlinear associations, interactions, and overall effect of the mixture of arsenic species were also studied using Bayesian Kernel Machine Regression (BKMR) (Bobb et al., 2014). It is important to use both linear and nonlinear models to accurately capture the complex relationships and interactions between different metabolites. Here, mixture refers to the combined exposure to multiple organic and inorganic arsenic metabolites, whose joint effects on health outcomes may differ from individual effects. Specifically, all modeling used a Gaussian kernel, with a burn-in period of 1,000 iterations, followed by 10,000 Monte Carlo iterations using a Markov chain approach. The posterior inclusion probabilities (PIPs) were calculated to evaluate the contribution of each arsenic metabolite within the joint association between the arsenic metabolite mixture and growth indicators. More details on the BKMR approach have been described in detail in previous studies (García-Villarino et al., 2022, 2021; Signes-Pastor et al., 2020). A p-value < 0.05 was used to define associations as statistically significant. We performed all statistical analyses and graphing with R version 3.5.2. (R Core Team, 2022).

3. Results

3.1. Study population characteristics

The enrolled children had a median age of 9 years, and the median concentrations of MMA, DMA, iAs, ∑As, and AsB were 0.56 μg/L, 4.07 μg/L, 1.33 μg/L, 6.40 μg/L, and 1.08 μg/L, respectively (Table 1). The

Table 1
Selected characteristics of the study population.

Characteristics	Total sample (n = 1,792)	Female Children (n = 880)	Male Children (n = 912)
Age (years)	9.0 (7.0–11.0)	9.0 (6.0–11.0)	9.0 (7.0–11.0)
Height (cm)	137.1 (127.4–148.4)	138.0 (127.2–150.1)	136.1 (127.6–147.1)
Weight (kg)	34.7 (26.7–45.0)	35.8 (27.3–46.9)	34.0 (26.2–43.1)
BMI (kg/m^2)	18.0 (16.0–21.5)	18.5 (16.2–22.3)	17.5 (15.9–20.8)
Waist circumference (cm)	64.0 (57.3–74.0)	65.5 (57.8–75.2)	62.3 (56.8–72.2)
Arm circumference (cm)	29.3 (26.7–32.0)	29.4 (26.7–32.4)	29.1 (26.7–31.8)
Total calorie intake (kcal/ per day)	1821 (1399–2321)	1732 (1300–2196)	1918 (1525–2457)
Seafood consumption (last 30 days)			
Yes	836 (46.7 %)	394 (44.8 %)	442 (48.5 %)
No	951 (53.1 %)	485 (55.1 %)	466 (51.1 %)
Shellfish consumption (last 30 days)			
Yes	650 (36.3 %)	329 (37.4 %)	321 (35.2 %)
No	1139 (63.6 %)	549 (62.4 %)	590 (64.7 %)
Poverty income-ratio (PIR)			
≤ 1.54	896 (50.0 %)	439 (49.9 %)	452 (49.6 %)
> 1.54	896 (50.0 %)	441 (50.1 %)	460 (50.4 %)
Race			
Mexican American	440 (24.6 %)	245 (27.8 %)	195 (21.4 %)
Other Hispanic	186 (10.4 %)	93 (10.6 %)	93 (10.2 %)
Non-Hispanic white	512 (28.6 %)	238 (27.0 %)	274 (30.0 %)
Non-Hispanic black	416 (23.2 %)	199 (22.6 %)	217 (23.8 %)
Other Race	238 (13.2 %)	105 (11.9 %)	133 (14.6 %)
Cycles*			
2007–2008	315 (17.6 %)	164 (18.6 %)	151 (16.6 %)
2009–2010	307 (17.1 %)	145 (16.5 %)	162 (17.8 %)
2013–2014	295 (16.5 %)	138 (15.7 %)	157 (17.2 %)
2015–2016	276 (15.4 %)	147 (16.7 %)	129 (14.1 %)
2017–2018	230 (12.8 %)	108 (12.3 %)	122 (13.4 %)
2017–2020	369 (20.6 %)	178 (20.2 %)	191 (20.9 %)
Cotinine (ng/mL)	0.04 (0.01–0.17)	0.04 (0.01–0.18)	0.03 (0.01–0.16)
Creatinine (mg/dL)	87.0 (48.0–131.0)	83.0 (43.0–130.0)	90.0 (51.2–132.3)
Urine Arsenic (μg/g creatinine)			
MMA	0.56 (0.30–0.98	0.57 (0.31–1.04)	0.56 (0.29–0.93)
DMA	4.07 (2.75–6.18)	4.08 (2.81–6.25)	4.05 (2.74–6.14)
iAs	1.33 (0.84–2.25)	1.36 (0.89–2.38)	1.31 (0.80–2.07)
Arsenite (As^{III})	0.52 (0.22– 0.99)	0.54 (0.23– 1.03)	0.51 (0.20– 0.96)
Arsenate (As^{V})	0.72 (0.47– 1.30)	0.75 (0.47– 1.42)	0.70 (0.47– 1.18)
ΣAs	6.40 (4.35– 9.47)	6.44 (4.38– 9.71)	6.28 (4.28– 9.17)
AsB	1.08 (0.54– 2.83)	1.12 (0.54– 2.93)	1.07 (0.53– 2.73)

