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Urinary arsenic species and methylation efficiency during pregnancy: Concentrations and associated factors in Spanish pregnant women

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ABSTRACT

Background: Arsenic (As) is considered to be toxic for humans, the main routes of exposure being through drinking water and the diet. Once ingested, inorganic arsenic can be methylated sequentially to monomethyl and dimethyl arsenicals. Several factors can affect both As exposure and methylation efficiency.
Objectives: To describe the urinary concentrations of the different As species and evaluate the methylation efficiency during pregnancy, as well as their associated factors in a birth cohort of pregnant Spanish women.
Methods: Participants in this cross-sectional study were 1017 pregnant women from two areas of Spain who had taken part in the INMA (Environment and Childhood) project (2003–2008). Total As (organic and inorganic compounds) and its main metabolites (monomethylarsonic acid, [MMA], dimethylarsinic acid, [DMA], inorganic As [iAs]) and arsenobetaine [AB]) were measured in urine samples collected during the first trimester. Socio-demographic and dietary information was collected through questionnaires. Multivariate linear regression models were used to explore the association between As species concentrations and covariates. Arsenic methylation efficiency was determined through the percentages of the metabolites and using As methylation phenotypes, obtained from principal component analysis.
Results: Median urine concentrations were 33.0, 21.6, 6.5, 0.35 and 0.33 μg/g creatinine for total As, AB, DMA, MMA and iAs, respectively. Daily consumption of rice and seafood during the first trimester of pregnancy were positively associated with the concentration of As species (i.e., β [CI95%] = 0.36 [0.09, 0.64] for rice and iAs, and 1.06 [0.68, 1.44] for seafood and AB). TAs, AB and iAs concentrations, and DMA and MMA concentrations were associated with legume and vegetable consumption, respectively. The medians of the percentage of As metabolites were 89.7 for %DMA, 5.1 for %MMA and 4.7 for %iAs. Non-smoker women and those with higher body mass index presented a higher methylation efficiency (denoted by a higher %DMA and lower %MMA).

Abbreviations: AB, Arsenobetaine; As, arsenic; AS3MT, Arsenic [+3 oxidation state] methyltransferase gene; BMI, body Mass Index; Cd, cadmium; CI, confident intervals; DMA, dimethylarsinic acid; EFSA, European Food Safety Authority; FFQ, food frequency questionnaire; GM, geometric mean; GSH, glutathione; HPLC, high-performance liquid chromatography; iAs, inorganic arsenic; ICP-MS, inductively coupled plasma mass spectrometry; INMA, INfancia y Medio Ambiente (Environment and Childhood); LOD, limit of detection; MMA, monomethylarsonic acid; Mn, manganese; oAs, organic Arsenic; OCM, one-carbon metabolism; PCA, principal component analysis; SAM, S-adenosylmethionine; SD, standard deviation; Se, selenium; TAs, total arsenic; ΣAs, sum of iAs DMA and MMA; w.w., wet weight; WHO, World Health Organization; Zn, zinc.

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Discussion: Certain dietary, lifestyle, and environmental factors were observed to have an influence on both As species concentrations and methylation efficiency in our population. Further birth cohort studies in low exposure areas are necessary to improve knowledge about arsenic exposure, especially to inorganic forms, and its potential health impact during childhood.

1. Introduction

Arsenic (As) is a toxicant that appears naturally in the soil; however, several human activities also contribute to the presence of As in the environment (European Food Safety Authority, 2009; Fowler et al., 2015). Arsenic can be classified into two groups: organic and inorganic compounds, the inorganic forms being the more toxic species. In some areas of Bangladesh, India, Vietnam, China, Argentina, Chile, Mexico, Australia and USA, the levels of As in drinking water are above the maximum guideline value recommended by the World Health Organization (WHO) in 2003 (10 μg/L) (World Health Organization, 2017), water consumption being the main exposure route for iAs. In regions with low levels of As in water, such as Spain, the main source of exposure to inorganic As (iAs) is rice consumption (European Food Safety Authority, 2014). Regarding organic As (oAs), the main contributor is the consumption of seafood. Arsenobetaine (AB) is usually the major form of arsenic in fish and other seafood. This compound and other oAs forms, such as arsenosugars and arsenolipids, are generally considered less toxic than iAs, although in vitro studies revealed cytotoxic effects of certain arsenic-containing hydrocarbons (Bornhorst et al., 2020).

Arsenic absorbed by the gastrointestinal tract is biotransfomed mainly in the liver (Drobná et al., 2010). Currently, the biotransformation mechanism is not entirely clear, and several possible pathways have been described (Cullen, 2014). The classic pathway proposed by Challenger (1945) was based on the enzymatic reduction and oxidative methylation of As. In this scheme, ingested iAs is reduced (from arsenate -iAs^{V}- to arsenite -iAs^{III}-) and methylated to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), S-adenosylmethionine (SAM) being the main donor of the methyl group (Cullen, 2014; Howe et al., 2014). After this biotransformation process, iAs is excreted through the urinary system. In general, the most frequent proportions of the metabolites observed in urine are 60–80% of DMA, 10–20% of MMA and 10–30% of iAs (Vahter, 1999). These relative concentrations reflect the iAs methylation efficiency, indicated by a high %DMA and lower %MMA and %iAs, especially in populations exposed to high levels of iAs through drinking water (Vahter, 1999). This methylation process is considered to be a detoxification mechanism, although high reactivity and toxicity of intermediate compounds, such as MMA, has been demonstrated (Vahter, 2002). Several factors can influence the methylation efficiency, such as age, sex, alcohol and tobacco consumption (Shen et al., 2016; Tseng, 2008). Some nutritional factors seem to increase the efficiency of iAs methylation, especially micronutrients involved in one-carbon metabolism (OCM), such as vitamins B_6 and B_{12}, betaine, choline and folic acid (Bozack et al., 2019; Gamble et al., 2006; Heck et al., 2007; Howe et al., 2017a; Kurzius-Spencer et al., 2017; Laine et al., 2018; Spratlen et al., 2017). Likewise, other experimental and epidemiological studies have shown that some elements, such as selenium (Se), manganese (Mn), zinc (Zn), or cadmium (Cd) can have an influence on As metabolism or interact with this metalloid (Nordberg et al., 2005; Rahman et al., 2019; Sun et al., 2014; Valeri et al., 2016). Regarding organic forms, AB is excreted unchanged in urine, but other organic forms, such as the arsenosugar and arsenolipid species present in seafood, seem to be metabolized and produce DMA (European Food Safety Authority, 2009; Molin et al., 2012; Taylor et al., 2017a).

The effects of As exposure have been studied extensively. In 2009, As and iAs compounds were classified as carcinogenic to humans (International Agency for Research on Cancer, 2012). Other health effects described in adults have been an increased risk of respiratory, cardiovascular, and metabolic diseases (Agency for Toxic Substances and Disease Registry, 2016; Moon et al., 2012; Sanchez et al., 2016). Furthermore, a lower As methylation efficiency has been related to a higher risk of skin lesions, bladder, lung and skin cancer, and peripheral vascular disease (Gamboa-loira et al., 2017; Tseng, 2007). The fetus could be exposed to this toxicant due to transfer of maternal As across the placenta (Gossai et al., 2015). Methylation efficiency has been observed to be augmented during pregnancy, thus increasing the excretion of DMA (Hopenhayn et al., 2003). Nevertheless, several studies have identified adverse effects on fetal and child development caused by prenatal exposure to As, such as miscarriage, low birth weight, skin lesions, respiratory effects, and impairment of neuropsychological development (Freire et al., 2018; Hamadani et al., 2011; Parajuli et al., 2013; Quansah et al., 2015; Sanchez et al., 2016).

