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The influence of diet on intra and inter-individual variability of urinary excretion of arsenic species in Italian healthy individuals

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ABSTRACT

To study the effect of eating foods with a high arsenic (As) content on the intra and inter-individual variability of urinary concentrations of the As species, daily urine samples were collected for 10 consecutive days from 12 healthy male subjects. A daily food diary was kept throughout the study period. Personal exposure to airborne As was measured once during the study. As^3, As^5, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine were determined in all urine samples by inductive coupled plasma mass spectrometry, and the sum of As^3 + As^5 + MMA + DMA (iAs) by hydride generation-atomic absorption spectrophotometry. Exposure to airborne As was below the limit of detection in all samplings. As^3 was found in only 19.2% and As^5 in only 3.3% of the urine samples, whereas high urinary concentrations of arsenobetaine were observed. With the exception of arsenobetaine, expressed as a percentage, a significant inter-individual variability was observed for all species of As, for iAs and for the MMA/DMA ratio ($p < 0.001$). Instead, the intra-individual variability was significant only for the MMA/DMA ratio ($p < 0.001$). Among foods with a high As content, only a heavy consumption of seafood was shown to influence inter-individual variability of DMA%, arsenobetaine expressed as $\mu g\, g^{-1}$ creatinine and iAs. In conclusion, even in populations with a high intake of organic As through foods, the finding of a significant inter-individual but no significant intra-individual variability of urinary species confirms the usefulness of urinary As speciation for biological monitoring of exposure to As.

1. Introduction

Environmental exposure to inorganic arsenic (As), arsenite (As^3) and arsenate (As^5), can derive from both natural and anthropic sources. Even today, natural contamination of groundwater and hence of drinking water is still the main source of exposure to inorganic As in many parts of the world (Bangladesh, India, China, United States, Argentina, Chile), despite the fact that already by 1993 the World Health Organization (WHO) recommended standard values in drinking water not exceeding $10\ \mu g\ L^{-1}$ (WHO, 1993). In contrast, the main sources of exposure to organic forms of As, such as arsenobetaine, arsenosugars, arsenolipids and dimethylarsinic acid (DMA), are marine products, including seafood (mollusks and crustaceans), fish and seaweed, followed by rice, poultry and mushrooms (EFSA, 2009). These foods can also contain inorganic As in concentrations of up to 42% of the total As in seafood and up to 93% in rice (Sloth and Julshamn, 2008; Torres-Escribano et al., 2008).

In man, after being rapidly and almost completely absorbed during digestion, inorganic As undergoes a biotransformation process consisting of two successive reduction and oxidative methylation steps (Vahter, 2002). As^3, ingested as is or derived from the rapid reduction of As^5 in the blood and liver, then undergoes biotransformation in the liver to monomethylarsonic acid $(MMA)^5$, that in turn can be metabolized to DMA^5 via the formation of MMA^3, a highly reactive trivalent intermediate product (Gregus and Nemeti, 2005). DMA^5 can also form a reactive trivalent intermediate product (DMA^3), whereas the methylation process to trymethylarsinic oxide (TMAO) does not appear to be relevant in man (Thomas et al., 2001). Ingested inorganic As is excreted in the urine in the proportion of 10–30% as As^3 and As^5, 10–20% as MMA and 60–70% as DMA, although the relative distribution of the different species has shown a great variability among both different subjects and different population groups (Vahter, 1999). Instead, arsenobetaine and DMA absorbed in the digestive tract do not seem to undergo biotransformation processes and are therefore excreted in the urine unchanged, whereas arsenosugars and arsenolipids seem to be metabolized to form DMA and other little known As compounds (Marafante et al., 1987; Brown et al., 1990; Francesconi et al., 2002; Schmeisser et al., 2006).

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In the past, the oxidative methylation processes were regarded as a detoxification process of inorganic forms, since only the inorganic As forms were considered to have toxic and carcinogenic effects, inducing peripheral arterial disease, hypertension, skin lesions and tumors, bladder and lung cancer (Straif et al., 2009). More recent studies have shown that one of the intermediate methylated forms, i.e., MMA^3, also has toxic effects in *in vitro* models that may be even greater than those induced by As^3 (Petrick et al., 2000; Styblo et al., 2000). Thus, the onset of toxic As effects may be linked to the methylation status and valence state of inorganic As metabolites and hence to the methylation capacity of the individual, that is partly dietary and partly genetic in origin (Gamble et al., 2005). The latter factor seems to play an essential role in causing hypersusceptibility of an individual or a population to the negative effects of As on human health (Tseng, 2009).

Previous studies employed the relative distribution of MMA (MMA%) and DMA (DMA%) or the MMA/DMA ratio in the urine as markers of the individual inorganic As methylation pattern. An association was observed between the onset of toxic As effects and an increased MMA% or MMA/DMA ratio, or a reduced DMA% (Tseng, 2007). The validity of these markers has been confirmed in populations with a high, stable intake of inorganic As through contaminated drinking water, since they also demonstrate a minor intra-individual versus a significant inter-individual variability, suggesting that a predominant role is played by genetic rather than environmental factors (Concha et al., 2002). Little study has yet been made of the influence of the intake of foods with a high content of organic and/or inorganic As on the intra- and inter-individual variability of urinary excretion of the As species, especially in populations whose main source of exposure to As is through foods.