Categorical variables are presented as *n* (%). Continuous variables are presented as median (Q1-Q3). PIR is categorized using the median as the cutoff.BMI = body mass index. DMA = dimethylarsinic acid, MMA = monomethylarsonic acid, AsB = arsenobetaine, iAs = Arsenite (As^{III}) + Arsenate (As^{V}). ΣAs = iAs + MMA + DMA. *Note: The 2011–2012 NHANES cycle lacks speciation data on arsenic due to the unavailability of such records during those years, with data solely pertaining to exposure starting at the age of 20. BMI = Body mass index.

observations below the LOD were 558 (31 %) for As^{III}, 1730 (96 %) for As^{V}, 553 (30 %) for AsB, 446 (22 %) for DMA and 593 (33 %) for MMA and they were evenly distributed by sex. The arsenic species concentrations in urine were paired correlated and had a Spearman ρ ranging from − 0.51 to 0.95 (Fig. S3). On the other hand, the children's indicators of body size were highly paired correlated (ranging from 0.49 to 0.95), with particularly high Spearman rho values of 0.90 between BMI and waist circumference (Fig. S3).

3.2. Linear regression analyses

In linear regression models for the total sample, it was observed that an increase of one IQR in DMA concentration in urine was associated with a change of −0.09 in arm circumference Z-score (95 % confidence interval (CI): −0.16; −0.01) and a change of −0.08 in height Z-score (95 % CI: −0.15; −0.01) (Fig. 1). An increase in the IQR in MMA concentration in urine was associated with a decrease of −0.01 in waist circumference/height ratio (95 % CI: −0.02; −0.01) and a decrease of −0.18 in BMI Z-score (95 % CI: −0.29; −0.06). Similarly, MMA was negatively associated with weight Z-score (−0.18; 95 % CI: −0.29; −0.06) (Fig. 1). The results of the sex-stratified linear regression models for the association between the concentrations of different arsenic metabolites and indicators of infant growth were similar to those of the linear regressions observed for the total sample. Specifically, in males, it was observed that an increase in the IQR in urinary MMA concentration was associated with a change of −0.20 in BMI Z-score (95 % CI: −0.36; −0.04), a change of −0.02 in waist circumference/height ratio (95 % CI: −0.03; −0.01) and a change of −0.19 in weight Z-score (95 % CI: −0.35; −0.02). There was a change of −0.02 (95 % CI: −0.02; −0.00) in waist circumference/height ratio for each increase in the IQR urinary iAs concentration Among female children, DMA in urine was associated with a change of −0.01 in arm circumference Z-score (95 % CI: −0.11; −0.21) and a change of −0.11 in height Z-score (95 % CI: −0.21; −0.01) in linear regression models (Fig. 1). The sensitivity analysis revealed that for the total sample, higher urinary MMA concentrations were associated with reduced odds of overweight and obesity. Stratified by sex, the pattern was similar, with MMA showing reduced odds of obesity and overweight for both males and females, although only statistically significant for obesity in males and for overweight in females. In addition, in the case of males, it is associated with an increase in the odds of underweight. For iAs and ΣAs concentrations in the total sample, they were associated with reduced odds of overweight, as they were for women, while the same trend was observed for men, but the results were not statistically significant. No significant associations were observed for other arsenic species (**Fig. S7**).