Therefore, the aim of this study is to describe the concentrations of total As (TAs) and the different urinary As species (DMA, MMA, AB and iAs) and the methylation efficiency in a birth cohort of pregnant Spanish women. Additionally, we have studied the factors (nutritional, sociodemographic and lifestyle variables) associated with both As exposure and methylation efficiency.

2. Materials and methods

2.1. Study population

In this cross-sectional study, subjects were pregnant women participating in the INMA (Environment and Childhood) Project, a multicentre birth cohort study that aims to investigate the effect of environmental exposures and diet during pregnancy and childhood on fetal and child development in different geographical areas of Spain (http://www.proyectoinma.org).

The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, 1465 pregnant women were recruited during their first antenatal visit (2003–2008) in two regions of Spain: Gipuzkoa (north of Spain, n=638) and Valencia (east of Spain, n=855). The inclusion criteria were: at least 16 years of age, 10–13 weeks of gestation, singleton pregnancy, intention of undergoing follow-up and delivery in the corresponding centre of reference, and no impediment for communication. These women were monitored during pregnancy. The final study population was made up of 1017 mothers with available urinary arsenic species concentrations at the first trimester of pregnancy (69.4% of total recruited participants). These mothers were selected taking into account two criteria: 1) availability of longitudinal information about their children until 2 years old (in order to assess the potential health effects of prenatal As exposure in further studies), and 2) from the women who met the first criterion we randomly selected a subsample of 1017 due to limited funding for the As analysis.

The study protocol was approved by the Ethics Committee of the university hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa). Informed consent was obtained from all participants in each phase.

2.2. Study variables and sources of information

2.2.1. Outcome variable: urinary arsenic speciation analysis

Concentrations of total As and its metabolites were determined in spot urine samples collected in the first trimester of pregnancy (mean [SD]= 13.0 [1.2] weeks of gestation). Urine samples were kept frozen at −80 °C until analysis.

The total As concentrations were determined with an inductively coupled plasma tandem mass spectrometer (ICPMS/MS, 8800, Agilent Technologies, Waldbronn, Germany) with oxygen as the reaction gas at m/z 75 → 91. An external calibration was used for quantification, from 0.05 to 100 μg As/L. The certified reference materials (CRM) SRM 1640a (Trace elements in natural water, NIST, Gaithersburg, USA, n=12) and SRM 2669 I and II (Arsenic Species in Frozen Human Urine, n=14) were used for quality control. A calibration standard was re-measured after every 10th sample to monitor the stability of the measurement.

Chromatographic separation of the arsenic compounds was carried out in accordance with a previously validated method (Scheer et al., 2012). An external calibration was used for quantification. It contained arsenate, MMA, DMA and AB in the concentration range 0.05–100 μg As/L. Hydrogen peroxide (10% v/v) was added to oxidize the species. For quality control, the CRMs SRM 1640a (n= 5), SRM 2669 I (n = 8) and SRM 2669 II (n = 22) were prepared similar to the urine samples and also investigated. The 1.0 μg As/L calibration standard was injected regularly to control the stability of the measurement. Every 10th sample was also re-measured for the same purpose. Of all the samples analysed (1017), 102 were re-analysed, which represents 10%. These second measurements matched the first in 100 ± 3% of the cases.

HPLC (1200, Agilent Technologies) coupled to ICPMS/MS (8800, Agilent Technologies) was employed for speciation analysis. The arsenic signal was again recorded in oxygen reaction mode at m/z 75 → 91, with the addition of CO2 for signal enhancement.

TAs concentrations and the sum of all As species were compared. When the difference was larger than 15%, either the total arsenic or the speciation analysis was repeated (124 samples).

Limits of detection (LOD) of AB, DMA, MMA and iAs for the samples from Valencia were 0.02, 0.02, 0.03 and 0.03 μg/L and for the Gipuzkoa samples they were 0.02, 0.03, 0.03 and 0.02 μg/L. When samples were below LOD, ½ LOD was assigned for the statistical analysis (2.8% of samples for MMA levels and 2.3% of samples for iAs levels).

2.2.2. Covariates

2.2.2.1. Sociodemographic variables. Women filled in two questionnaires during their pregnancy, at the first and the third trimesters of gestation (mean [SD]= 13.1 [1.5] and 32.5 [2.2] weeks of gestation, respectively). The questionnaires were administered by trained interviewers and focused on sociodemographic, environmental and lifestyle information. In both cohorts, the questionnaires were offered in Spanish and, additionally, in the co-official language (Basque) in the Gipuzkoa cohort. We selected the covariates used in this study from the previous literature on this topic (European Food Safety Authority, 2014; Nigra et al., 2019; Saxena et al., 2018; Shen et al., 2016; Tseng, 2008): age at conception (years), education level (up to primary, secondary, university), place of birth (Spain, Latin America, other), body mass index (BMI, kg/m^2) before pregnancy (continuous and categorized by low and healthy weight [<25], overweight [25 ≤ 30], obesity [≥30]), parity (0, ≥1), working status at first trimester of pregnancy (non-worker, worker), area of residence (rural, non-rural), tobacco and alcohol consumption in the first trimester of pregnancy (yes, no), and season of sample collection (spring, summer, winter, autumn). Subjective variables about the proximity of the residence to industrial or agricultural areas (yes, no) and frequency of traffic near the residence (continuous, quite frequent, infrequent/never) were collected.

We defined parental social class from the maternal or paternal occupation during pregnancy with the highest social class, according to the Spanish adaptation of the International Standard Classification of Occupations coding system approved in 1988 (ISCO88). Class I + II included managerial jobs, senior technical staff, and commercial managers; Class III included skilled non-manual workers; and class IV + V included manual and unskilled workers.

2.2.2.2. Dietary variables. Information on diet during the first trimester of pregnancy was obtained from a 100-item semi-quantitative food frequency questionnaire (FFQ) completed at the time of sampling. The dietary information covered the time from the last menstruation to the first prenatal visit, which occurred between weeks 10 and 13 of pregnancy. This FFQ was validated with good reproducibility for nutrient and food intake (Vioque et al., 2013). The items had nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. A commonly used serving size was specified for each food item in the FFQ. This was converted to average daily intake in grams for each individual participant. We obtained data on the intake of dairy products, eggs, meat, seafood and shellfish, fruits, vegetables, legumes, nuts, potatoes, cereals and bread, and coffee and other infusions. Some foods were explored in a more comprehensive manner due to their clear relationship with As concentrations, as is the case of rice, other cereals and bread, fish split into categories (lean fish, oily fish and shellfish and molluscs), and meat split into categories (red and white meat) (European Food Safety Authority, 2009). Moreover, we obtained information about consumption of tap water (less than 1 glass [250 cc] a day, 1 or more glasses a day).

Additionally, dietary folate, folic acid, vitamins B_{12} and B_6, iron (Fe) and Zn intake were estimated using the food composition tables of the US Department of Agriculture (U.S. Department of Agriculture: Agricultural Research Service USDA, 2007) and with Spanish sources (Palma et al., 2008). Energy-adjusted intakes were computed using the residual method (Willet, 2013). Information on the intake of supplements (brand name, dose and composition) was also collected, converted into nutrient intake daily dose, and added to the calculation of total daily nutrient intake (Vioque et al., 2013). Moreover, categorical variables were created for estimated vitamins B_{12} and B_6, folate, Fe and Zn intake (<or ≥ the Population Reference Intake for Fe, Zn and vitamin B_6 and adequate intake for folate and vitamin B_{12}) (European Food Safety Authority, 2019).

