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Urinary excretion of arsenicals following daily intake of various seafoods during a two weeks intervention ☆

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

[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] Atlantis Medical University College, P.O. Box 4290 Nydalen, N-0402 Oslo, Norway
[d] National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, N-5817 Bergen, Norway
[e] Institute for Chemistry-Analytical Chemistry, Stremayrgasse 16, 8010 Graz, Austria
[f] National Food Institute, Technical University of Denmark, DK-2860 Søborg, Denmark
[g] Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, N-0403 Oslo, Norway
[h] Statistics Norway, P.O. Box 8131 Dep, N-0033 Oslo, Norway

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ABSTRACT

The excretion pattern of arsenic (As) species after seafood intake varies widely depending on species ingested and individual handling. We have previously reported the 72 h urinary excretion of arsenicals following a single dose of seafood. Here, we report the excretion patterns in the same 37 subjects following 15 days daily consumption of either 150 g cod, salmon, blue mussels or potato (control), followed by a 72 h period with a low-As diet. In all seafood groups, total As (tAs) in plasma and urinary excretion of tAs, arsenobetaine (AB) and dimethylarsinate (DMA) increased significantly after the intervention. Confirming the single dose study AB and DMA excreted were apparently endogenously formed from other arsenicals ingested. Total tAs excretion was 1386, 763 and 303 μg in the cod, blue mussel and salmon groups, respectively; about twice the amounts after the single dose study indicating accumulation of arsenicals. In the cod group, rapid excretion after the single dose was associated with lower total As in blood and less accumulation after two weeks with seafood indicating lower accumulation. In the blue mussels group only, inorganic As (iAs) excretion increased significantly, whilst methylarsonate (MA) strongly increased, indicating a possible toxicological concern of repeated mussel consumption.

1. Introduction

While it is well documented that a diet rich in seafood promotes health (Alexander et al., 2006; Mozaffarian and Rimm, 2006), seafood may also contain potentially harmful compounds, like arsenic (As) (Alexander et al., 2006; Sirot et al., 2012). In addition to the well-known toxicant inorganic As (iAs), thio-arsenicals, which have been little studied so far, constituted 10% of urinary As excreted after a bolus dose of blue mussels (Molin et al., 2012b). Thio-arsenicals are considered potentially toxic (Naranmandura et al., 2011; Ochi et al., 2008; Rehman and Naranmandura, 2012). High concentrations of methylarsonate (MA) and dimethylarsinate (DMA) may also be of concern (EFSA, 2009; Fowler et al., 2007). For most of the population within the EU, As exposure from drinking water is insignificant, but the possible risks of As exposure from a seafood rich diet are little studied (Borak and Hosgood, 2007; Choi et al., 2010; EFSA, 2009).

Abbreviations: AB, arsenobetaine; AC, arsenocholine; As (III), arsenite; As (V), arsenate; As, arsenic; As3MT, arsenic methyl transferase; CR, creatinine; CRM, certified reference material; CRP, C-reactive protein; DMA, dimethylarsinate; HPLC, high performance liquid chromatography; iAs, inorganic arsenic; ICP-MS, inductively coupled plasma mass spectrometry; ∠LOQ, limit of quantification; MA, methylarsonate; tAs, total arsenic; TETRA, tetramethyl arsonium ion.

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* Corresponding author. Address: Atlantis Medical University College, P.O. Box 4290 Nydalen, N-0402 Oslo, Norway. Tel.: +47 930 90 250; fax: +47 23 00 73 51.

E-mail addresses: molinmarianne@gmail.com (M. Molin), sulven@hioa.no (S.M. Ulven), Lisbeth.Dahl@nifes.no (L. Dahl), walter.goessler@uni-graz.at (W. Goessler), Daniel.Fliegel@tine.no (D. Fliegel), marianneholck@yahoo.no (M. Holck), jjsl@food.dtu.dk (J.J. Sloth), arne.oshaug@hioa.no (A. Oshaug), Jan.Alexander@fhi.no (J. Alexander), Helle.Margrete.Meltzer@fhi.no (H.M. Meltzer), Trond.Arild.Ydersbond@ssb.no (T.A. Ydersbond).