3.3. Bayesian kernel Machine regression analyses

In BKMR, we observed a positive linear association between DMA concentrations and BMI Z-score and waist-to-height ratio. Additionally, the effect estimates for DMA were more pronounced when there were high urinary concentrations of other arsenic metabolites in the mixture (Fig. 2**C**). For MMA, we identified an inverse relationship with BMI Z-score and weight Z-score, with the associations appearing to follow a linear dose–response pattern (Fig. 2**A**). No other statistically significant associations were found between the remaining components of the mixture and child growth indicator. Interaction effects and quantile-specific trends further emphasized the complex relationships between arsenic species and anthropometric indicators. In our sex-stratified analyses (**Fig. S4** and **Fig. S5**), the results suggested that male children may be more susceptible than female children to the effects of arsenic species. Among males, our analysis revealed notable patterns in the associations between urinary arsenic species and growth indicators. DMA showed positive associations with BMI Z-score and waist-to-height ratio, with stronger effects observed when other arsenic metabolites in the mixture were elevated. MMA exhibited inverse relationships with BMI Z-score, weight Z-score, and waist-to-height ratio, with trends suggesting a negative dose–response. iAs showed a positive association with height Z-score, particularly at moderate concentrations (**Fig. S4**). In contrast, no statistically significant associations were observed between arsenic species and growth indicators in girls, highlighting potential sex-specific differences in the effects of arsenic exposure on growth outcomes (**Fig. S5**). The Posterior Inclusion Probabilities (PIPs) indicate that DMA had the highest contribution across most growth indicators, particularly for waist circumference/height and BMI Z-score, while MMA generally showed a lower contribution. Stratified by sex, the patterns differed: in males, DMA remained the most significant contributor to BMI Z-score and waist circumference/height, whereas in

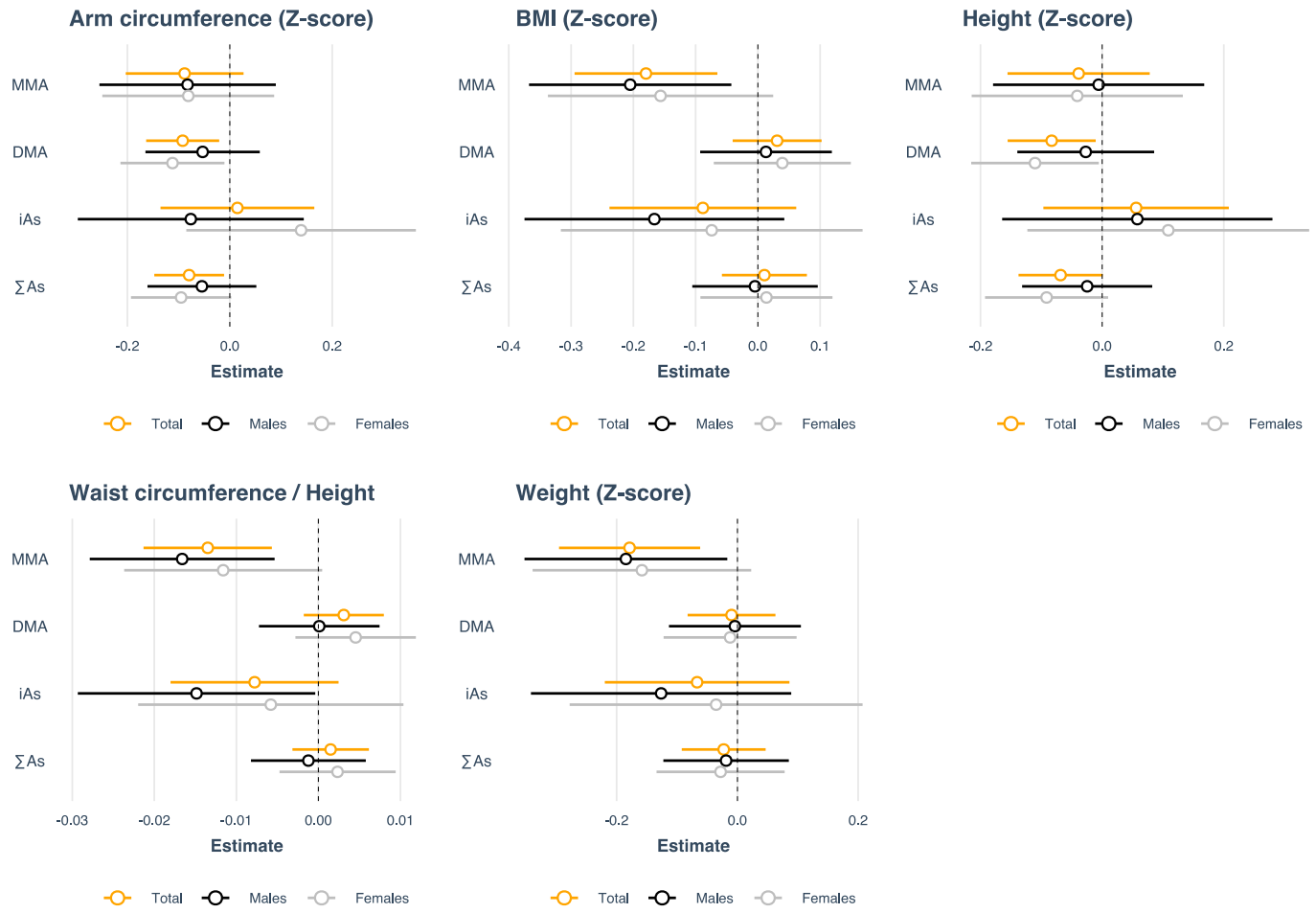

Fig. 1. Difference in children's growth indicators for each interquartile range (IQR) increase in population levels of arsenic species (n = 1,792). Linear regression models adjusted for age and sex of children, race, total calorie intake, seafood and shellfish consumption, poverty-income-ratio and cotinine. BMI = body mass index. DMA = dimethylarsinic acid, MMA = monomethylarsonic acid, iAs = Arsenite (As^{III}) + Arsenate (As^{V}). ΣAs = iAs + MMA + DMA. Sample size: males = 912 and females = 880.