2.2.2.3. Other elements. Mn and Se concentrations were determined in serum samples taken at the first trimester of pregnancy. More information about the methodology has been reported in detail elsewhere (Lozano et al., 2020; Soler-Blasco et al., 2020). Cd concentrations were determined by ICPMS in urine samples taken at the first trimester of pregnancy. Creatinine concentrations were measured in the same urine samples at the first trimester of pregnancy by DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter. The quantification of maternal plasmatic levels of ferritin was performed by fluoroimmunoassay (DELFIA Ferritin kit A069-101), in the Gipuzkoa Public Health Laboratory. In Valencia, the quantification was performed at La Fe hospital through immunoturbidimetry in Beckman Coulter AU analysers (Arija et al., 2019).

2.3. Statistical analysis

Descriptive and bivariate analyses were performed using Fisher's Exact Test for categorical variables and the Kruskall Wallis Test for continuous variables in order to detect any differences between the included and the excluded populations.

In populations with moderate-high fish/seafood consumption, the ΣAs could not properly reflect the exposure to inorganic As, because oAs from seafood contributes to DMA levels and TAs (Navas-Acien et al., 2011). To eliminate the influence of seafood arsenicals, we calibrated the methylated and non-methylated species concentrations using a mathematical method proposed by Jones et al. (2016). In brief, AB concentrations were used as a marker of seafood consumption. Using linear regression models, calibrated iAs, DMA and MMA concentrations were estimated by regressing the measured concentrations of iAs, MMA, and DMA on AB and creatinine concentrations (all measures were log-2 transformed) in three separate models. The new calibrated iAs, MMA

and DMA concentrations were calculated by adding the residual of each metabolite model to a constant (mean level of each metabolite estimated from participants with AB<1 μg/L).

We calculated the geometric mean (GM) and 95% confidence intervals (95%CI) of the urinary TAs, AB, DMA, MMA, iAs, and ΣAs (as the sum of iAs, DMA and MMA]) for both measured and calibrated concentrations. Additionally, GM and 95%CI of the measured concentrations were calculated according to sociodemographic, environmental and dietary characteristics of the study population. Concentrations were expressed in μg/L and corrected to creatinine content (μg/creatinine). The GMs were compared using the ANOVA F-test. For further analysis, we used the log2 and probit-transformed values of the urinary As species concentrations and the percentage of the individual metabolites, respectively, to approach normality.

Bivariate and multivariate linear regression models were built in order to study the relationship between urinary As concentrations and the sociodemographic, environmental, and dietary factors. Beta coefficients (β) and 95%CI were obtained. A two-step procedure was used to construct the multivariate models. First, core models were built using all the sociodemographic and environmental covariates associated with a p value < 0.2 in the bivariate analysis. Following a backward elimination procedure, the covariates associated with each species at a level of p value < 0.1 in the likelihood ratio test were retained in the model. Second, each food group was adjusted in the core model individually. The final multivariate models were built using the core model and all the food groups associated with each species at a level of p value < 0.1 in the likelihood ratio test. Although food intake or estimated nutrients variables were mutually correlated (Fig. S1 and Fig. S2), we found no collinearity among them in the final models. The area of study (Valencia or Gipuzkoa) and urinary creatinine levels in the first trimester of pregnancy were included in all the models regardless of their statistical significance.

Methylation As efficiency was determined through two approaches. First, by calculating the percentage of the individual calibrated metabolites (iAs, MMA and DMA) over the sum of those species (ΣAs). Second, a principal component analysis (PCA) of the three calibrated, untransformed and un-rotated percentages was performed. The main reason for carrying out a PCA was to avoid the high correlation between the percentages of the three metabolites, by transforming the three interrelated variables into two independent measures of As metabolism phenotypes (Balakrishnan et al., 2016; Gribble et al., 2015; Jansen et al., 2016; Spratlen et al., 2017). The results obtained through the PCA analysis were two principal components (PC1 and PC2) that explained 100% of the original variance. Therefore, these PC were used as As methylation phenotypes.

We analysed the factors associated with the methylation efficiency using the same procedure as described above. In particular, we performed five different models by using the probit-transformed calibrated %DMA, %MMA, %iAs and untransformed PC1 and PC2 as outcome variables. In these models, the variables used in the second step were the estimation of nutrients and vitamin intake described above and Se, Mn and Cd concentrations. The area of study was also included.

To check whether linear regression assumptions were met, we visually inspected model residuals for normality and homoscedasticity. No influential data were identified by Cook's distance. Variance inflation factors (VIFs) were used to test for collinearity among variables in the final models, all VIFs being <2.5. To deal with the possibility of minor deviations from normality and homoscedasticity, confidence intervals were calculated on the basis of robust standard error in the final results.

Statistical analyses were carried out using R statistical package version 3.5.1 (R Core Team, 2017).

3. Results

3.1. TAs and As metabolite concentrations and associated factors

Differences between included and excluded subjects are shown in Table S1. Among the participants, there was a higher percentage of slightly older women with a higher level of education and higher social class than among the excluded women. There were no differences in rice consumption. Participants showed higher fish consumption than the excluded women. Among the participants, there was a slightly lower percentage of women who did not take any vitamin supplement during the period of the study (4% vs. 7%).

The GM [95%CI] of measured urinary TAs, ΣAs and AB concentrations were 35.55 [33.10–38.19], 7.74 [7.41–8.09] and 20.17 [18.34–22.19] μg/g creatinine, respectively. Table 1 shows measured and calibrated As concentrations and the metabolite percentages. The calibrated concentrations of metabolites were lower in all cases, especially for DMA concentrations (GM [95%CI] 6.82 [6.52, 7.14] μg/g creatinine and 2.98 [2.85, 3.09] μg/g creatinine, in measured and calibrated DMA concentrations, respectively). Correlations between measured and calibrated metabolite concentrations were high (Pearson correlation coefficient of 0.85 for DMA, and 0.99 for MMA and iAs, p value < 0.01 in all cases). The correlation between DMA and TAs decreased from 0.69 to 0.23 when the metabolite was calibrated **(Fig. S3).**

Table 1
Geometric mean and 95% confidence intervals of measured and calibrated[a] urinary As concentrations and percentages. INMA Project (Valencia and Gipuzkoa, Spain. 2003–2008).

	Measured concentrations		Calibrated concentrations[a]	
As concentrations	μg/L	μg/g creatinine	μg/L	μg/g creatinine
TAs	28.89 (26.81, 31.13)	35.55 (33.10, 38.19)		
AB	16.40 (14.88, 18.08)	20.17 (18.34, 22.19)		
ΣAs	6.28 (5.98, 6.59)	7.74 (7.41, 8.09)	2.94 (2.82, 3.06)	3.62 (3.48, 3.76)
DMA	5.54 (5.27, 5.82)	6.82 (6.52, 7.14)	2.41 (2.31, 2.51)	2.97 (2.85, 3.09)
MMA	0.28 (0.26, 0.29)	0.34 (0.32, 0.36)	0.19 (0.18, 0.20)	0.23 (0.22, 0.24)
iAs	0.27 (0.25, 0.29)	0.33 (0.31, 0.35)	0.21 (0.20, 0.23)	0.26 (0.25, 0.28)
Metabolite percentages				
%DMA[b]	89.7 (89.3, 90.2)		84.4 (83.9, 84.9)	
%MMA[b]	5.1 (4.8, 5.3)		7.1 (6.7, 7.3)	
%iAs[b]	4.7 (4.5,5.0)		7.6 (7.3, 8.0)	

Note: TAs, Total As; ΣAs, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As.
The percentages of each metabolite were calculated: levels of the metabolite/(DMA + MMA + iAs)*100; μg/L: micrograms per litre. μg/g creat: micrograms per gram of creatinine.

[a] As metabolite concentrations corrected by arsenobetaine concentrations;
[b] Median (95% confidence intervals).