The aim of the present study was to evaluate intra- and inter-individual variability of urinary excretion of the different As species, and to investigate the effect of a high intake of foods considered to have high As contents on this variability.

2. Materials and methods

2.1. Subjects

The investigation was conducted in 12 healthy subjects over 10 consecutive days. All subjects were administrative workers with a normal nutritional status, six smokers and six non smokers, and all Caucasian males resident in a coastal area of southern Italy (Manfredonia-Apulia). All subjects gave prior written informed consent to take part in the study.

Firstly, a questionnaire was administered to all subjects, inquiring into personal data, current or previous occupational exposure to arsenic, lifestyle with particular reference to a smoking habit, and diet, especially relative to the intake of foods with a high As content (mollusks, crustaceans, fish, rice, poultry, mushrooms), the type and quantity of drinking water, and the medical history, to exclude liver and kidney disease. The subjects were asked to keep a daily food diary starting 3 d before the start of the study and continuing throughout the study period. No dietary limitations were imposed, and they were not asked to abstain from eating any specific foods or type of drinking water.

2.2. Environmental monitoring

Although the study subjects were office workers with no occupational exposure to As, to exclude any possible exposure to airborne As at the workplace, airborne As was sampled on the 5th of the 10 d of study, using active personal samplers applied in the respiratory zone on each subject. All sampling was done in the morning during the subject's normal activities and lasted 8 h. Airborne particulate matter was collected on cellulose ester membranes to assess the total As concentration in the inhalable dust fraction. The analysis was carried out by inductively coupled plasma mass spectrometry (ICP-MS) ELAN 5000 (Perkin–Elmer SCIEX). The limit of detection (LOD) was 0.001 $\mu g\ m^{-3}$.

2.3. Biological monitoring

A urine sample was collected from each of the 12 study subjects every morning for 10 consecutive days, between 8 and 9 a.m. All the urine samples were assigned a code number, immediately frozen to −20 °C and kept at this temperature until the time of analysis, carried out blind within 1 month of collection.

The urinary species As^3, As^5, MMA, DMA and arsenobetaine were measured by liquid chromatography coupled with mass spectrometry (HPLC-ICP-MS), using the analytical method described by Apostoli et al. (1997, 1999). The LOD for all the As species was 0.5 $\mu g\ L^{-1}$, and the coefficient of variation (CV) was 2.5% for As^3 and Arsenobetaine and 5.0% for As^5, MMA and DMA. The total content of inorganic As (As^3 + As^5) and its methylated forms, MMA and DMA (iAs), was determined by the extraction method with the atomic absorption spectrophotometer 5100 (Perkin–Elmer) using the hydride generation technique (HG-AAS). The LOD was 0.1 $\mu g\ L^{-1}$ and the CV 4.5%. The colorimetric method was used to determine urinary creatinine, value used in subsequent adjustments (Harmoinen, 1996). The LOD was 0.01 mg dL^{-1} and the CV 5%. All the urine samples showed urinary creatinine values ranging between 0.3 and 3.0 g L^{-1}, within the WHO recommended limits for biological samples to be judged acceptable (WHO, 1996).

The laboratory that conducted the analytical measurements is a member of the external quality control program organized by the Institute of Occupational Social and Environmental Medicine of the University of Erlangen, Nuremberg.

2.4. Statistical analyses

Statistical analyses were done with the SPSS program (version 14.0, Chicago, IL, USA). Analytical determinations below the LOD of the method were assigned a value of half the respective LOD. The relative proportion of MMA (MMA%) and DMA (DMA%) was calculated by dividing the respective urinary concentrations by the sum of the species As^3 + MMA + DMA, while the relative proportion of arsenobetaine (arsenobetaine%) was calculated by using the total As, defined as the sum of the species As^3 + MMA + DMA + arsenobetaine, as the denominator. A normal distribution of the variables was ascertained using the Kolmogorov–Smirnov test, and non normally distributed variables were analyzed after logarithmic transformation. The intra and inter-individual variability was assessed on a one-way random effects model (ANOVA) for repeated measures, using the day as the within-subjects factor. The intraclass correlation coefficient (ICC) was calculated as the ratio between the variance among subjects and the total variance, ranging between 0 (maximum intra-individual variability) and 1 (maximum inter-individual variability). Study of the influence of eating foods considered to have a high As content on the intra- and inter-individual variability of the As species was done using linear mixed effect models. In these models, the 12 subjects were classified as high or low consumers of each of the different foods, taking as the categorical factor whether during the 10 d of the study they ate four or more meals of fish (high intake) versus less (low intake), three or more meals (high intake) of seafood versus less (low intake), and more than one meal of rice (high intake) versus less (low intake). Determination of the serial intra-individual correlation was calculated by first-order autocorrelation analysis for all the As species. The level of significance was set at $p < 0.05$.