females, iAs and MMA showed relatively higher contributions to certain indicators, such as height Z-score (**Fig. S6**). In the sensitivity analysis using the maximum possible sample size with median/imputed data, the associations observed in the BKMR models tended to follow a more linear pattern, reinforcing the relationships identified in the main analysis (see Supplementary Material: **Figs. S8-S13**).

3.4. Arsenic methylation index regression analyses

Fig. 3 presents the linear regression estimates of the mean percent differences in arsenic methylation indices per IQR-unit differences in anthropometric measures, analyzed for the total sample and stratified by sex. The analysis revealed no statistically significant associations between the PMI and any of the anthropometric measures, including arm circumference Z-score, BMI Z-score, weight Z-score, waist circumference/height ratio, and height Z-score, across the total sample or in the sex-stratified groups. Similarly, the SMI did not show statistically significant associations with any growth indicator in either the total sample or among females and males.

4. Discussion

Our findings from a study of 1,792 American children aged 6–12 years suggest that internal exposure to a mixture of arsenic species, including MMA, DMA, iAs, and ∑As, may affect body size and growth, even at relatively low levels of iAs exposure during childhood. Using a flexible approach to environmental mixture modeling, BKMR indicated that the effects varied according to the exposure concentration of each metabolite in the mixture. Specifically, MMA in urine was associated with a reduction in BMI Z-score and waist circumference/height ratio when the concentrations of other metabolites were high, which was corroborated by linear regression analyses. Furthermore, exposure to DMA, when the concentrations of other metabolites were low in the mixture, was positively associated with BMI Z-score and waist circumference/height ratio. Additionally, the linear regression analyses revealed that higher urinary MMA concentrations were consistently associated with reductions in multiple growth indicators, while increased DMA levels were linked to decreased height outcomes. Our observations indicate that increased arsenic exposure could have an impact on growth in young children in US.

Urine samples are frequently used in environmental studies, such as NHANES or the German Environmental Survey for Children (Esteban and Castaño, 2009; Padilla et al., 2010; Shao et al., 2017). In this study, we used the concentrations of arsenic species measured in urine as valid biomarkers of arsenic and its metabolites exposure (Castiello et al., 2020; Esteban and Castaño, 2009; García-Villarino et al., 2021; Shao et al., 2017). ∑As is a short-term biomarker of iAs exposure; however, it can also reflect long-term exposure to iAs among individuals who have consistent exposure patterns. This is particularly relevant for children, whose diets are typically less diverse compared to adults (Kile et al., 2009; Navas-Acien et al., 2011; Signes-Pastor et al., 2017a). In addition to diet and other environmental factors, drinking water is a contributor

