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Concentrations of urinary arsenic species in relation to rice and seafood consumption among children living in Spain

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A B S T R A C T

Inorganic arsenic (i-As) has been related to wide-ranging health effects in children, leading to lifelong concerns. Proportionally, dietary i-As exposure dominates in regions with low arsenic drinking water. This study aims to investigate the relation between rice and seafood consumption and urinary arsenic species during childhood and to assess the proportion of urinary i-As metabolites. Urinary arsenic species concentration in 400 4-year-old children living in four geographical areas of Spain, in addition to repeated measures from 100 children at 7 years of age are included in this study. Rice and seafood products intake was collected from children's parents using a validated food frequency questionnaire (FFQ). At 4 years of age, children's urine i-As and monomethylarsonic acid (MMA) concentrations increased with rice product consumption (p-value = 0.010 and 0.018, respectively), and urinary arsenobetaine (AsB) with seafood consumption ($p = 0.002$). Four-year-old children had a higher consumption of both rice and seafood per body weight and a higher urinary %MMA (p-value = 0.001) and lower % dimethylarsinic acid (DMA) (p-value = 0.017). This study suggests increased dietary i-As exposure related to rice product consumption among children living in Spain, and the younger ones may be especially vulnerable to the health impacts of this exposure also considering that they might have a lower i-As methylation capacity than older children. In contrast, seafood consumption did not appear to influence the presence of potentially toxic arsenic species in this population of children.

1. Introduction

There is sufficient evidence in humans for carcinogenicity due to i-As oral exposure (IARC, 2012). Other than cancer, exposure to i-As has been associated with a wide range of adverse health outcomes including neurological, cardiovascular, respiratory and metabolic diseases (Medrano et al., 2010; Naujokas et al., 2013; Tsuji et al., 2014). Fetal and early life exposure to i-As is of particular concern due to the specific vulnerability to the adverse health effects of i-As, which may have a marked impact throughout the lifespan even at low to moderate levels of exposure (Farzan et al., 2016; Gilbert-Diamond et al., 2016; Rodríguez-Barranco et al., 2016; Vahter, 2009). The metabolic pathway of i-As involves a series of reduction and oxidative methylations, in which i-As is sequentially transformed to MMA and DMA. The metabolic process is incomplete such that i-As along with MMA and DMA metabolites are excreted in urine (Tseng, 2009). Health risks associated with exposure to organic arsenic compounds such as DMA and MMA are less certain than i-As, and AsB, which is excreted in the urine

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unchanged and regarded as non-toxic (Cubadda et al., 2016; Feldmann and Krupp, 2011; Molin et al., 2015).

Diet is the main source of i-As exposure when relatively low arsenic drinking water is available, the maximum level of which is regulated at 0.01 mg/l in the EU (Cubadda et al., 2016; EFSA, 2009; The Council of the European Union, 1998). The 0.01 mg/l is also the WHO guideline value for arsenic in drinking water and thus the legal limit in many countries around the world (WHO, 2011). Rice and rice-based products contain higher levels of i-As compared to other foodstuff, in addition to DMA and traces of MMA, and therefore they are an important i-As dietary source (Meharg et al., 2008; Signes-Pastor et al., 2016a, b; Williams et al., 2007). This is an increasingly recognized issue for infants and young children whose food consumption per body weight is higher compared to adults, and rice products generally predominate their diet due to the putative organoleptic and nutritional quality, and relatively low allergic potential of these products (Burló et al., 2012; Da Sacco et al., 2013; Karagas et al., 2016; Meharg et al., 2008; Signes-Pastor et al., 2016b). New regulations regarding maximum levels of i-As in rice and rice-based products have been recently established in the European Union (EU) with the most restrictive level of 0.1 mg/kg set for rice-based foods for infants and young children (EC, 2015). The 0.1 mg/kg i-As threshold is also a draft “action level” in the United States (U.S.) (FDA, 2016). As far as seafood is concerned, AsB dominates in fish, whereas other organic arsenic compounds such as arsenosugars and arsenolipids are also found in seafood products, including i-As in bivalves, crustaceans and algae (Feldmann and Krupp, 2011; Fontcuberta et al., 2011; Krishnakumar et al., 2016; Molin et al., 2015; Navas-Acien et al., 2011; Taylor et al., 2016). Thus, if relatively low arsenic drinking water is available, rice and seafood products are principal total arsenic, including potentially toxic arsenic species, dietary sources (Cubadda et al., 2016; EFSA, 2009). However, limited information is as yet available regarding the contribution of these key food items to urinary arsenic species concentration as exposure biomarker during childhood, especially among those with increased and consistent rice and seafood product consumption, such as those living in Spain, whose consumption of rice and seafood products is enhanced by the Spanish gastronomic culture.

