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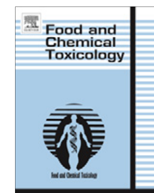

Major and minor arsenic compounds accounting for the total urinary excretion of arsenic following intake of blue mussels (*Mytilus edulis*): A controlled human study ☆

M. Molin [a,b,*], T.A. Ydersbond [c], S.M. Ulven [a], M. Holck [a], L. Dahl [d], J.J. Sloth [d,e], D. Fliegel [d], W. Goessler [f], J. Alexander [g], H.M. Meltzer [g]

[a] Department of Health, Nutrition and Management, Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, P.O. Box 4, St. Olavs Plass, NO-0130 Oslo, Norway
[b] Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, P.O. Box 1110, Blindern, NO-0317 Oslo, Norway
[c] Statistics Norway, P.O. Box 8131, Dep, N-0033 Oslo, Norway
[d] National Institute of Nutrition and Seafood Research, P.O. Box 2029, Nordnes, N-5817 Bergen, Norway
[e] National Food Institute, Technical University of Denmark, DK-2860 Søborg, Denmark
[f] Institute for Chemistry–Analytical Chemistry, Stremayrgasse 16, 8010 Graz, Austria
[g] Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, N-0403 Oslo, Norway

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A B S T R A C T

Blue mussels (*Mytilus edulis*) accumulate and biotransform arsenic (As) to a larger variety of arsenicals than most seafood. Eight volunteers ingested a test meal consisting of 150 g blue mussel (680 μg As), followed by 72 h with an identical, low As controlled diet and full urine sampling. We provide a complete speciation, with individual patterns, of urinary As excretion. Total As (tAs) urinary excretion was 328 ± 47 μg, whereof arsenobetaine (AB) and dimethylarsinate (DMA) accounted for 66% and 21%, respectively. Fifteen minor urinary arsenicals were quantified with inductively coupled plasma mass spectrometry (ICPMS) coupled to reverse-phase, anion and cation-exchange high performance liquid chromatography (HPLC). Thio-arsenicals and non-thio minor arsenicals (including inorganic As (iAs) and methylarsonate (MA)) contributed 10% and 7% of the total sum of species excretion, respectively, but there were large individual differences in the excretion patterns. Apparently, formation of thio-arsenicals was negatively correlated to AB formation and excretion, possibly indicating a metabolic interrelationship. The results may be of toxicological relevance since DMA and MA have been classified as possibly carcinogenic, and six of the excreted As species were thio-arsenicals which recently have been recognized as toxic, while iAs toxicity is well known.

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1. Introduction

Chronic exposure to inorganic arsenic (iAs) is associated with numerous adverse health effects, such as cancer in the lung, urinary bladder and skin, as well as cardiovascular diseases and diabetes (EFSA, 2009; FAO/WHO, 2010). The arsenic (As) species dimethylarsinate (DMA) and methylarsonate (MA) were recently classified as "possibly carcinogenic" to humans by the International Agency

Abbreviations: AB, arsenobetaine; AC, arsenocholine; As (III), arsenite; As (V), arsenate; As, arsenic; CRM, certified reference material; CRP, C-reactive protein; DMA, dimethylarsinate; DMAB, dimethylarsenobutanate; HPLC, high performance liquid chromatography; iAs, inorganic arsenic; ICPMS, inductively coupled plasma mass spectrometry; LOQ, limit of quantification; MA, methylarsonate; oxo-DMAB, dimethylarsenobutanate; oxo-DMAE, dimethylarsenoethanol; tAs, total arsenic; TETRA, tetramethylarsonium ion; Thio-DMA, thio-dimethylarsinate; Thio-DMAA, thio-dimethylarsenoethanol; Thio-DMAE, thio-dimethylarsenoethanol; Thio-DMAB, thio-dimethylarsenobutanate; Thio-DMAP, thio-dimethylarsenopropionate; Thio-Gly, thio-arsenosugar-glycerol; Thio-phosphate, thio-arsenosugar-phosphate; TMAO, trimethylarsine oxide; TMAP, trimethylarsoniopropionate; TMAS, trimethylarsine sulfide.

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* Corresponding author at: Department of Health, Nutrition and Management, Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, P.O. Box 4, St. Olavs Plass, NO-0130 Oslo, Norway. Tel.: +47 930 90 250; fax: +47 64 84 90 02.