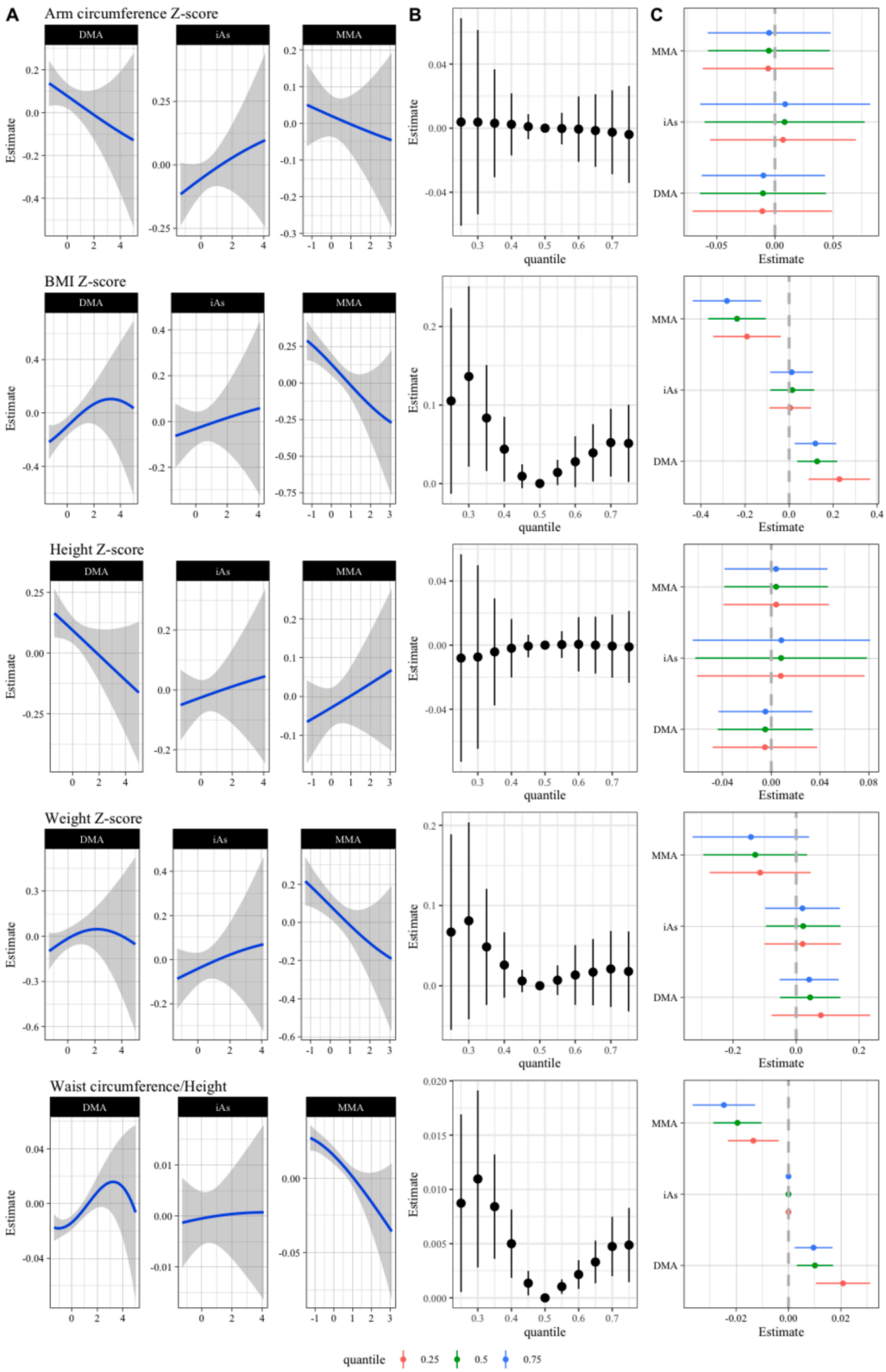

Fig. 2. Arsenic exposure and anthropometric indicators in 6–12-year-old children. BKMR dose–response function and interaction within the arsenic mixture (n = 1,792). Models adjusted for age and sex of children, race, total calorie intake, seafood and shellfish consumption, poverty-income-ratio and cotinine. **A**, Univariate exposure–response functions and 95 % confidence bands for each metabolite with the other species fixed at the median. **B**, Joint effect (95 % credible interval) of the mixtures on growth indicators. All arsenic species in a particular quantile were compared to all arsenic mixture at their median. **C**, Single pollutant association (estimates and 95 % credible intervals, gray dashed line at the null). This plot compares children' size when a single pollutant is at 75th versus 25th percentile, when all the other exposures are fixed at either the 25th, 50th, or 75th percentile. Notice that the scale of the x- and y-axis vary in order to facilitate the visualization of the estimates in each plot. DMA = dimethylarsinic acid, MMA = monomethylarsonic acid, iAs = Arsenite (As^{III}) + Arsenate (As^{V}).