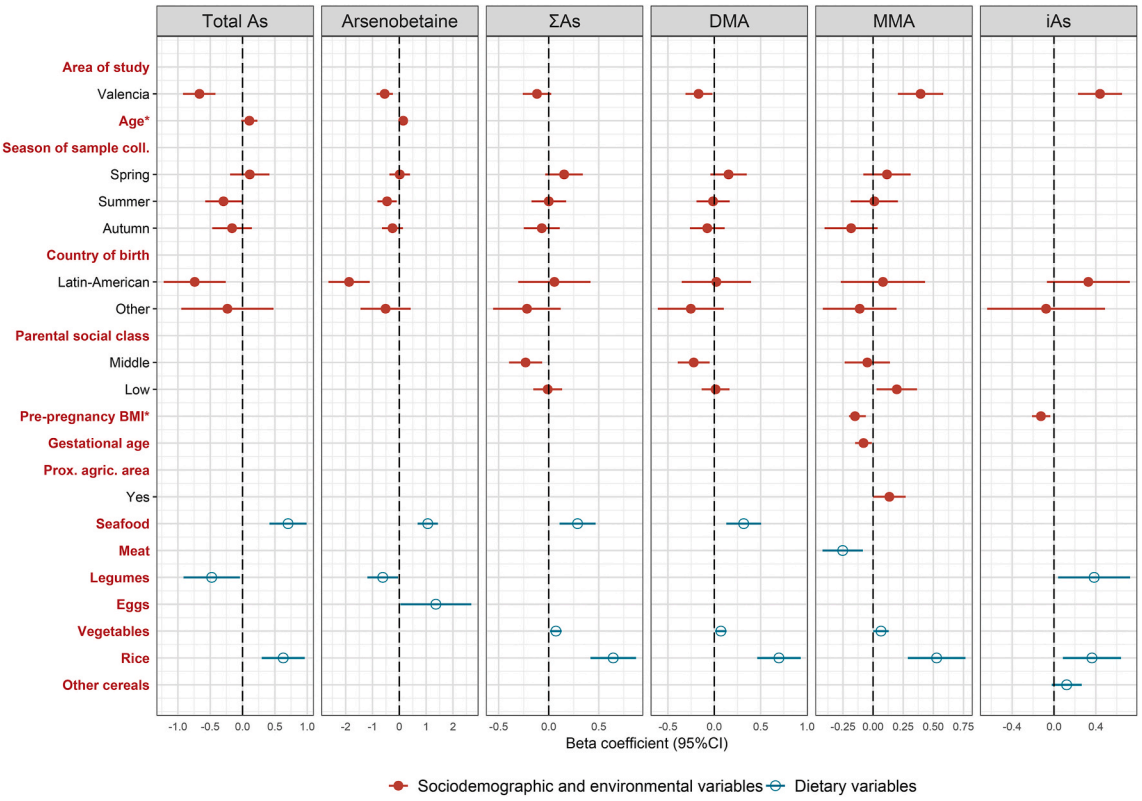

Fig. 1. Beta coefficients (CI95%) of the multivariate linear regression between measured levels of arsenic metabolites in maternal urine, and sociodemographic and dietary factors. INMA Project (Valencia and Gipuzkoa, Spain, 2003–2008). Note: TAs, Total As; ∑As, sum of DMA, MMA and iAs; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, inorganic As; ref, category reference; Coll, collection: Prox.agric.area, proximity to agricultural area. Dietary factors are expressed in 100 g per day at first trimester of pregnancy, adjusted for calories. Reference categories: area of study (Gipuzkoa); season of sample collection (winter); maternal country of birth (Spain); parental social class (high); proximity to agricultural area (no). Samples used in all models were n=1005, except for log2 MMA model (n=994). TAs, AB, ∑As, DMA, MMA and iAs concentrations were log2-transformed.*Age and pre-pregnancy BMI variables were expressed as increments of 5 years and 5 kg/m^2, respectively.

Table S2 shows the total As and metabolite concentrations by participant characteristics. Women from Gipuzkoa presented higher TAs and AB concentrations and lower levels of MMA and iAs than those from Valencia. Latin-American women presented higher concentrations of iAs and MMA than the Spaniards, and very low AB levels. The urine samples collected during spring presented higher levels of TAs, ΣAs, DMA, MMA and iAs. Consumption of more than 1 serving of rice per week was associated with an increase in DMA, MMA and iAs concentrations. Consumption of fish (<1 serving per week, 1 serving per week, > 1 serving per week) showed a clearly increasing trend with AB and TAs concentrations.

The multivariate models for the factors associated with the concentrations for each of the measured As species can be observed in Fig. 1. The area of study was significantly related to all As measures, except for ΣAs concentrations. Women from Valencia presented higher concentrations of MMA (β [95%CI]: 0.51 [0.35, 0.68], p value < 0.01) and iAs (β [95%CI]: 0.44 [0.23, 0.65], p value < 0.01), but lower concentrations of DMA (β [95%CI]: 0.17 [-0.31, −0.02], p= 0.03) and AB (β [95%CI]: 0.55 [-0.85, −0.24, p value < 0.01). Latin-American mothers showed lower concentrations of AB and the TAs (β [95%CI]: 1.88 [-2.65, −1.10], p value < 0.01, and −0.74 [-1.22, −0.26], p value =0.01, respectively). Parental social class was related to ΣAs and DMA concentrations, lower levels being observed in middle social class mothers (β [95%CI]: 0.23 [-0.40, −0.07], p value =0.01, and −0.22 [-0.39, −0.05], p value =0.01, respectively). Pre-pregnancy BMI was negatively associated with MMA and iAs concentrations (β [95%CI]: 0.03 [-0.04, −0.01], p value < 0.01, and −0.03 [-0.04, −0.01], p =0.01, respectively). Proximity of residence to an agricultural area was positively and significantly related to MMA concentrations (β [95%CI]: 0.14 [0.001, 0.27], p value =0.05).

Regarding dietary variables, a positive and significant association between seafood consumption and all As measures was observed, except for MMA and iAs concentrations, with a stronger association with AB concentrations (β [95%CI]: 1.06 [0.69, 1.43], p value < 0.01).

Similarly, rice consumption during pregnancy was positively associated with all As measures (β [95%CI]: 0.63 [0.27, 0.99], p value < 0.01 for TAs; 0.64 [0.46, 0.86], p value < 0.01 for ΣAs; 0.70 [0.47, 0.92], p value < 0.01 for DMA; 0.50 [0.26, 0.73], p value < 0.01 for MMA; and 0.36 [0.09, 0.64], p value =0.01 for iAs), except for AB concentrations. Consumption of other cereals was positively associated with iAs concentrations (β [95%CI]: 0.12 [-0.01, 0.25], p value =0.07). The association between the intake of legumes and TAs and AB concentrations was negative (β [95%CI]: 0.48 [-0.90, −0.05], p value =0.03; and −0.62 [-1.17, −0.07], p value =0.03, respectively), but positive for iAs (β [95%

Table 2
Summary of principal components analysis of calibrated[a] percentage As metabolites.

	PC1	PC2
Standard deviation	0.13	0.05
Proportion of variance	0.88	0.12
Weight for calibrated[a] %DMA	0.77	0.27
Weight for calibrated[a] %MMA	−0.15	−0.80
Weight for calibrated[a] %iAs	−0.62	0.53

Note: PC1, principal component 1; PC2, principal component 2; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As.

[a] As metabolite concentrations corrected for arsenobetaine and creatinine concentrations.

Table 3
Beta coefficient (95%CI) of the multivariate linear regression between methylation efficiency (measured by calibrated[1] percentage of As metabolites in maternal urine and principal component 1 and 2 of PCA) and sociodemographic, estimated nutrients intake (adjusted for calories) and essential and toxics elements factors. INMA Project (Valencia and Gipuzkoa, Spain, 2003–2008).