E-mail addresses: Marianne.Molin@hioa.no (M. Molin), Trond.Arild.Yders bond@ssb.no (T.A. Ydersbond), StineMarie.Ulven@hioa.no (S.M. Ulven), Marianne. Holck@hioa.no (M. Holck), Lisbeth.Dahl@nifes.no (L. Dahl), Daniel.Fliegel@nifes.no (D. Fliegel), walter.goessler@uni-graz.at (W. Goessler), Jan.Alexander@fhi.no (J. Alexander), Helle. Margrete.Meltzer@fhi.no (H.M. Meltzer).

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for Research on Cancer (IARC), while arsenobetaine (AB) was "not classifiable" (Straif et al., 2009). However, exposure to arsenolipids, arsenosugars, thio-arsenicals and other minor arsenicals and their potential impact on human health is not clearly established (EFSA, 2009).

Fish and seafood, including shellfish and algae, are main contributors of As in the diet, and the dominating species is AB (Dahl et al., 2010; EFSA, 2009). Blue mussels (*Mytilus edulis*) are filter feeders that accumulate metals and metalloids in their soft body parts from water and particulate matters. In addition, blue mussels have the capacity to biotransform such compounds (Airas et al., 2004; Burger and Gochfeld, 2006). In contrast to e.g. marine fish, where AB may constitute up to 95% of total arsenic (tAs), blue mussels may contain only 30–40% AB of tAs. Blue mussels can contain large amounts of iAs; although in most cases less than 5%, amounts up to 42% iAs of tAs has been reported in one single study (Lai et al., 2004; Sloth and Julshamn, 2008). A large part of the increase in tAs content in blue mussels along an As contamination gradient was found to be due to iAs, and at low contamination, sulphur-bound As accounted for as much as 36% of tAs (Whaley-Martin et al., 2012). DMA and minor arsenicals seem to account for 10–20% of tAs, seemingly somewhat less than the share of arsenosugars, which are mainly found in algae and algal-consuming animals like mussels (EFSA, 2009; Lai et al., 2004). Hence, blue mussels may contain appreciable amounts of a much wider range of As compounds than most other dietary sources of As.

A major pathway for elimination of As compounds in humans seems to be by urinary excretion, with AB and DMA as the dominating urinary metabolites. The extent of biotransformation depends both on the chemical form of the As ingested and inter-individual variations (EFSA, 2009; Raml et al., 2009; WHO, 2001). Although no precise quantitative data exist on AB absorption in humans, AB is generally considered to be metabolically stable and of no known toxicological concern. It is readily absorbed and rapidly excreted, seemingly unchanged, with more than 50% eliminated through urine after 24 h (Brown et al., 1990; EFSA, 2009; Freeman et al., 1979; Tam et al., 1982). Inorganic As is readily absorbed (ATSDR, 2007) and seems mostly to be methylated to DMA and/or MA before being excreted in urine (Caldwell et al., 2009; Vahter, 2002). MA, DMA and arsenosugars may be other sources of urinary MA and DMA after mussel ingestion (Buchet et al., 1994, 1996; Lai et al., 2004), but currently, these sources are far from completely identified.

During the last decade, great improvement has been made in the determination and quantification of a range of As species by high performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (HPLC–ICPMS) (Raml et al., 2005, 2006; Schmeisser et al., 2005, 2006; Taleshi et al., 2010). The improved analytical methods are a means for further characterization of As metabolism. Thio-arsenicals, the sulphur analogues of oxo-arsenicals, have recently been reported in both shellfish and human urine (Raml et al., 2005, 2007; Schmeisser et al., 2004; Soeroes et al., 2005), and have shown cytotoxic effects *in vitro* (Ochi et al., 2008) in addition to a toxicity similar to that of DMA (III) (Naranmandura et al., 2011).

Here we report in-depth analyses of urinary As compounds after ingestion of a bolus dose of blue mussels, as part of a randomized controlled seafood study carried out in 38 healthy subjects allocated into four different groups (salmon, cod, blue mussel and potato) (Molin et al., 2012). Eight of the participants in this study consumed a breakfast meal of blue mussels followed by an identical controlled diet and full urine sampling for 72 h. The main focus in this article is on the minor arsenicals, among them the recently described thio-arsenicals in blue mussels (Whaley-Martin et al., 2012), and the individual variation of their excretory patterns. To the best of our knowledge, this is the first report to provide an essentially complete speciation of urinary As compounds after a bolus dose of blue mussels. The aim of the present study is to use the controlled study setting to document the urinary excretion of minor arsenicals and study individual variations in excretory patterns, in conjunction with the major arsenicals excreted after a blue mussel meal.