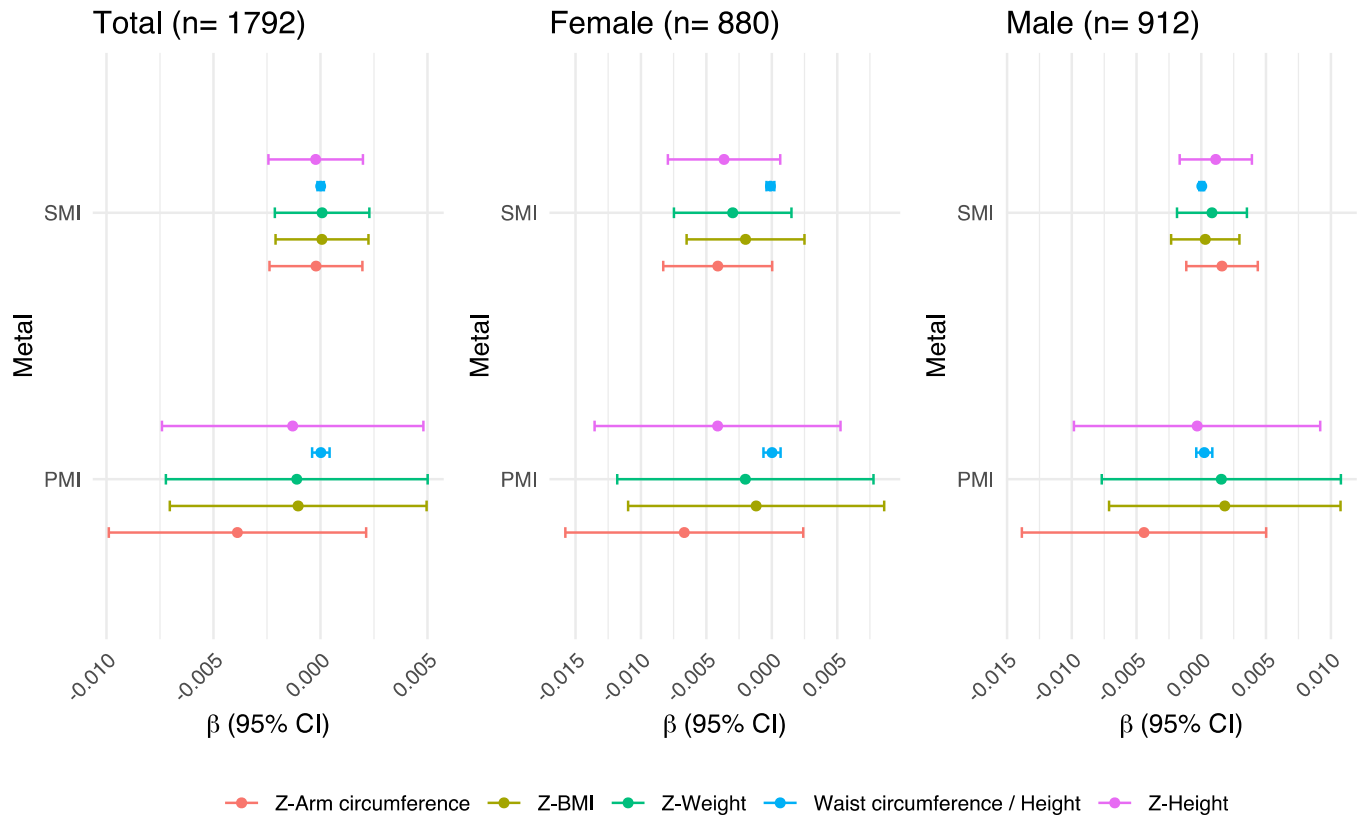

Fig. 3. Linear regression estimates of mean percent differences in arsenic methylation indices per interquartile range (IQR)-unit differences in anthropometric measures among children. DMA = dimethylarsinic acid, MMA = monomethylarsonic acid. iAs = Arsenite (As^{III}) + Arsenate (As^{V}). PMI = primary methylation index (MMA/iAs). SMI = Secondary methylation index (DMA/MMA). Models adjusted for age and sex of children, race, total calorie intake, seafood and shellfish consumption, poverty-income-ratio and cotinine.

to metalloid exposure in our study population (Buckley et al., 2020; Signes-Pastor et al., 2019a). The median urinary ΣAs levels of the children in our study were approximately 10 times lower than those reported for 4.5- and 9-year-old children from the MINIMat cohort, who were exposed to arsenic-contaminated drinking water (Rahman et al., 2009; Saha et al., 2012). However, the urinary concentrations in children enrolled in NHANES cycles 2007–2020 were similar to those of other child populations who consume water that meets the maximum arsenic levels of < 10 μg/L set by the EU (Castiello et al., 2020; Freire et al., 2018; García-Villarino et al., 2021), US (Pace et al., 2018; Padilla et al., 2010; Shao et al., 2017), and WHO.

This study examines the impact of exposure to a mixture of arsenic metabolites on infant growth. While many studies have explored the relationship between prenatal arsenic exposure and birth outcomes (Claus et al., 2016; Gilbert-Diamond et al., 2016; Howe et al., 2020; Laine et al., 2015; Muse et al., 2020; Rahman et al., 2009), the effects on fetal, infant, and child growth remain inconclusive. Notably, research from Bangladesh indicated a sex-dependent impact on child growth, affecting only girls (Saha et al., 2012). This contrasts with our findings, where MMA exposure was more strongly associated with BMI Z-score and waist circumference/height ratio in total sample and males. Differences in exposure levels may explain these discrepancies, with our study having ten times lower exposure levels. Other studies, such as Gardner et al.'s cohort in Bangladesh, found negative correlations between arsenic exposure and growth indicators in children, aligning with our results (Gardner et al., 2013). Another investigation in rural Bangladesh carried out by Alao et al. showed that urinary As concentrations in the highest tertile were meaningfully associated with underweight and acute malnutrition (Alao et al., 2021). Moreover, some studies, such as those by Ma et al. and Wang et al., have reported negative correlations between arsenic exposure and child growth, which are consistent with the associations observed for MMA in our findings (Ma et al., 2023; Wang et al., 2007). The sex-specific effects observed in our study align with prior evidence indicating that males and females may exhibit differential susceptibility to arsenic exposure, potentially driven by hormonal influences, differences in methylation capacity, and other metabolic factors (Naujokas et al., 2013; Vahter et al., 2007). This distinction underscores the need to consider sex as a modifier in arsenic-related health outcomes. Toxicological studies have also suggested that estrogen may play a protective role in arsenic metabolism, further supporting the plausibility of our findings (Che et al., 2019).