	Calibrated %DMA[a,b] (n= 1005)		Calibrated %MMA[a,b] (n= 998)		Calibrated %iAs[a,b] (n= 999)		PC1 (n= 992)		PC2 (n= 992)	
	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]	Beta (95%CI)	P[c]
Sociodemographic, environmental and lifestyle variables										
Area of study (ref. Gipuzkoa)										
Valencia	−8.90 (−13.95, −3.85)	<0.01	12.41 (7.22, 17.60)	<0.01	6.55 (0.73, 12.37)	0.02	−0.02 (−0.04, 0.00)	0.03	−0.02 (−0.02, −0.01)	<0.01
Place of birth (ref. Spain)										
Latin America	14.24 (1.50, 26.97)	0.03	−18.28 (−26.98, −9.59)	<0.01					0.03 (0.01, 0.04)	<0.01
Other	−3.87 (−18.13, 11.19)		0.55 (−9.78, 10.88)						0.01 (−0.01, 0.02)	
Working status (ref. non-worker)										
Worker			−4.84 (−9.34, −0.34)	0.05					0.01 (0.01, 0.02)	0.03
Parental social class (ref. I + II high)										
III							0.002 (−0.020, 0.025)	0.08		
IV + V (low)							0.020 (−0.001, 0.040)			
Tobacco consumption (ref. no)										
Yes					6.07 (−0.07, 12.20)	0.08	−0.022 (−0.042, −0.002)	0.03		
Body mass index	1.16 (0.60, 1.72)	<0.01	−0.83 (−1.31, −0.35)	<0.01	−0.94 (−1.52, −0.36)	<0.01	0.003 (0.002, 0.005)	<0.01	0.001 (0.000, 0.001)	0.02
Gestational age at sampling			−1.95 (−4.24, 0.35)	0.05						
Estimated daily intake of nutrients										
Estimated maternal zinc intake (mg/day)[d,e]	7.71 (−1.31, 16.81)	0.09								
Estimated maternal iron intake (mg/day)[d,e]							−0.016 (−0.036, 0.004)	0.02		
Estimated maternal folate ntake (μg/day)[d]									0.002 (0.000, 0.004)	0.01
Other toxic elements										
Maternal urine cadmium (μg/L)[e]			1.89 (0.29, 3.49)	0.02					−0.002 (−0.005, −0.00)	0.03

Note: 95%CI, 95% confidence intervals; %DMA, percentage of dimethylarsinic acid; %MMA, percentage of monomethylarsonic acid; %iAs, percentage of inorganic As. PCA, principal component analysis; PC1, principal component 1; PC2, principal component 2.
The percentages of each metabolite were calculated: levels of calibrated metabolite/(calibrated DMA + calibrated MMA + calibrated unmethylated iAs.
[a] Calibrated percentages were calculated with As metabolite concentrations corrected by arsenobetaine and creatinine concentrations;
[b] Probit-transformed appears
[c] p value from ANOVA F- test.
[d] Estimated daily intake of nutrients from the diet and supplementation.
[e] log2-transformed.Each model was simultaneously adjusted by all the presented variables.

Table 4
Geometric mean urinary arsenic concentrations and their metabolites measured in pregnant women in other published studies.

Study	Location	Gestational Age	N	Year of sampling	ΣAs	iAs	MMA	DMA	AB	%iAs	%MMA	%DMA
Present study	Spain (GIP-VAL)	1st trimester (13 weeks)	1017	2003–2008	7.7 (7.4, 8.1)[c]	0.33 (0.31, 0.359)[c]	0.34 (0.32, 0.36)[c]	6.8 (6.5, 7.1)[c]	20.17 (18.34, 22.19)[c]	4.3 (4.0, 4.5)[c]	4.4 (4.2, 4.6)[c]	88.2 (87.6, 88.7)[c]
Farzan (2020)	US	≤20 wk	241	2015–2019	ND	1.0 (0.6, 1.3)[b,f]	0.5 (0.2, 0.7)[b,f]	4.1 (3.0, 5.6)[b,f]	0.5 (0.2, 1.9)[b,f]	15.8 (10.0, 23.3)[b,f]	8.0 (6.0, 10.3)[b,f]	74.8 (65.9, 81.6)[b,f]
Gao (2019)	Bangladesh	4–16 weeks	1425	2008–2011	ND	6.5 (0, 98.9)[c,i]	3.8 (0, 57.9)[c,i]	65.1 (17.5, 530)[c,i]	ND	8.5 (0, 24)[c,i]	4.9 (0, 13.2)[c,i]	85.7 (66.6, 100)[c,i]
Howe (2020)	US	Early pregnancy (6–24 weeks)	167	2015	5.66 (1.96, 28.75)[a,g]	0.93 (0.17, 9.82)[a,g]	0.44 (0.12, 4.96)[a,g]	4.24 (0.82–21.11)[a,g]	0.50 (0.04, 478.82)[a,g]	15.3 (2.6, 63.4)[a,g]	8.0 (22.5, 94.1)[a,g]	75.1 (22.5, 94.1)[a,g]
Stajnko (2019)	Croatia-Slovenia	3rd trimester	136	2006–2011	3.23 (2.84, 3.68)[b]	As^{III}: 0.11 (0.10, 0.13)[b] As^{V}:<LOD	0.15 (0.13, 0.35)[b]	2.43 (2.06, 2.85)[b]	19.8 (14.8, 26.5)[b]	As^{III} 3.83 (3.16, 4.62)[b]	5.13 (4.40, 5.98)[b]	82.7 (77.2, 88.5)[b]
Ettinger (2017)	Canada	1st trimester	1933	2008–2011	ND	<50% below the LOD	<50% below the LOD	2.57 (2.49, 2.65)[b]	ND	ND	ND	ND
Laine (2015)	Mexico	At delivery	200	2011–2012	23.3 (4.3, 319.7)[b,g]	1.3 (0.14, 23.0[b,g]	1.4 (0.12, 18.2)[b,g]	20.6 (1.4, 292.5)[b,g]	ND	5.3 (0.77, 45.1)[b,g]	6.0 (0.68, 24.9)[b,g]	88.5 (32.7, 96.7)[b,g]
Neamtiu (2015)	Romania		10[j]	2011–2013	5.0 (3.7, 8.9)[b,h]	0.4 (0.4, 1.1)[b,h]	0.5 (0.2, 1.1)[b,h]	4.2 (2.0, 7.2)[b,h]	ND	ND	14.5 (5.9, 28.3)[b,h]	79.1 (52.7, 88.3)[b,h]
			10[k]		6.6 (3.9, 10)[b,h]	0.4 (0.3, 1.1)[b,h]	0.5 (0.4, 1.7)[b,h]	5.5 (3.1, 8.8)[b,h]	ND	ND	12.0 (9.1, 18.5)[b,h]	80.9 (77.2, 87.6)[b,h]
Chou (2014)	China	3rd trimester	299	2001–2002	22.6 (8.21, 36.72)[c,f]	0.79 (0.38, 1.49)[c,f]	0.46 (0.18, 2.03)[c,f]	20.01 (7.48, 32.30)[c,f]	ND	ND	ND	ND
Gilbert-Diamond (2011)	USA	24–28 weeks	229	2009	3.78 (1.80, 6.10)[a,f]	0.24 (0.13, 0.40)[a,f]	0.30 (0.14, 0.50)[a,f]	3.25 (1.51, 5.53)[a,f]	0.67 (0.07–5.47)[a,f]	ND	ND	ND
Gardner (2011)	Bangladesh	8 weeks	324	2001–2003	94 (3.0)[b,d]	ND	ND	ND	ND	16 ± 10[e]	11 ± 4.2[e]	73 ± 11[e]
		14 weeks			112.0 (3.0)[b,d]	ND	ND	ND	ND	14 ± 6.4[e]	7.6 ± 3.1[e]	79 ± 7.5[e]
		30 weeks			103 (2.9)[b,d]	ND	ND	ND	ND	10 ± 4.9[e]	6.5 ± 2.8[e]	83 ± 6.0[e]
Jay Christian (2006)	Chile	During pregnancy (not specified)	93	1998–2000	55.8 (41.6)[a,g]	5.6 (5.5)[a,g]	3.2 (4.0)[a,g]	46.9 (36.1)[a,g]	ND	11.0 (9.7)[a,g]	5.2 (4.7)[a,g]	83.8 (11.0)[a,g]

Note: ND, No data available; LOD, limit of detection; TiAs, Total inorganic iAs (sum of DMA, MMA and iAs); DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; iAs, unmethylated inorganic As.