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The water-soluble specie arsenobetaine (AB) is the main arsenical in most seafood, usually constituting up to 95% of tAs in marine fish and up to 30–40% in blue mussel (EFSA, 2009). The iAs content is generally low in most seafood; fin-fish like cod and salmon usually contain <1% but bivalves like blue mussels in most cases contain up to 5% although amounts up to 42% iAs of tAs (corresponding to 5.8 mg iAs/kg wet weight) has been reported (EFSA, 2009; Sloth and Julshamn, 2008). Arsenosugars are the major arsenicals in marine algae (usually 2–50 mg As/kg dry mass) and they are found in animals feeding on algae, like e.g. mussels, which typically contain 0.5–5 mg As/kg dry mass (Borak and Hosgood, 2007). Scarce documentation show that arsenolipids accounts for 10–30% of tAs (Sele et al., 2012) and their presence have been reported in cod liver oil, capelin and tuna (Rumpler et al., 2008; Schmeisser et al., 2005; Taleshi et al., 2010, 2008) and they are most likely present also in other fish species, especially fatty fish (Sele et al., 2012).

Ingested arsenicals seem to be readily absorbed (30–95%) in the gastro-intestinal tract, and urinary excretion is the major pathway of elimination of As from the body (Fowler et al., 2007; WHO, 2001). AB and DMA are the major arsenicals excreted (Buchet et al., 1996, 1994; Heinrich-Ramm et al., 2002; Lai et al., 2004; Le et al., 1994; Molin et al., 2012a). AB is assumedly not metabolized, and thus excreted unchanged in urine (Brown et al., 1990; Freeman et al., 1979; Tam et al., 1982; Vahter et al., 1983; Yamauchi and Yamamura, 1984). Arsenosugars and arsenolipids seem to be metabolized with DMA as one of the main metabolites (Francesconi et al., 2002; Ma and Le, 1998; Molin et al., 2012b; Raml et al., 2005; Schmeisser et al., 2006).

Inorganic As seems mostly to be methylated and excreted as DMA and MA (Caldwell et al., 2009; Vahter, 2002), and this bioconversion process was previously viewed as a detoxification mechanism. The current view, however, is that the methylation pathway probably is related to the toxic action of iAs (Tseng, 2009), possibly via the production of highly reactive intermediate products, MA (III) (methylarsonite) and DMA (III) (dimethylarsinite) (Challenger, 1947; Hayakawa et al., 2005). Furthermore, the iAs metabolites DMA and MA were recently classified as "possibly carcinogenic" to humans by the International Agency for Research on Cancer (IARC) (IARC, 2009). Similar intermediates may be formed during the biotransformation of arsenosugars and arsenolipids to DMA and thus the toxicology of these, previously considered non-toxic arsenicals requires further investigation (Sele et al., 2012).

To obtain more information about the effects of As intake with seafood and the subsequent urinary arsenical excretion, including possible health risks, a controlled dietary study consisting of two parts was conducted. In the first part the urinary As excretion after a single dose of either 150 g cod, salmon, blue mussel or control (potato), respectively, was assessed (Molin et al., 2012a). High urinary DMA excretion was found following a blue mussel and salmon intake, indicating that DMA is not only a result of the iAs methylation, but a result of biotransformation of organoarsenicals present in these types of seafood species as well. The results after the single dose intervention also indicated a possible endogenous formation of AB from other arsenicals present in the seafood (Molin et al., 2012a,b).

Here the results from the second part of the study, assessing the urinary As excretion after 15 consecutive days with daily intake of seafood, similar to that consumed as one single dose, is reported. The aims of the present study were (1) to compare the excretion pattern after 15 consecutive days with seafood with that after a bolus dose of seafood and (2) to explore whether a repeated intake of seafood for 15 consecutive days resulted in accumulation of arsenicals of possible health concern. Ultimately, the aim of the study was to improve the understanding of the human metabolism of arsenicals in seafood.

