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Arsenic species and selected metals in human urine: validation of HPLC/ICPMS and ICPMS procedures for a long-term population-based epidemiological study

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Abstract

Exposure to high inorganic arsenic concentrations in drinking water has been related to detrimental health effects, including cancers and possibly cardiovascular disease, in many epidemiological studies. Recent studies suggest that arsenic might elicit some of its toxic effects also at lower concentrations. The Strong Heart Study, a large epidemiological study of cardiovascular disease in American Indian communities, collected urine samples and performed medical examinations on 4,549 participants over a 10-year period beginning in 1989. We used anion-exchange HPLC/ICPMS to determine concentrations of arsenic species (methylarsonate, dimethylarsinate and arsenate) in 5,095 urine samples from the Strong Heart Study. We repeated the chromatography on a portion of the urine sample that had been oxidised, by addition of H_2O_2, to provide additional information on the presence of As(III) species and thio-arsenicals, and by difference, of arsenobetaine and other non-retained cations. Total concentrations for As, Cd, Mo, Pb, Sb, Se, U, W, and Zn were also determined in the urine samples by ICPMS. The dataset will be used to evaluate the relationships between the concentrations of urinary arsenic species and selected metals with various cardiometabolic health endpoints. We present and discuss the analytical protocol put in place to produce this large and valuable dataset.

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Introduction

Arsenic in drinking water is a major global environmental health problem. Early epidemiological evidence showed that inorganic arsenic increases the risk of various cancers.[1] More recent research has linked inorganic arsenic exposure to a range of health effects including cardiovascular disease,[2,3] respiratory disease,[4] and type 2 diabetes.[5,6] Those health problems were first identified in populations exposed to high arsenic levels (>100 μg L^{-1}). Recently, attention has shifted to investigating the health effects of moderate to low arsenic exposure levels (<50 μg L^{-1}).

Human exposure to inorganic arsenic is commonly assessed by determining arsenic concentrations in urine. Total arsenic values, however, can also reflect exposure to organoarsenicals from food, in particular seafood. Arsenic in seafood is present predominantly in the form of arsenobetaine, arsenosugars and arsenolipids, and these compounds or their metabolites such as thio-arsenicals are also excreted in the urine.[7] To assess inorganic arsenic exposure reliably, the individual arsenic species must be measured in urine. The most common method for determining urine arsenic species is the coupled technique of high performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC/ICPMS). Several recent studies have demonstrated the strength of this approach for quantitative measurement of arsenic species in urine.[8,9]

The Strong Heart Study, a large epidemiological study of cardiometabolic diseases in American Indian communities from Arizona, Oklahoma and the Dakotas, collected socio-demographic and medical data from 4,549 participants during three visits over a 10-year period beginning in 1989.[10] Urine samples were collected and stored frozen at the time of the examinations. The arsenic content of the drinking water for the three study populations ranged from <10 to 61 μg L^{-1} in Arizona and from <1 μg L^{-1} to 21 μg L^{-1} in North and South Dakota. No data are available for the Oklahoma communities, although arsenic levels in drinking water are generally <10 μg L^{-1} in Oklahoma (U.S. EPA (U.S. Environmental Protection Agency). Drinking Water Data & Databases, 2011, http://water.epa.gov/scitech/datait/databases/drink/sdwisfed/index.cfm). These stored urine samples provide an excellent opportunity to investigate the relationship between arsenic exposure and cardiovascular disease and diabetes in communities with low to moderate levels of inorganic arsenic exposure in their drinking water.

Here, we report the analytical protocol, including the quality assurance and quality control measures, put in place to determine arsenic species by HPLC/ICPMS in 5,095 urine samples from participants in the Strong Heart Study. The urine samples, comprising samples from all participants in the first visit (1989–1991) as well as from a subset of 500 participants at visits 2 and 3, were analysed over a two-year period. We discuss the rationale for the procedures adopted taking into consideration sample age and storage conditions, small sample volume (usually <1 mL), the arsenic species essential for toxicological assessment, and the levels of precision and limits of detection. Additionally, we determined total concentrations for selected elements (As, Cd, Mo, Pb, Sb, Se, U, W, and Zn) in the urine samples by ICPMS. The analytical protocol defines the databases for arsenic species and those selected elements that will be used to investigate the relationships between metal concentrations in urine and the development of cardiovascular disease and diabetes.

Materials and Methods

Chemicals, standards and reference materials

The following commercial chemicals were used: ortho phosphoric acid (p.a., or TraceSELECT Ultra) from Fluka (Buchs, Switzerland); pyridine from Merck (Merck,

Darmstadt, Germany); and hydrogen peroxide 30 % (p.a.), aqueous ammonia 25 % (suprapure), 65 % nitric acid (p.a.), and formic acid (p.a.) from Roth (Carl Roth, Karlsruhe, Germany). Chemicals were used without further purification except for the nitric acid which was distilled in a quartz sub-boiling distillation unit. Water used throughout was from a Milli-Q Academic water purification system (Millipore GmbH, Vienna, Austria) with a specific resistivity of 18.2 MΩ*cm.