The catch-up growth hypothesis could provide a meaningful framework for interpreting the observed associations between arsenic exposure and growth indicators in our study. Catch-up growth refers to the rapid growth observed in children who initially experience growth retardation but subsequently accelerate their growth to achieve their genetic growth potential (Wit and Boersma, 2002). This phenomenon is particularly relevant when examining early-life exposures, such as in utero or during infancy, which may result in initial growth deficits followed by compensatory growth patterns later in childhood. If arsenic exposure influences early growth retardation, as suggested by previous study (Rahman et al., 2017), it is possible that catch-up growth mechanisms could mitigate or mask the longer-term impacts of exposure in our observed data. Moreover, the interplay between nutritional factors, metabolic processes, and arsenic methylation capacity could further modulate these growth patterns (Abuawad et al., 2021a). Future research should explore this hypothesis in longitudinal designs, as such studies could provide clearer insights into the potential role of catch-up growth in mediating or modifying the effects of early arsenic exposure on growth trajectories.

The observed positive associations between DMA and some growth indicators, alongside the negative associations for MMA, may reflect

differences in the toxicity and metabolic pathways of these arsenic metabolites. MMA is recognized as more toxic than DMA, and the latter is typically associated with more advanced methylation and detoxification processes (Tseng, 2009; Vahter, 2002). This suggests that higher DMA levels could signal greater methylation efficiency, potentially mitigating arsenic's adverse effects on growth (Naujokas et al., 2013; Thomas, 2021). PIP analysis supports this observation, showing that DMA consistently exhibited higher contributions across most outcomes, particularly in males, while MMA had lower contributions overall but was more prominent among females for certain indicators. However, this hypothesis requires further exploration, as toxicological evidence indicates that MMA and DMA may exert distinct biological effects (Engström et al., 2011). Additionally, our study is, to our knowledge, the first to focus exclusively on the impact of arsenic metabolite mixtures on growth indicators in children, which adds novelty to the findings. Nevertheless, this uniqueness also highlights a limitation: we cannot yet fully understand how interactions between arsenic metabolites behave in other populations with varying exposure levels or methylation capacities (Kile et al., 2014; Thomas, 2021). The results of the sensitivity analysis using obesity categories align with the findings of the linear regression and BKMR models, particularly regarding the association between MMA and lower growth indicators. The consistent association of MMA with increased odds of underweight and reduced odds of overweight or obesity further reinforces its potential impact on childhood growth and nutritional status, highlighting. The robustness of our findings.

Our study also explored the relationship between the PMI and SMI with the growth indicators in children (Kuo et al., 2017). These indexes are commonly used to assess the efficiency of arsenic methylation, with higher SMI indicating greater methylation efficiency (Wei et al., 2016). Additionally, methylation indices are often used as indicators of the body's detoxification capacity, as more efficient methylation of arsenic is generally associated with a reduced toxicity burden. Despite conducting comprehensive analyses, we did not find any substantial associations between either PMI or SMI and the growth indicators. Nevertheless, there are plausible biological mechanisms suggesting that adiposity could promote arsenic methylation (Smith et al., 2023). The process of arsenic methylation involves multiple interconnected pathways associated with one-carbon metabolism (Vahter, 2009). Since adipose tissue produces estrogens (Kershaw and Flier, 2004), it is proposed that adiposity could lead to increased estrogen, which boosts choline and thus arsenic methylation (Bustaffa et al., 2020). However, choline might also promote adiposity (Zeisel, 2013), and the temporal relationship among these factors remains unclear (Abuawad et al., 2021b). More longitudinal studies with specific adiposity measures, such as body fat percentage, waist circumference/height ratio, skinfold thickness, or advanced imaging techniques, are needed to clarify these mechanisms. Studies with repeated measures of adiposity and arsenic methylation biomarkers could provide valuable insights into the causal relationships between these factors. Alternatively, it could indicate that other factors, such as genetic variability, nutritional status, or concurrent exposure to other environmental toxins, may play a more crucial role in influencing these growth indicators. Previous research has shown mixed results regarding the relationship between arsenic methylation and health outcomes, with some studies indicating a protective effect of efficient methylation and others finding no substantial associations (Concha et al., 1998; Kuo et al., 2017; Smith et al., 2023). Our findings align with those studies suggesting that the complexity of arsenic metabolism and its health effects cannot be fully captured by methylation indices alone. Cross-sectional studies face limitations in capturing the temporal relationship between arsenic exposure, methylation, and health outcomes, which may vary over time. This constraint could underlie the lack of statistically significant results in our study. Longitudinal research would more effectively examine these changes and elucidate arsenic methylation's impact on health.