[a] μg/L (unadjusted).
[b] adjusted by specific gravity.
[c] adjusted by creatinine (μg/g creatinine).
[d] geometric standard deviation.
[e] AM ± standard deviation.
[f] Median (percentile 25, percentile 75).
[g] Median (range).
[h] Median (CI95%).
[i] Median (percentile 5, percentile 95).
[j] Pregnant women exposed to average iAs water concentration <0.5 μg/l.
[k] Pregnant women exposed to average iAs water concentration 10.24 μg/l

CI]: 0.38 [0.06, 0.71]). Meat consumption was negatively associated with MMA concentrations (β [95%CI]: 0.26 [-0.43, −0.09], p value =0.02). When seafood consumption was replaced by the different fish categories (lean fish, oily fish, and shellfish and mollusc consumption), a positive and significant association among the consumption of shellfish and molluscs and TAs, AB, ΣAs, DMA and iAs concentrations was observed (see Fig. S4). Similarly, the meat intake variable was split into two different categories (red and white meat), and only red meat was associated with the MMA metabolite (β [95%CI]: 0.39 [-0.61, −0.18], p value < 0.01) (see Fig. S4).

3.2. Arsenic methylation efficiency and factors associated

The medians (95%CI) of the percentage of As metabolites were 89.7 (89.3, 90.2) for %DMA, 5.1 (4.8, 5.3) for %MMA, and 4.7 (4.5, 5.0) for %iAs. When the percentage of As metabolites was calculated with the calibrated concentration, we observed a decrease in %DMA (84.4 [83.9, 84.9]) and an increase in %MMA and %iAs (7.1 [6.7, 7.3] and 7.6 [7.3, 8.0], respectively) (see Table 1). The variability of these three variables can be summarized by two principal components (Table 2). Principal component 1 (PC1) explained 88% of the variance and reflected higher %DMA, and lower %MMA and %iAs. Principal component 2 (PC2) explained the remaining 12% of the variance and reflected higher %iAs and lower %MMA.

The variables associated with the percentage of each calibrated metabolite in the multivariate model can be observed in Table 3. The area of Valencia was associated with a lower methylation (higher % MMA and %iAs and lower %DMA). Latin-American mothers presented higher %DMA and lower %MMA (β [95%CI]: 14.2 [1.5, 26.3 and −18.5 [-27.3, −9.8], respectively]), but this factor did not remain in the %iAs model. Mothers who worked during pregnancy showed lower %MMA. Tobacco consumption during the first trimester of pregnancy was associated with higher %iAs (β [95%CI]: 6.1 [-0.07, 12.2]). Pre-pregnancy BMI was associated with a higher methylation (lower %MMA and % iAs and higher %DMA). Furthermore, gestational age at sampling was inversely related to %MMA (β [95%CI]: 0.84 [-1.32, −0.36]). Regarding the estimated nutrients, only the estimation of daily intake of Zn was positively associated with %DMA in the bivariate analysis (Table S3), and this association continued to be present in the multivariate model, although in a marginal way (β [95%CI]: 7.71 [-1.39, 16.81]). The micronutrients involved in the one-carbon metabolism evaluated (estimated folate and vitamins B_6 and B_{12} intake) and other elements evaluated (serum Se and Mn concentrations) were not related to As metabolite percentages in the bivariate or the multivariate analyses **(Table S3)**. Higher levels of urinary Cd were associated with higher % MMA (β [95%CI]: 1.89 [0.28, 3.49]).

Finally, the variables associated with the As PC1 and PC2 methylation phenotypes in the multivariate model can be observed in Table 3. Regarding PC1, the area of Valencia, tobacco consumption during pregnancy and a higher estimated Fe intake were negatively associated with PC1. Pre-pregnancy BMI was directly associated with PC1, reflecting a better capacity to produce DMA. The factors associated with PC2 were the area of study (lower among the Valencian women), the place of birth (higher in Latin-American women), working situation during pregnancy (higher among workers) and higher BMI.

4. Discussion

In the present study, we measured the urinary concentrations of As metabolites in a large sample of pregnant women from Spain, a country without high levels of environmental iAs exposure, and high rice and seafood consumption. The main factor contributing to the As concentrations was diet, rice being the main source of exposure to inorganic species (iAs, MMA and DMA) while seafood contributed mainly to DMA and the organic form (AB). We also estimated the methylation efficiency by two different approaches (the relative percentages of each As metabolite and a principal component analysis). The factors that were most clearly associated were area of study, women's place of origin, body mass index and tobacco consumption. When the percentages of calibrated iAs, MMA and DMA were combined into principal components, 88% of the variance was explained by the first of them.

4.1. Concentrations of As and its metabolites during pregnancy: comparison with previous studies

Concentrations of urinary inorganic As species in our study population were lower than those observed in other areas. For example, in Bangladesh or Chile the mean ΣAs concentrations [SD] were 112.0 [3.0] and 55.8 [41.6] μg/L (Gardner et al., 2011; Jay Christian et al., 2006), compared with the GM of 7.7 μg/g creatinine observed in the present study (Table 4). Other populations with higher levels of ΣAs are those from Mexico and China, with median concentrations of 23.3 μg/L and 22.6 μg/g creatinine, respectively (Chou et al., 2014; Laine et al., 2015). These areas present high levels of As in groundwater, due to geochemical natural processes, and also anthropogenic processes, such as mining or smelting (Litter et al., 2020). In our study, urinary concentrations of iAs and MMA were higher than in other studies carried out on regions with low levels of iAs in drinking water, such as some areas of USA, Croatia, Slovenia and Canada (Howe et al., 2020; Stajnko et al., 2019; Vaughan Watson et al., 2020). Conversely, in our study urinary AB concentrations were higher than in other studies conducted in USA, the main reason probably being the high fish and seafood consumption of the Spanish population.

4.2. Factors associated with As exposure: dietary factors

In our study, rice consumption was the main predictor of TAs and all As species, except for AB. A recent study conducted in Valencia (Spain) showed that rice was a foodstuff with high total As and iAs content (0.15 and 0.06 mg/kg, respectively) (Marín et al., 2018). In that study, all the samples were below the limits established by the European regulation (0.20–0.25 mg/kg for iAs) (European Commission, 2015). In another study carried out on the Iberian Peninsula (Spain and Portugal), the As species concentrations were analysed according to the type of rice and geographical region (Signes-Pastor et al., 2016). The results showed that 26% of the rice samples exceeded the levels of iAs established for rice-based food for infants and young children (0.10 mg/kg). The same study found that commercial brown rice was the type that presented the highest levels of iAs and DMA species (0.16 and 0.084 mg/kg, respectively), compared with polished rice, both produced in the Iberian Peninsula region. Spain is the European country with the second highest consumption of rice, after Portugal (11 kg per capita per year) (Food and Agriculture Organization of the United Nations, 2017).