Individual standard solutions (1000 ± 3 μg L^{-1} in 2 % nitric acid) for total element determinations of As, Cd, Mo, Pb, Sb, Se, U, W, and Zn (in the urine samples) and Ge, In, and Lu (internal standards) were obtained from CPI International (Santa Rosa, CA, US). For arsenic speciation, stock solutions containing 1000 mg As L^{-1} of each of the following species were prepared in water: arsenite (As(III) and arsenate (As(V)) prepared from $NaAsO_2$ and $Na_2HAsO_4.7\ H_2O$, respectively, purchased from Merck (Darmstadt, Germany); dimethylarsinate (DMA) prepared from sodium dimethylarsinate purchased from Fluka (Buchs, Switzerland); methylarsonate (MA) prepared in-house from sodium arsenite and methyl iodide (Meyer reaction); and arsenobetaine (AB), as the bromide salt, prepared in-house following the method of Cannon et al.[11] The purity of the synthesized standards (MA and AB) was established by NMR and HPLC/mass spectrometry. Other arsenic standards (trimethylarsine oxide, arsenocholine, tetramethylarsonium ion, oxo and thio-dimethylarsinylethanol and oxo- and thio-dimethylarsinylacetic acid) were prepared as previously reported;[12,13] these standards were used to check the identity of minor peaks which occasionally appeared in the chromatograms.

The certified reference materials for total element measurements were NIST 1643e, trace elements in water (National Institute of Standards & Technology, Gaithersburg, Maryland, US) certified for As, Cd, Mo, Pb, Sb, Se, & Zn; and NIES No. 18, human urine (National Institute for Environmental Studies, Tsukuba, Japan) certified for As, Se & Zn. In addition, Seronorm™ control urine (Sero AS, Billingstad, Norway) and an in-house urine sample served as non-certified reference materials. The certified reference material for determining arsenic species was NIES No 18, human urine, certified for AB and DMA. Our in-house reference urine was used as a control for iAs, MA, DMA, and AB.

Instrumentation

Total element determinations and arsenic speciation analyses were performed with an Agilent 7700x ICPMS (Agilent Technologies, Waldbronn, Germany) equipped with a Micro Mist nebulizer (Glass Expansion, Melbourne, Australia) and a Scott double pass spray chamber. For total element analysis, the integrated auto sampler G3160B from Agilent Technologies was used. The sample cups (1.5 mL capacity, made from polystyrene) were purchased from Sarstedt AG & Co (Sarstedt, Nurnbrecht, Germany). Data acquisition and evaluation was performed with ICPMS Masshunter B.01.01 software.

For arsenic speciation analyses, an Agilent 1100 Series HPLC system consisting of a solvent degassing unit, a quaternary pump, an autosampler and a thermostated column compartment was used as the chromatographic system. The outlet of the HPLC column was connected via PEEK capillary tubing (0.125 mm i.d.) to the nebulizer of the ICPMS. The ion intensity at m/z 75 (^{75}As) was monitored using the ICPMS Masshunter B.01.01 software. Additionally, the ion intensities at m/z 53 ($^{40}Ar^{13}C/^{40}Ar^{12}C^{1}H$) and 77 ($^{40}Ar^{37}Cl$) were monitored. Instrumental settings used throughout this work were optimized before each run using the software autotune function. Typical values were: forward power 1600 W; carrier gas flow 1.00 L min^{-1}; nebulizer pump 0.1 rps (1.02 mm inner diameter tubing); extraction lens 1 0 V, extraction lens 2 −195 V; omega bias −80 V; omega lens 10 V; cell entrance −30 V; cell exit −50 V; deflect 17 V; plate bias −50 V; octopole RF 170 V octopole bias −8 V; quadrupole bias −3 V. With these settings the performance of the instrument was ^{7}Li >5*10^{4}

cps/μg L^{-1}, ^{89}Y >1.3*10^5 cps/μg $L^{-1;}$ ^{205}Tl >1.3*10^5 cps/μg L^{-1} with a $^{156}CeO/^{140}Ce$ ratio < 0.01.

Cd (*m/z*=111), Sb (*m/z*=121), W (*m/z*=182), Pb (*m/z*=208), U (*m/z*=238) and the internal standards Ge (*m/z*=74), In (*m/z*=115) and Lu (*m/z*=175) were measured in the normal mode. One channel was measured per isotope. In the collision gas mode, 4.0 mL min^{-1} He was used and the instrument settings were changed to cell entrance −40 V; cell exit −60 V; deflect 3 V; plate bias −60 V; octopole RF 190 V octopole bias −18 V; quadrupole bias −15 V. Under these conditions, the ratio of $^{156}CeO/^{140}Ce$ was typically < 0.006. Zn (*m/z*=66), As (*m/z*=75), Se (*m/z*=78), Mo (*m/z*=95), and the internal standards Ge (*m/z*=74) and In (*m/z*=115) were measured in the helium collision gas mode. One channel was measured per isotope. From each sample, five replicate measurements were recorded.

Sample collection and storage

Urine was collected from the Strong Heart Study participants on three occasions from 1989 to 1999 (1989–1991, 1993–1995, and 1998–1999). Following a physical examination conducted in the morning, participants were asked to void urine into a plastic cup, and approximately 8 mL was transferred to a 14 mL polypropylene screw-cap tube. These samples were frozen within 1–2 hours of collection and shipped on dry ice to the Medstar Health Research Institute, Hyattsville, MD, USA where they were stored at −80 °C. In the subsequent years, the samples had been thawed briefly and sub-samples removed for analysis of albumin and creatinine. During 2009 and 2010, portions (0.5 – 1.0 mL) of the samples were shipped on dry ice to Graz University, Austria where they were stored at −80 °C until analysis.