This study has some limitations. One important challenge is the assessment of arsenic species in populations with low exposure levels, which often results in a high percentage of non-detectable values due to the constraints of current measurement methods and equipment. This issue underscores the need for further methodological advancements to better address low-level exposures and their health implications, as this is an increasingly important area of research in environmental health. In our study, it should be noted that some arsenic species had a considerable proportion of values below the LOD, which were imputed using a fixed value (LOD divided by the square root of two). This approach may reduce the variability of the resultant estimates, potentially leading to narrower confidence intervals and overstated precision of our findings (Helsel, 2006; Hornung and Reed, 1990; Lubin et al., 2004). Although its simplicity compared to other methods facilitates the application of complex analysis techniques such as BKMR. Secondly, our analyses were cross-sectional, meaning that exposure levels and anthropometric indicators were measured simultaneously, which prevents us from determining the directionality or establishing a temporal relationship between iAs metabolite exposure and growth indicators in children. Future studies should replicate these associations using longitudinal data with repeated measurements over time. Third, the use of creatinine to standardize urinary arsenic concentrations. While specific gravity would provide a more accurate adjustment for urine dilution, this measure was not available in the NHANES dataset. Fourth, urinary arsenic species were used to evaluate arsenic methylation. After absorption, arsenic undergoes methylation mainly in the liver. Previous studies in Bangladesh have shown a strong correlation between arsenic concentrations in urine and blood (Hall et al., 2006). However, the proportions of arsenic species in urine may not exactly reflect their respective concentrations in blood or tissues. Despite this, the use of urine samples as biomarkers of internal arsenic exposure is also one of the major strengths of our study. This method offers distinct advantages over other biomarkers, such as nails or hair, as it provides a more accurate reflection of long-term arsenic exposure. The use of urine samples is particularly beneficial as it captures the cumulative exposure over time, rather than just a snapshot, which is crucial for understanding the impact on children's health. Additionally, the data spanning several years allows for a comprehensive analysis of how these exposures affect the pediatric population. The relatively low fish and seafood consumption among the study participants supports our focus on iAs metabolites as indicators of exposure. This approach provides robust evidence to support our findings on the relationship between arsenic exposure and its effects on body size and growth in children. The study's strengths include its novel focus on evaluating the combined effects of arsenic metabolite mixtures (iAs, MMA, DMA) on child growth indicators, utilizing advanced BKMR models to capture complex, non-linear interactions. The use of age-specific Z-scores ensures precise standardization of outcomes, enhancing comparability across age groups. Stratified analyses by sex provide insights into potential gender-specific effects, while the integration of linear regression and BKMR offers a comprehensive view of both individual and combined associations. Lastly, the study's use of NHANES data, spanning multiple cycles (2007–2020), ensures broad applicability and relevance to diverse populations.

5. Conclusions

Childhood exposure to a mixture of arsenic species appears to influence growth indicators in children enrolled in NHANES, indicating that even low levels of arsenic exposure can adversely affect physical development. These findings underscore the importance of mitigating arsenic exposure to protect child health and support optimal growth. Longitudinal studies are required to evaluate the factors influencing changes in arsenic methylation throughout childhood.

CRediT authorship contribution statement

Miguel García-Villarino: Writing – original draft, Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rocío Fernández-Iglesias:** Writing – original draft, Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Ana Victoria García:** Writing – review & editing, Investigation. **Elsa Villa-Fernández:** Writing – review & editing, Investigation. **Lucía Fernández-Arce:** Writing – review & editing, Methodology, Investigation. **Isolina Riaño-Galán:** Writing – review & editing, Methodology, Investigation. **Carmen Lambert:** Writing – review & editing, Investigation. **Vicente Martín:** Writing – review & editing, Funding acquisition. **Margaret R. Karagas:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Elías Delgado-Álvarez:** Writing – review & editing. **Ana Fernández-Somoano:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition. **Antonio J. Signes-Pastor:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109347.

Data availability

Data will be made available on request.

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