2. Subjects and methods

2.1. Subjects and study design

The general design of the study, has been reported elsewhere (Molin et al., 2012). The blue mussel group included in the present study consisted of 8 non-smoking subjects (5 women and 3 men) (Table 1), aged 23–37 (mean 27.9) years. The control group ingested a non-seafood diet low in As and consisted of 10 (6 women and 4 men) non-smoking individuals, aged 20–40 (mean 24.5). Following a 1 week wash-out period where the participants were instructed to refrain from eating foods containing As (seafood, rice, rice products, mushrooms), the subjects ingested a bolus dose of blue mussel (150 g) in the form of a pie (test meal) served as breakfast. During the following 72 h, the subjects were given an identical, controlled diet designed to be devoid of As.

The study was approved by the National Committee for Research Ethics and was carried out in accordance with The Code of Ethics of the World Medical Association. Written informed consent was obtained from each participant.

2.2. Blood and urine sampling

Fasting blood samples were collected at baseline (day-7) and on day 0 before ingestion of the test meal. In addition blood samples were collected 2, 4, 24 and 48 h after ingestion of the test meal. All urine was collected 72 h following the test meal; in three intervals during the first 24 h (after test meal-2 pm, 2–7 pm, 7 pm-morning urine next morning) and the following 2 days in 24 h urine batches. All urine volumes registered were within the normal range (mean urine volume 2.03–2.51 L/day, range 0.49–7.73), indicating that essentially all urine was collected. The urine samples were stored at −70 °C until analysis.

2.3. Analytical methods

The tAs in the food, plasma and urine was determined using ICPMS as previously described (Julshamn et al., 2007; Sloth et al., 2005b). The As speciation analysis in the food was performed using ICPMS coupled to HPLC as previously described (Sloth et al., 2003, 2005b). The As speciation analysis in the urine was measured with anion-, cation- and reversed-phase HPLC–ICPMS, as previously described (Raml et al., 2006). Prior to sample measurements, the samples were filtrated (0.2 μm) and subsequently injected into the HPLC system. For the separation of As (V), DMA and MA, initially all As (III) was converted into As (V) using H_2O_2 (10%) for 2 h at >30 °C. Then the compounds were separated with anion exchange-chromatography on a PRP-X100 (4.6 × 150 mm) column. A 20 mM $NH_4H_2PO_4$ solution at pH 6.0 and a flow rate of 1.5 mL min^{-1} was used as mobile phase. The injection volume was 10 μL and the column temperature was 40 °C. The compounds AB, TMAO/DMAE, TETRA, AC and TMAP were determined with cation-exchange chromatography on a Zorbax 300 SCX (4.6 × 250 mm) column. A 10 mM aqueous pyridine solution at pH 2.3 and a flow rate of 1.0 mL min^{-1} was used as mobile phase. The injection volume was 5 μL and the column temperature was 30 °C. The compounds Oxo-DMAB, TMAS, thio-Gly, thio-DMAA, thio-DMAE, thio-DMAP, thio-DMAB, oxo-DMAB were measured with reversed phase chromatography on an Atlantis dC18 (4.6 × 150 mm) column. A 20 mM $NH_4H_2PO_4$ at pH 3.0 and a flow rate of 1.0 mL min^{-1} was used as mobile phase. The injection volume was 5 μL and the column temperature was 30 °C. For quality assurance the CRM (certified reference material) NIES No 18 human urine was used. For AB, we determined a concentration of: 67.5 ± 1.8 μg As/L (certified: 69 ± 12 μg/L) and for DMA we measured: 42.0 ± 1.5 μg As/L (certified: 36 ± 9 μg/L). The concentration of our lowest standard solution (0.5 μg As/L) was set as the LOQ for the measurement. The column recoveries were 89 ± 15% for the anion-exchange results and 94 ± 13% for the cation-exchange chromatography.

Results from the As analyses were reported in μg/L and not normalized to specific gravity or creatinine. Since all urine was collected 72 h following the test meal, the urine volume was known and the results therefore calculated in μg. All chemical structures, abbreviations and generic names of the arsenicals detected after the bolus dose of blue mussel are given in Fig. 1.

2.4. Statistical analyses

The SPSS 14.0 software package (SPSS Inc., Chicago, IL, USA) and R 2.12.0 (http://cran.r-project.org/) were used for the statistical analyses. The non-parametric Spearman Rank Correlation test was used to measure correlations, except for the cases where linear ANOVA/ANCOVA models were applied, in which case the model's estimated *r*, adjusted for degrees of freedom, was used. The *p*-values for group

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