Total element measurements

A portion (170 μL) of the thawed urine sample was transferred to a Plastibrand® microtube (Brand GmbH + Co KG, Wertheim, Germany) and diluted to 1.76 g with 10 % v/v nitric acid containing the internal standards Ge, In, Lu at a concentration of 44 μg L^{-1}. The mixture was centrifuged for ten minutes at 1.3 × 10^4 g. The resultant supernatant was then analysed for As, Cd, Mo, Pb, Sb, Se, U (only for some samples), W, and Zn by ICPMS.

Determination of arsenic species

The remainder of the thawed urine sample was filtered through a 0.2 μm Nylon filter (Whatman GmbH, Dassel, Germany) into a 250 μL polypropylene crimp vial (Agilent Technologies). This filtered sample was analysed directly by anion-exchange HPLC/ICPMS. Additionally, a portion (90 μL) of the filtered sample was removed from the HPLC vial and 10 μL of H_2O_2 were added, to convert any trivalent- and thio-arsenicals to their pentavalent and/or oxygenated forms, and the mixture was allowed to stand for at least two hours at a temperature > 23°C before analysis by anion-exchange HPLC/ICPMS.

The anion-exchange HPLC conditions (identical for both non-oxidised and oxidised urine samples) were: PRP-X100 column (4.6 mm × 150 mm, 5 μm particles; Hamilton Company, Reno USA) at 40°C with a mobile phase of 20 mM aqueous phosphoric acid adjusted with aqueous ammonia to pH 6 at a flow rate of 1 mL min^{-1}. Injection volume was 20 μL. A carbon source (1% CO_2 in argon) was introduced directly to the plasma, as previously described for selenium,[14] to provide a 4-5-fold increase in sensitivity. The CO_2 was introduced via the T-piece of the high matrix sample introduction kit and the optional gas was set to 0.17 L min^{-1}. Under these chromatographic conditions, As(III) elutes near the void volume, very close to AB and most other cationic arsenic species. This void-volume peak was assigned as AB + As(III) in the non-oxidised sample, and as AB in the oxidised sample (Fig. 1), based on the premise that AB is the only arsenic cation found in significant

quantities in urine (see below).[15] The total iAs content [As(III) + As(V)] was obtained from the As(V) peak in the oxidised sample. For all HPLC runs, peaks were quantified against the respective standard. Calibration was usually performed in the range 0.10 to 20.0 μg As L^{-1} (six-point calibration curve); limit of detection was 0.1 μg As L^{-1} for iAs [As(V) peak], MA, DMA and AB, and the intra-assay coefficient of variation was better than 5 % for all species.

The premise that AB was essentially the only cationic arsenic species in the urine samples was tested by performing cation-exchange HPLC/ICPMS on 188 samples that had shown a significant peak at the void volume during anion-exchange HPLC/ICPMS of the oxidized samples. A Zorbax 300-SCX column (4.6 mm × 150 mm, 5 μm particles; Agilent Technologies) at 30°C was used with a mobile phase of 10 mM pyridine at pH 2.3 (adjusted with formic acid) at a flow rate of 1.5 mL min^{-1}. The injection volume was 10 μL. ICPMS was used as a detector with the settings described above for anion-exchange HPLC/ICPMS.

Quality assurance/quality control

Before dispatch to the analytical laboratory in Graz, each sample was assigned a unique six digit code. A total of 5,095 urines samples were analysed over a two-year period in batches of 79 samples. In each batch, samples were run together with calibration blanks and checks (every ten samples), sample preparation blanks and spikes, Reference Materials, and “in-house” reference urine under the QA/QC regime described below. A typical batch sample sequence is summarised in Table 1.

Calibration blanks and calibration standards were used to construct the primary calibration curves for quantification of total element content in the samples. They were prepared in the same acid matrix as the urine samples. The standard curve was constructed from blank + five concentrations of the element spanning the range of sample element concentrations. Analysis of samples proceeded only when the correlation coefficients of the standard curves were >0.999. Calibration checks included the re-analysis of selected calibration standards every ten samples throughout each sample analytical run. They served as a check of the calibration curve to account for changes in instrument sensitivity during the analysis run.

Sample preparation blanks were portions of Milli-Q water which underwent the same sample handling procedure applied to the urine samples. They were used to monitor and correct for procedural and analytical contamination resulting from reagents, glassware/plastic-ware, handling, etc. Sample preparation spikes were sample preparation blanks that were spiked with known quantities of arsenic compounds. They were used to evaluate sample preparation and analysis performance (recovery, reproducibility, etc.).

Reference Materials – namely NIST 1643e, NIES No 18 and the control material Seronorm™ for total element measurements, and NIES No 18 and the in-house urine reference sample for both total element and arsenic species measurements – were analysed with every batch of urine samples.

Unbeknown to the analytical laboratory, 47 samples were supplied in duplicate which provided an unbiased check on the repeatability of the methods. Additionally, the reference samples were independently measured for total arsenic and arsenic species by an external laboratory in Norway at the National Institute of Nutrition and Seafood Research (NIFES). A further check was made by our participation in a round robin exercise organized by Recipe Chemicals + Instruments GmbH (Munich, Germany).