In this study, the relations between the arsenic species and their summation (excluding AsB) concentrations in urine from 4-year-old children participating in a mother-child prospective cohort study, and rice and seafood product intake were determined. Further, the percentage of urinary i-As, MMA, and DMA were assessed in children at 4 and 7 years of age.

2. Methods

2.1. Study population

The study population here belongs to the mother-child pair participants in the INMA–*INfancia y Medio Ambiente* - Environment and Childhood project, a prospective population-based birth cohort study conducted in various Spanish regions, www.proyectoinma.org (Guxens et al., 2012). Women participants of the INMA project were recruited at the beginning of their pregnancy (2003–2006) at their reference primary health care centers or public hospitals, and were followed-up until delivery (n = 2625). All women met the inclusion criteria of ≥ 16 years old, singleton pregnancy, non-assisted conception, delivery scheduled at the reference hospital, and adequate knowledge of the Spanish language to understand and complete the questionnaires accurately. Their children were enrolled at birth and were followed-up during infancy and childhood.

2.2. Samples collection

Spot urine samples were collected from a study population of 400 children of 4 years of age comprised of four groups of 100 children evenly distributed between boys and girls, and randomly chosen from each of the Spanish sub-cohorts of Asturias, Gipuzkoa, Sabadell, and Valencia, situated in the north and east of the country; Bay of Biscay and Mediterranean coast, respectively. Additionally, urine samples of the same children from the Valencia sub-cohort were collected at 7 years of age (n = 100).

Informed consent was obtained from all participants in each phase, and the hospital ethics committees in the participating regions approved the study.

2.3. Anthropometric data and questionnaires

Parental sociodemographic and socioeconomic characteristics were collected through a maternal questionnaire in their 1st trimester of pregnancy including living area, parents education, and social class according to maternal and paternal occupation coded using the International Standard Classification of Occupations (ISCO88) (International Labor Office (ILO), 2012). Trained staff measured children's weight and height following standard protocols. Children's body mass index (BMI) was calculated in kg/m^2. Four-year-old children's dietary intake was assessed with a validated FFQ asking about consumption of 105 food items in the previous year (Vioque et al., 2016). Parents or children's guardians provided the information regarding children's diet, and it was recorded in the FFQ during personal interviews administered by trained nutritionists. The usual daily total energy intake for each child was calculated by multiplying the frequency of the use of each food item by the nutrient content of the portion size specified in the FFQ. Total energy values were primarily obtained from food composition tables from the U.S. Department of Agriculture (USDA, 2016), and other published sources reporting information on content in Spanish food (Palma et al., 2008). Rice products included 3 food items referring to cooked rice and breakfast cereal consumption. Seafood products gathered several food items (n = 11) including i) surimi products such as seafood sticks and crab sticks, ii) white fish such as hake, sole and sea bream fried or battered, iii) white fish boiled or grilled, iv) swordfish, v) big oily fish such as tuna, bonito, and salmon, vi) small oily fish such as anchovy, sardine and mackerel, vii) canned tuna in oil, viii) canned tuna in brine, ix) squid, cuttlefish, and octopus, x) clams, mussels, and cockles, and xi) prawns, crab, and lobster.

2.4. Sample preparation and chemical analysis

Children's urinary arsenic speciation included AsB, DMA, MMA, and i-As, and the levels of which were previously reported in Signes-Pastor et al. (2017) with a median of 9.71 µg/l, 3.97 µg/l, 0.44 µg/l, and 0.35 µg/l for the 400 4-year-old children, respectively. The urine samples were stored at or below −20 °C until analysis. Then, they were centrifuged and analytical grade hydrogen peroxide was added to convert any arsenite to arsenate to facilitate subsequent chromatographic detection by IC-ICP-MS. Replicate samples of the urine lyophilized material ClinChek® - Control level I (Recipe Chemicals + Instruments GmbH in Munich, Germany), and blank samples were included in each analysis batch as quality control. The specific gravity was measured with a clinical refractometer to normalize urine dilution. The recovery percentages of arsenic speciation based on several replicate samples of the urine lyophilized material ClinChek® - Control level I (n = 33) were 115 ± 2% for i-As, 97 ± 2% for MMA, 94 ± 2% for DMA, and 90 ± 2% for AsB. The mean and range concentrations of the arsenic species reference values in the urine lyophilized material ClinChek® - Control level I are as follows: 4.55 (2.73–6.37) µg/l for i-As, 2.50 (1.50–3.50) µg/l for MMA, 9.8 (5.88–13.7) µg/l for DMA, and 16.8 (12.6–21.0) µg/l for AsB. The LOD for arsenic speciation, calculated from DMA calibration, was 0.011 µg/l (Signes-Pastor et al., 2017).