In our population, the consumption of 100 g of seafood increased the urinary concentrations of AB by 109%. It is well-established that AB is the major As species present in fish (European Food Safety Authority, 2009). This organoarsenical is considered non-toxic, because it is rapidly excreted without undergoing any change (European Food Safety Authority, 2009). Additionally, we observed that fish consumption during pregnancy was positively associated with urinary DMA levels. The explanation for this finding could be related to some more complex forms of As, such as arsenosugars and arsenolipids, detected in fish, marine algae and filter feeders (European Food Safety Authority, 2009; Taylor et al., 2017b). It has been suggested that these As forms are biotransformed in the human body into species like DMA (Molin et al., 2012). In relation to the inorganic forms, iAs levels in fish and shellfish are thought to be low (European Food Safety Authority, 2014). Nevertheless, Spain has recently been reported as one of the European countries with the highest iAs intake through fish and seafood, especially among Mediterranean high consumers of molluscs (Ferrante et al., 2019). Indeed, in our population, the consumption of 100 g of shellfish and molluscs was associated with an increase of 146% in urinary iAs

concentrations (CI95%: 38,341%). This increase was stronger than the association with rice consumption (26%, CI95%: 4,52%).

Other food groups associated with the As species concentrations in our study were meat, legumes and vegetables. We observed that the daily intake of 100 g of meat was associated with a 16% reduction in MMA, and when the different categories of meat were studied, only red meat remained statistically significant. This finding seems to be in agreement with the Strong Heart Family Study, where the authors observed a negative relationship between red meat consumption and ΣAs (Nigra et al., 2019). These associations may reflect nutrients found in red meat that have been associated with As metabolism, such as Zn and Fe, and certain OCM nutrients, such as vitamin B_{12}, choline and methionine (Kurzius-Spencer et al., 2017).

The consumption of legumes was also negatively associated with TAs and AB concentrations, but positively related to iAs concentrations, with an effect size similar to that of rice consumption (an increase of 30% in the iAs levels per daily intake of 100 g of any of these foods). Overall, legumes showed low levels of TAs (European Food Safety Authority, 2009), but the content of iAs was relatively high (66% of the TAs was iAs) (Agencia Catalana de Seguretat Alimentaria, 2017). In addition, Spain is the third highest consumer of legumes in the European Union (Food and Agriculture Organization of the United Nations, 2017). In our population, vegetable consumption was directly associated with ΣAs, DMA and MMA levels, each metabolite increasing by 5% per 100 g of vegetable intake. The iAs concentrations seem to vary with the type of vegetable. For example, the iAs levels measured in vegetables marketed in Valencia ranged between 0.0001 mg/kg of fresh mass in tomatoes to 0.02 mg/kg in aubergines, courgette and cucumber (Marín et al., 2018). In addition, the relatively high frequency of consumption of this type of food in our population (around 200 g per day) could explain the positive relationship with some As species.

4.3. Factors associated with As exposure: other factors

In our study, we have found differences in all As species concentrations, except for ΣAs, according to the study area. In Valencia, urinary MMA and iAs concentrations were higher than in Gipuzkoa. The dietary pattern in each area seems to be different. For example, women from Valencia consumed more rice (mean [SD] = 61 [32] grams per day) than those from Gipuzkoa (mean [SD] = 33[20] grams per day) (Table S4). Women from Valencia also consumed more shellfish and molluscs than women from Gipuzkoa. Ferrante (2019) reported that molluscs sampled on the Mediterranean coasts presented higher iAs concentrations (0.50 mg/kg wet weight, w.w.) than those sampled on the Atlantic coast (0.01 mg/kg w.w). These differences in the dietary habits could explain the geographical variability in iAs exposure observed in this study.

In our study, women born in Latin America presented lower concentrations of AB. This observation could be related to their lower consumption of fish in comparison to Spanish women (mean [SD] = 384 [210] grams per week vs. 570 [251] grams per week]). In fact, when the model was adjusted for the total fish intake, the coefficients for the women's place of origin were attenuated. Similarly, results from the US National Health and Nutrition Examination Survey showed slightly lower levels of urinary AB in a Mexican American population, compared to the Non-Hispanic white and the black population (Caldwell et al., 2009).

4.4. Evaluation of As methylation

The process underlying iAs metabolism is still not fully understood. Nowadays, the proposed iAs metabolism pathways include an enzymatic reduction (from pentavalent to trivalent forms) and a methylation process, giving rise to MMA and DMA forms, in a first and second step, respectively (Cullen, 2014). The MMA form is considered more toxic than DMA and so this methylation process is thought to be a detoxification mechanism, although it has been shown that intermediate compounds can be highly toxic (Vahter, 2002). The relative proportion of each urinary metabolite (iAs, MMA and DMA) over the sum of those species has been used to reflect the individual iAs methylation efficiency (Agency for Toxic Substances and Disease Registry, 2007). However, this method seems to be inappropriate for populations with low exposure to iAs through water and with high fish and seafood consumption, such as Spain (Navas-Acien et al., 2011), because, as a metabolite from the As species of fish, DMA could be overestimating the iAs exposure. In order to solve this problem, some strategies have been proposed, such as the residual-based method developed by Jones et al. (2016). In the present study, we have applied this approach to obtain a more accurate assessment of iAs metabolism. In fact, we have observed differences between the calibrated and non-calibrated percentages.

Several studies have used the percentages method to assess the iAs metabolism efficiency. However, a limitation of this approach is that the three percentages are highly correlated with each other, which hinders the interpretation of the results. In order to minimize this problem, some authors have proposed the use of a principal component analysis by transforming the three interrelated variables into two independent measures of As metabolism phenotypes (Balakrishnan et al., 2016; Gribble et al., 2015; Jansen et al., 2016; Spratlen et al., 2017). The first component, PC1, has been suggested to show the capacity of producing DMA (higher methylation efficiency) and explains the highest percentage of the original variance. That is, PC1 seems to represent the second step of metabolism, or the overall metabolism efficiency. In our study, PC1 agreed with these previous studies, indicating an inverse relation between %iAs and %DMA. Regarding the pattern for PC2, it has been interpreted as the first step of metabolism, or the capacity to transform iAs into MMA. In our results, PC2 showed a negative correlation between the %MMA and %iAs, regardless of %DMA. Until now, there has been no consensus on which is the most accurate approach to evaluate the efficiency of As methylation, bearing in mind that As metabolism is still under study and that the metabolites could also reflect dietary sources.

For this reason, interpretation of the results is complex and they should be taken with caution.

4.5. Factors associated with iAs methylation efficiency

One factor related to As methylation efficiency is the women's place of origin. Latin-American women presented higher methylation of iAs, represented by a higher %DMA and lower %MMA. This association remained even after adjusting for diet and other variables that can affect As exposure. This result is consistent with those of other studies, which have shown differences in metabolism depending on the ethnicity of the participants. A study carried out in USA showed a slightly higher methylation efficiency among Hispanic people than non-Hispanic white, African American and Chinese American people (Balakrishnan et al., 2018). In the same way, a recent study has reported a more efficient methylation pattern (denoted by higher %DMA and lower %iAs) in US-born and foreign-born pregnant Hispanic women, compared with their non-Hispanic counterparts (Farzan et al., 2020). Other studies found that ethnicity was the strongest factor associated with As metabolism, even if the models were adjusted for water exposure (De Loma et al., 2019; Hopenhayn-Rich et al., 1996). It has been observed that some South American populations exposed to high levels of iAs for generations have a higher iAs methylation efficiency (higher DMA excretion) that has been acquired by a higher presence of the protective genetic variants in the *Arsenic [+3 oxidation state] methyltransferase* (*AS3MT*) gene, considered to be the major contributor to As methylation efficiency (Schlebusch et al., 2015; Vahter et al., 1995).

Our results indicated a positive relationship between the women's BMI and methylation efficiency, i.e. women with higher BMI present a decrease in %iAs and %MMA and an increase in %DMA. This pattern has also been observed in previous studies (Bommarito et al., 2019; Shen et al., 2016). The mechanism underlying the relationship between As

methylation and BMI is still unclear; however, some studies postulate that worse kidney function or the intake of certain proteins, both associated with an increasing BMI, could be associated with an increased DMA excretion (Duan et al., 2019; Peters et al., 2015; Vahter, 2007).