Results and Discussion

The availability of urine samples from a large well-characterized population provide the unique opportunity to investigate relationships of arsenic, arsenic species, and other elements, with a range of cardiovascular and metabolic health effects that have been evaluated as part of the Strong Heart Study. The analytical methods reported here produced the trace element and arsenic species data necessary for such an assessment. Below we discuss the analytical protocol and quality assurance/quality control measures as well as some limitations of the data, as the Strong Heart Study was not originally designed to analyse trace elements or species.

Total element measurements: As, Cd, Mo, Pb, Sb, Se, U, W, and Zn

Quality assurance/quality control—Analysis of the NIST 1643e reference water over the course of the study demonstrated generally good accuracy and precision for those seven elements with certified concentrations (Table 2).

NIES human urine No 18 has certified values only for As, Se, and Zn. Although we had good precision for all three elements (Table 2), our mean values were higher than the certified values for arsenic (18 % higher) and selenium (29 %). This is possibly a consequence of our sample preparation - because the sample size was small (<1 mL) we did not mineralise (digest with acid) the samples before ICPMS analysis. Consequently, the urine samples contained small amounts of naturally occurring organic material which could contribute to a slightly increased signal (carbon-enhancement effect) for both arsenic and selenium.[16] It is also possible that traces of volatile metabolites of arsenic and selenium could be present leading to high values because volatile species would be transported to the plasma more efficiently than the calibration standards (which are inorganic species). Importantly, the precision of our data for arsenic and selenium (both about 8 %) was good and consistent throughout the whole two years of the analytical work, as illustrated for arsenic in Figure 2.

The Strong Heart Study samples – storage, contamination and interferences—Long-term storage could have consequences for total element measurements. A major urinary selenium metabolite is a selenosugar, which can degrade in part to volatile selenium species such as dimethyldiselenide.[17] These volatile species are transported efficiently to the plasma during ICPMS analysis - depending on the type of nebulizer, much more efficiently than inorganic selenium in solution which is the usual calibration standard used in conventional ICPMS analysis. Thus, falsely high values of selenium might be recorded for urine samples that have spent periods in an unfrozen state. It is possible that some other elements could also produce volatile species on long-term storage, with similar ensuing analytical problems.

During the course of the analyses, contamination problems with Pb, Sb and Zn in some of the samples became apparent. The source of contamination was found to be particular types of sample vials and caps. Up to ten different types of sample vials had been used during the ten-year collection period of the Strong Heart Study. Contamination of some of the samples was clearly observed when Pb concentrations in urine samples from the Strong Heart Study were categorized according to the type of storage vial; samples from one vial type with an orange cap returned Pb data 3–4 fold higher than the overall mean value (Figure S1 in Electronic Supplementary Information). Analysis of the vials and caps showed that those with an orange cap contained about 40 mg g^{-1} Pb and 0.2 mg g^{-1} Sb; these values were about 1000-fold (Pb) and 100-fold (Sb) higher than those for the other types of vials and caps.

We also encountered Sb contamination in the filters initially tested as part of the sample preparation for total element analysis. We changed from filtration to centrifugation to avoid this problem. Contamination with Zn was more widespread with values up to 15 mg g^{-1} recorded for those vials with rubber gaskets in the caps. Additionally, natural latex rubber gloves were a source of Zn contamination during sample preparation. This was quickly identified and overcome by using thin polyethylene gloves similar to the type commonly used for handling fruit at supermarkets.

We did not experience significant problems with spectral interferences. ICPMS measurements were made in collision cell mode for As, Se and Zn. Significant polyatomic interference by MoO on the Cd signal has been reported by others.[18] We also observed this interference, although in our case it was minor because our ICPMS operating conditions produced very low oxide/element ratios (MoO/Mo was 0.0016). Nevertheless, the Cd data were corrected in the data-set using the relationship:

$$\text{Corrected CD counts} = [\text{measured counts at } m/z\ 111] - [\text{measured counts at } m/z\ 95]^{*}0.0016.$$

Element concentrations in urine samples from the Strong Heart Study—

Summary statistics for the nine elements measured in the Strong Heart Study urine samples are shown in Table 3. The total number of data points was 5,095 for six elements (As, Cd, Mo, Se, W and Zn), 3,470 for Pb and Sb (after excluding those samples in orange cap vials) and 900 for U, as this element was only analysed during the last three months of the project.

Arsenic species measurements

Long-term storage – the consequences for arsenic species determinations—

Although the urine samples were frozen within 1–2 hours of collection (and stored at −80 °C), in the intervening years between collection and analysis they were briefly thawed and re-frozen on several occasions. Based on the study by Feldmann et al.,[19] the arsenic species measured in our study (iAs, MA, DMA and AB) were unlikely to be affected by the storage/handling conditions. Such handling conditions, however, precluded the measurement of the methylated As(III) species, methylarsonite (MA(III)) and dimethylarsinite (DMA(III)), because these species are reactive and they quickly convert to MA and DMA when urine is not frozen. [20] To detect those reduced methylated arsenic species, special sample handling procedures are necessary, and analysis must take place shortly after the collection of urine.[21] But these species are so elusive that even with special handling they have escaped detection.[9,22] Similar problems occur with thio-arsenicals, in particular thio-DMA. This species was reported as a metabolite in urine from Bangladeshi women, but it converts to DMA on storage and handling, so reliable quantitative data are difficult to obtain.[23]

In view of these analytical difficulties, the value in determining labile arsenic species in urine in large epidemiologic studies is unclear. MA(III) and DMA(III) (and possibly thio-DMA) are thought to play important roles in the toxic action of inorganic arsenic. Certainly, they display high toxicity in experimental settings,[24] and there is substantial interest in evaluating the relationship between MA(III)/DMA(III) concentrations in urine and potential harmful effects. The extreme lability of these species, however, essentially precludes their use in large epidemiologic studies; because reliable quantitative results are not possible with analytes that are highly dependent on sample handling and sample matrix, correlations of their concentrations with health effects would be markedly biased and inaccurate. A feasible alternative for large epidemiologic studies is to focus on the stable, easily measured analogs, MA and DMA. Indeed, it might be preferable to encourage the transformation of the labile species to their respective stable analytes as discussed below.

Oxidation of As(III) to As(V) with H_2O_2—The ready and variable inter-conversion of the two oxidation states of inorganic arsenic suggests that there is little benefit in attempting to distinguish these two species in urine samples. In our analyses, we specifically introduced an oxidation step, addition of H_2O_2, to convert arsenite to arsenate, and performed anion-exchange HPLC on the original sample and the oxidised sample. This approach provided two advantages. First, the problem of interference with the As(III) measurement, caused by the presence of arsenobetaine in urine as a consequence of eating seafood, is overcome. Arsenite and arsenobetaine both chromatograph near the void volume of the column, and hence interfere with each other which can lead to confounded results. After the oxidation step, arsenite is converted to arsenate which is well resolved from arsenobetaine and the other arsenic species. The peak remaining at the void volume can now be assigned as AB + cations (see below). Second, addition of H_2O_2 converts thio-arsenicals and any methylated As(III) forms to their respective oxygenated/As(V) forms, which are then measured in the normal way. Thus, additional information about the thio-species is forthcoming by comparing chromatograms before and after the sample is oxidised (Electronic Supplementary Information, Fig. S2)

Based on previous reports on cationic arsenic species in urine, [15] we expected that the peak assigned to AB + cations (anion-exchange HPLC, oxidised samples) would be predominantly arsenobetaine. We tested this assumption by analysing a sub-set (n=188, ca 4% of total) of the urine samples specifically for AB by cation-exchange HPLC/ICPMS; the mean AB content, as a percentage of total cationic arsenic species, was 98%.

Quality assurance/quality control—Anion-exchange HPLC/ICPMS was performed directly on the urine sample, and on a sub-sample that had been treated with H_2O_2. The first analysis provided data for MA, DMA, As(V), and As(III) + As cations, whereas the second analysis provided data for arsenic cations (mostly AB), MA, DMA, and iAs (total inorganic As determined as As(V)). Figure 1 provides an example of a typical chromatogram for a non-oxidised and an oxidised urine sample.

The in-house reference urine and the NIES No 18 Human urine sample were run with every batch of samples over the two-year analysis time; these data are summarised in Table 4. The data for the in-house reference urine of the individual batches are presented as Figure S3 in Electronic Supplementary Information. The results for AB and DMA for NIES No 18 were very consistent throughout the whole study period, and agreed with the certified values within the certified uncertainties - obtained values were 78.4 ± 4.8 μg As L^{-1} (AB) and 43.9 ± 4.0 μg As L^{-1} (DMA), compared with the certified values (69 ± 12 μg As L^{-1} and 36 ± 9 μg As L^{-1}, respectively). The results obtained for the 47 duplicate unknown samples (Figure 3) demonstrate the stability of the measurement procedure. The intra-class correlation coefficient for duplicate samples was 0.996 for As(V), 0.992 for MA, 0.987 for DMA and 0.990 for arsenobetaine + cations, showing excellent reliability between unknown duplicate samples stored in separate vials and assayed at distinct times.

The column recovery was also evaluated from the data for sum of species (from the oxidised sample) and the total arsenic value obtained from ICPMS. Over the complete range of concentrations, column recovery varied from 28% to 161% (median value 91%; mean value 90 ± 13 %). The ratio of sum of species (oxidised sample) to sum of species (non-oxidised sample) had a median value of 105 % and a mean value of 107 ± 12 % (range was 51–281 %). In both cases (column recovery and ratio of sum of species), the extreme values were generally recorded for the samples with low arsenic concentrations (see also Electronic Supplementary Information, Figures S4 and S5).

An external check on the data was provided by further measurements in two ways. First, six samples of urine from our study were analysed for DMA by NIFES, Norway and 16 samples from Norway were analysed in our laboratory – the intra-class correlation coefficient for duplicate samples was 0.996. Second, we took part in a small round robin exercise (Recipe Control Urine) for AB, DMA, MA and iAs – our values matched the mean values of the round robin participants for all species (deviations ranged from 1–18 % with a mean deviation of 6 %).