The gestational age at sampling was inversely related to the %MMA. This result is consistent with the findings of previous studies, which show an increase in methylation efficiency throughout pregnancy, denoted by higher %DMA and lower %MMA and iAs (Gardner et al., 2011; Hopenhayn et al., 2003). It has been suggested that As metabolism is elevated during the course of pregnancy due to a more efficient maternal one-carbon metabolism that increases the endogenous synthesis of the methyl-donor choline so as to be able to supply the high fetal demand for correct development (Vahter, 2009). Nevertheless, in our study, gestational age was only related to %MMA, but it was not associated with an increase in %DMA. Unfortunately, in our study we measured As metabolite concentrations in one-spot urine samples during pregnancy, which prevents us from evaluating whether this decreasing trend in %MMA is due to an improvement in As methylation or a change in the maternal diet.

In our study, smoker women presented a lower iAs methylation efficiency by showing a decrease in PC1 (understood as the overall metabolism efficiency). This result is in agreement with previous studies (Shen et al., 2016; Tseng, 2008). Tobacco consumption has been observed to decrease concentrations of one-carbon nutrients, such as vitamin B_{12} and folate, which could be affecting the As methylation efficiency (Mouhamed et al., 2011). Another possible explanation could be related to the presence of other metals in the tobacco, such as Cd, which could modify As metabolism. This metal seems to bind to reduced glutathione (GSH) (Zalups and Ahmad, 2003), which is an antioxidant involved in the reduction from trivalent to pentavalent As species. In fact, in our population, urinary Cd concentrations were directly associated with an increase in %MMA.

We also observed an association between the estimation of Fe intake and the As metabolism efficiency, specifically with decreasing PC1. In experimental studies, this element seems to diminish the bioaccessibility of As in the gut (Yu et al., 2016). Nevertheless, in a randomized controlled trial study conducted with Mexican children, supplementation with Fe had no impact on the As metabolism (Kordas et al., 2017). In this same study, Zn supplementation was not related to better As metabolism. These results seem to be in disagreement with those found in our study, where estimated Zn consumption was positively associated with the %DMA, although the coefficients did not reach statistical significance. Similarly to our findings, in an observational study conducted on Mexican women, the estimated Zn intake was related to a decrease in %MMA and %iAs, and an increase in %DMA (López-Carrillo et al., 2016). Zn is a necessary cofactor of betaine homocysteine methyltransferase (BHMT; EC 2.1.1.5). This enzyme uses betaine as a methyl donor and Zn as a cofactor for the remethylation of homocysteine to methionine (Millian and Garrow, 1998).

Finally, we did not observe any statistically significant association between As methylation efficiency and the estimated intake of OCM nutrients. Previous studies have observed an influence of some of these nutrients on iAs methylation, such as folate and vitamins B_6 and B_{12}, which are involved in the synthesis of S-adenosylmethionine (SAM), the main donor of the methyl group in iAs methylation (Bozack et al., 2019; Gamble et al., 2005; Howe et al., 2017b; Kurzius-Spencer et al., 2017). However, the results from studies conducted on populations of pregnant women have been heterogeneous; thus, in a Mexican cohort, no association was observed between levels of vitamin B_{12} in serum and the percentages of the urinary arsenic metabolites (Laine et al., 2018). A study of pregnant women in Bangladesh showed a marginal negative association with plasma folate and %iAs, but no association with vitamin B_{12} was found (Li et al., 2008). Additionally, plasma folate was inversely associated with the urinary percentage of As^{+5} before delivery (Hall et al., 2007). However, in that same cohort, an increase in methylation efficiency throughout the pregnancy was observed regardless of the women's folate and vitamin B_{12} status (Gardner et al., 2011). It has been suggested that As metabolism is more efficient during pregnancy due to an increase in the endogenous synthesis of the methyl-donor choline, in order to meet the high fetal demand (Vahter, 2009). This specific process during pregnancy may lead to certain cofactors or methyl-donors, such as folate, having a marginal influence on As metabolism (Gardner et al., 2011). Moreover, it is possible that in well-nourished populations it is more difficult to observe the influence of the one-carbon nutrients than in populations with nutritional deficiencies. In fact, Howe (2014) revealed a positive association between blood SAM and %MMA in folate and cobalamin-deficient participants, but this association was not found in the micronutrient-sufficient group. In our populations, only 22%, 2% and 16% of women had estimated levels of vitamins B_6, B_{12} and folate intake, respectively, below the recommendations (European Food Safety Authority, 2019), and only 4% of the participants did not take folic acid supplements at the first trimester of pregnancy.

This study has several limitations: 1) around 30% of the recruited participants were not included in this study, and participants could have a more privileged socioeconomic profile than non-participants. This fact could be related to dietary habits that can lead to differences in exposure to different forms of As; 2) another limitation in our study could be related to the assessment of As exposure at only one time point during pregnancy. This could reflect only recent As exposure and it might not be representative of the period of pregnancy as a whole; 3) the present study lacks information on some important nutrients involved in OCM and related to As metabolism, such as choline and betaine, and thus the influence of these variables could not be tested; 4) finally, we used creatinine concentrations to control for urinary dilution. Creatinine concentrations seem to be associated with As metabolism. To control for this effect, we used the approach proposed by Barr et al. (2005), which involves including the creatinine concentrations in the multivariate models as a separate independent variable. Nevertheless, due to the complexity of the interrelations between As metabolism, micronutrients and creatinine, the interpretation of the factors associated with As metabolism should be taken with caution.

The major strength of the present study is the analysis of As speciation in a considerably large sample size. In fact, as far as we know, this is the largest European study describing As species concentrations and methylation efficiency in pregnant women. Another advantage is the analysis of As methylation efficiency through two approaches: using the relative percentages of each As metabolite and, in order to minimize the high correlation between the three percentages, using a principal component analysis. This allows comparability with previous studies that have used either of these two methods to evaluate the efficiency of arsenic metabolism.

5. Conclusions

The concentrations of the urinary As species in our study were slightly higher compared to other populations with low environmental exposure to As through water intake. Rice and seafood consumption, especially shellfish and molluscs, were the major contributors to the urinary concentrations of As species during pregnancy. Vegetables, legumes, eggs and other cereals contributed to the concentrations of different species of As during pregnancy. In the present study, the intake of nutrients and vitamins seemed to be weakly related to methylation efficiency. The consumption of tobacco in pregnancy, the women's place of origin and their body mass index were also associated with the methylation efficiency. Further birth cohort studies in low exposure areas are necessary to improve knowledge about prenatal arsenic exposure, especially of its inorganic forms, and its potential health impact during childhood. This information could be used to propose new strategies in public health.

Credit author statement

Raquel Soler-Blasco: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Mario Murcia: Methodology, Formal analysis, Writing – review & editing. Manuel Lozano: Writing – review & editing. Blanca Sarzo: Methodology, Writing – review & editing. Ana Esplugues: Writing – review & editing. Jesús Vioque: Writing – review & editing. Nerea Lertxundi: Writing – review & editing. Loreto Santa Marina: Conceptualization, Writing – review & editing, Funding acquisition. Aitana Lertxundi: Writing – review & editing, Funding acquisition. Amaia Irizar: Methodology, Writing – review & editing. Simone Braeuer: Writing – review & editing. Walter Goesler: Writing – review & editing. Ferran Ballester: Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition. Sabrina Llop: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition.

Policy and ethics

The study protocol was approved by the Ethics Committee of the university hospital La Fe (Valencia), the Ethics Committee of the Public Health Research Centre in Valencia (CSISP) and the Ethics Committee of Donostia Hospital (Gipuzkoa). Informed consent was obtained from all participants in each phase.

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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.envres.2021.110889.

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