Arsenic species concentrations in urine samples from the Strong Heart Study —The levels of arsenic species from the 5,095 urine samples are summarised in Table 5. The data are characterized by generally low AB values compared with other studies in US populations, [9,15,25] a result consistent with low seafood intake in our study population, and a wide range of levels for inorganic and methylated arsenic species, consistent with low to moderate arsenic exposure from drinking water in the study communities. The low detection limits attained by our methods ensured that the arsenic species could be quantified in almost all of the samples. The overall patterns and ratios of iAs, MA and DMA were similar to those reported in previous studies on arsenic urinary metabolites.[26]

Concluding comments—The Strong Heart Study is a major epidemiologic study investigating genetic and environmental determinants of cardiovascular disease and diabetes development. By measuring arsenic and metal exposure, this study may elucidate the possible influence of arsenic and metal exposure on a range of health effects, with a focus on cardiometabolic diseases. The exposure to arsenic and metals will be assessed based on their concentrations in urine samples of the study participants. Presented here are the analytical protocols followed to deliver these data. Future interpretation of the epidemiological data can be assessed and evaluated within the reported analytical limitations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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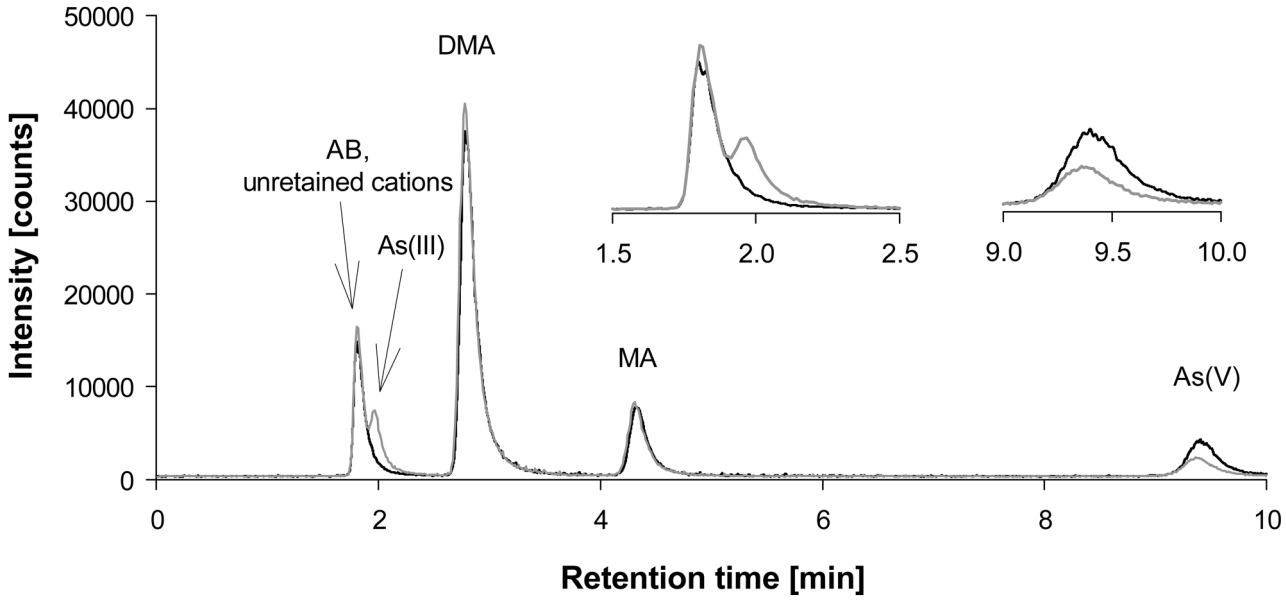

Figure 1.

Anion-exchange HPLC/ICPMS chromatograms of a urine sample (grey line) and the same sample after treatment with H_2O_2 (black line). The expanded regions show the two areas where changes occurred. HPLC conditions were: PRP-X100 column (4.6 mm × 150 mm, 5 μm particles) at 40°C with a mobile phase of 20 mM aqueous phosphoric acid adjusted with aqueous ammonia to pH 6 at a flow rate of 1 mL min^{-1}. Injection volume was 20 μL.

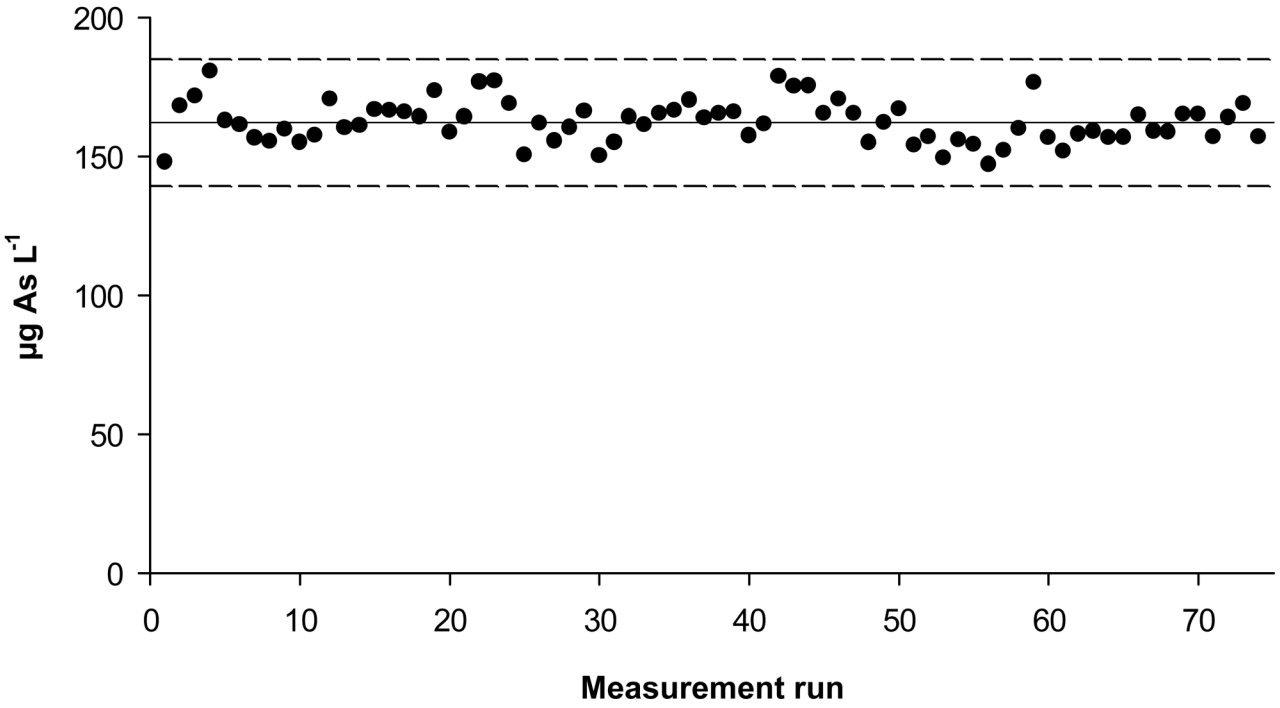

Figure 2.
Data obtained for total arsenic measurements of NIES No 18 Human urine obtained on 74 occasions over the course of the study. The solid line represents the mean value (162 μg L^{-1}) and the dashed lines represent the mean ± 3 SD.

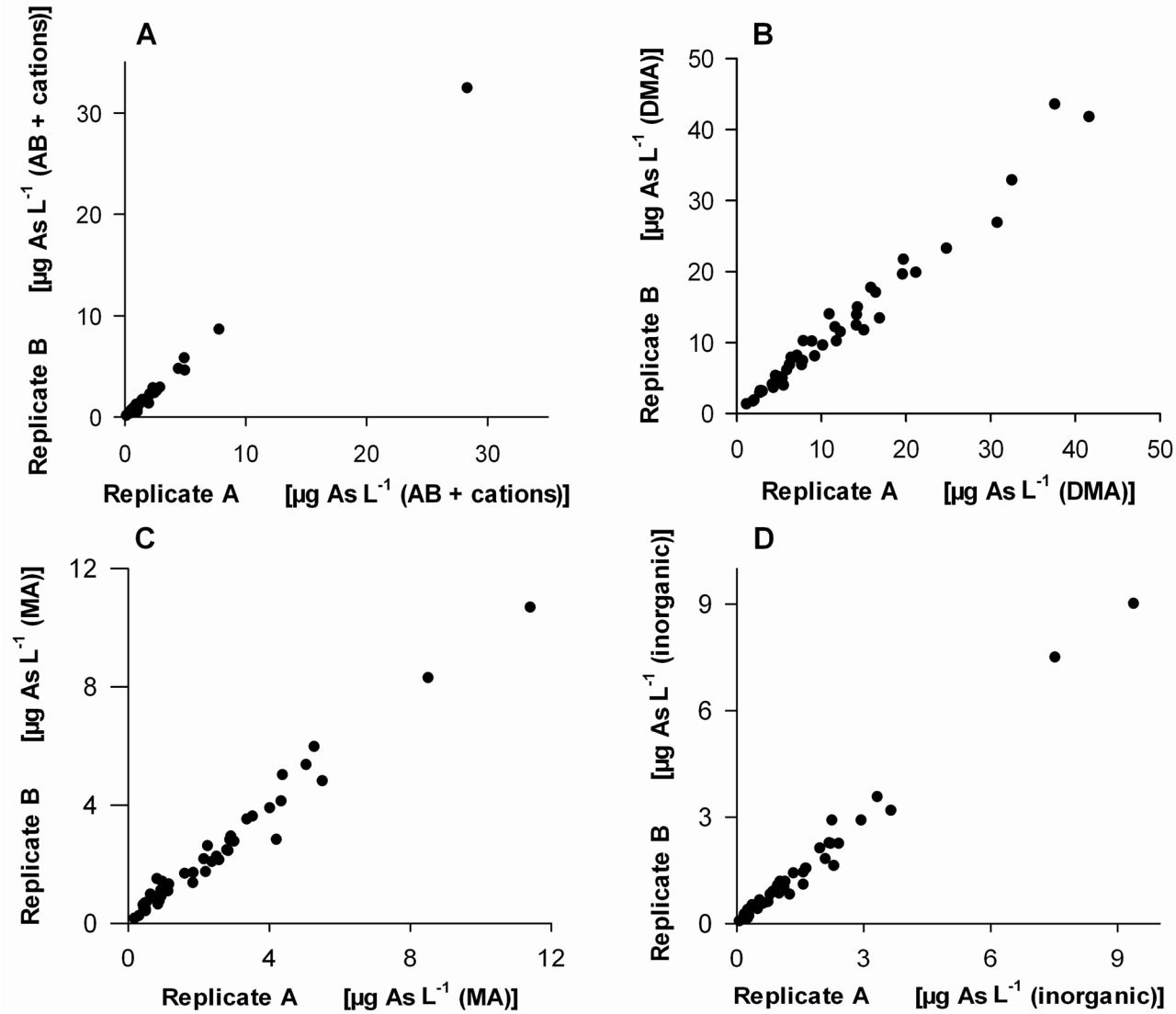

Figure 3.
Results obtained for the arsenic species in 47 (previously unknown) duplicate samples

Table 1

Analysis sequence for trace element and arsenic species analyses (typical batch of 79 samples)

Trace metal analysis (ICPMS)	As species analysis (HPLC/ICPMS)
Instrument warm up	Instrument warm up
Tune instrument for sensitivity	Tune instrument for sensitivity
Tune instrument for oxide ratios	Tune instrument for oxide ratios
Tune mass resolution, pulse/analogue factor	Tune mass resolution, pulse/analogue factor
Dummy 1,2	Calibration blank
Calibration blank	Calibration standards 1–6
Calibration standards 1–5	NIES No 18 urine
Delay_1	In-house reference urine
NIST 1643e	Preparation blank
In-house reference urine_1	Preparation blank spiked
Seronorm™ control urine_1	Samples 1–10
NIES 18	Blank_1
Delay_2	Standard 4_1
Preparation blank	In-house reference urine_1
Samples 1–10	Sample 1
Blank_1	Samples 11–20
Standard 3_1	Blank_2
In-house reference urine_2	Standard 4_2
Delay_3	In-house reference urine_2
Sample 1	Sample 10
Samples 11–20	Samples 21–30
Blank_2	Blank_3
Standard 3_2	Standard 4_3
Seronorm™ control urine_2	In-house reference urine_3
Delay_4	Sample 20
Sample 10	& so on…
Samples 21–30	Samples 71–79
Blank_3	Blank_8
Standard 3_3	Standard 4_8
In-house reference urine_3	In-house reference urine_8
Delay_5	Sample 70
Sample 20	Sample 79
& so on…	
Samples 71–79	
Blank_8	
Standard 3_8	
Seronorm™ control urine_5	
Delay_10	
Sample 70	

Trace metal analysis (ICPMS)	As species analysis (HPLC/ICPMS)
Sample 79	

Table 2

Concentrations of selected elements in NIST 1643e Reference water and NIES Nº 18 Human urine obtained during our two-year study.

Element	NIST 1643e Reference water		NIES No 18 Human urine	
	Certified value μg/L	Obtained value μg/L, n=74	Certified value μg/L	Obtained value μg/L, n=74
As	60.45 ± 0.72	59.65 ± 1.99	137 ± 11	161 ± 6
Cd	6.568 ± 0.073	6.42 ± 0.38	-	0.77 ± 0.1
Mo	121.4 ± 1.3	108.3 ± 3.8	-	115 ± 5
Pb	19.63 ± 0.21	17.74 ± 0.87	-	1.0 ± 0.2
Sb	58.30 ± 0.61	51.69 ± 2.2	-	0.16 ± 0.11
Se	11.97 ± 0.14	11.06 ± 1.03	59 ± 5	76 ± 5
W	-	0.07 ± 0.06	-	0.18 ± 0.03
Zn	78.5 ± 2.2	72.3 ± 5.7	620 ± 50	603 ± 33

Table 3

Summary of element concentrations in urine samples from the Strong Heart Study. The Pb and Sb data do not include the Pb/Sb-contaminated samples.

	Element concentrations (μg L^{-1}) in urine								
	As	**Cd**	**Mo**	**Pb***	**Sb***	**Se**	**U****	**W**	**Zn**
N	5095	5095	5095	3470	3470	5095	900	5095	5095
Nº. <LOD	1	1	0	0	74	4	81	675	0
min	< 0.1	< 0.05	0.38	0.32	< 0.05	< 2	< 0.01	< 0.05	46.5
max	1936	69.1	2520	254	13.7	707	1.76	31.1	11800
median	13.1	1.04	35.7	2.88	0.22	58.2	0.051	0.15	664
mean	20.5	1.51	46.3	3.96	0.30	70.1	0.13	0.31	839

*Contaminated samples were excluded

**Uranium was only analysed for the last three months of the study

Table 4

Precision of arsenic speciation analysis: data from analysis of in-house reference sample and NIES Nº 18 human urine over a two-year period

	In-house Reference urine				NIES Nº 18 Human urine			
	AB	**DMA**	**MA**	**iAs**	**AB**	**DMA**	**MA**	**iAs**
min [μg/L]	11.9	6.41	2.06	2.09	64.4	38.9	0.20	< 0.1
max [μg/L]	16.8	8.51	2.78	2.74	94.8	59.6	0.97	0.92
median [μg/L]	14.1	7.41	2.45	2.39	78.4	43.0	0.71	0.35
mean [μg/L]	14.0	7.48	2.45	2.4	79.0	43.9	0.66	0.40
SD [μg/L]	0.91	0.44	0.16	0.14	4.8	4.0	0.18	0.18
RSD (%)	6.5	5.9	6.5	6.0	6.1	9.0	27	46

Table 5

Summary of arsenic species concentrations in 5095 urine samples from the Strong Heart Study - data obtained from the HPLC/ICPMS of the oxidised samples.

	AB	DMA	MA	iAs
No. samples <LOD	192	1	41	286
min [μg/L]	< 0.1	< 0.1	< 0.1	< 0.1
max [μg/L]	2150	143	38.1	29.5
median [μg/L]	0.75	7.6	1.34	0.8
mean [μg/L]	3.90	10.9	2.04	1.36