2. Subjects and methods

2.1. Subjects

Thirty-seven healthy subjects (C-reactive protein (CRP) <10 mg/L), 10 men and 27 women, aged 20–40 years, recruited from Akershus University College, Norway, were part of this study. Exclusion criteria were smoking, pregnancy, lactation and the use of medical drugs other than contraceptives. Additionally, subjects with a habitual seafood consumption that was higher than generally recommended in Norway (>three servings/week) were excluded. All participants were compliant with the protocol throughout the study. Based on the amount of leftovers of the trial-food during the experimental period, compliance was estimated to be 97 (92–100)%, 97 (86–100)%, 93 (73–100)% and 98 (95–100)% in the cod, salmon, blue mussel and control group during the 14 day semi-controlled diet period. Further details on recruitment and baseline data regarding the subjects have previously been described (Molin et al., 2012a).

2.2. Study design

A randomized controlled, parallel group study, lasting 31 days in total, was conducted. Part of the design (day −7 to day 2) describing the urinary arsenical excretion after a single dose of seafood has been reported previously (Molin et al., 2012a). Four intervention groups ingested either cod (*Gadus morhua*) ($n = 9$), farmed salmon (*Salmo salar*) ($n = 11$), blue mussel (*Mytilus edulis*) ($n = 7$) or potato ($n = 10$) during the intervention (Fig. 1). In the first part of the controlled dietary study the groups received a single meal consisting of 150 g seafood/potato (test meal 1) served for breakfast (8–10 am), after a one-week run-in period. In the run-in period and throughout the study period of 31 days, the subjects were instructed to abstain from eating food items rich in As; seafood (except what was provided in the study), mushrooms, rice or rice products or take any dietary supplements. Consumption of cod liver oil, a food supplement commonly used in Norway, was prohibited, starting four weeks prior to test meal 1 and throughout the study period. One week after the single dose of seafood/potato (test meal 1), in the second part of the controlled dietary study, the subjects received a semi-controlled seafood diet for 14 consecutive days followed by a single meal consisting of 150 g seafood/potato (test meal 2) (Fig. 1). After intake of test meal 1 and 2, all subjects consumed a strictly controlled diet prepared and served at the University College the next 72 h (day 0–2 and day 21–23). The subjects were requested to eat all food served and to maintain their normal physical activity routines. Tap water (the As level in Norwegian groundwater is mostly below 0.2 μg As/L (Olsen and Morland, 2004)) and energy buns were provided with no restrictions during the strictly controlled diet periods.

2.3. Test meals, the 72 h controlled diet and the 14 days semi-controlled diet

The seafood/potato test meals consisted of pies made with identical recipes practically free from As (flour, wholemeal flour, quark, butter, water and salt) for all intervention groups except for the 150 g of seafood/potato ingredient. It was served as a breakfast between 8 and 9 pm on day 0.0 and on day 21.0 (Fig. 1). The two strictly controlled diet periods were identical; all food and drink were provided (day 0–2 and day 21–23) and the diet was designed to be low in tAs while meeting the Nordic recommended daily intake of energy (2100 kcal/8.8 MJ) (NNR, 2004). Further details on the test meals and the strictly controlled diet has been reported previously (Molin et al., 2012a).

The 14 days semi-controlled diet consisted of a 7-day menu with warm and cold dishes repeated twice (Fig. 1). All the dishes were identical for all intervention groups except for the 150 g seafood/potato ingredient and the portion size was a realistic daily portion size of seafood. During this period food and drink ingested, besides the seafood/potato meal, were free of choice as long as it was within the food restrictions. The test meals, the 72 h strictly controlled diet (except the supper that were brought in bags and eaten in the subjects homes) and the semi-controlled diet were served Monday–Friday at the University College. During the semi-controlled diet period lunch boxes to bring home were provided for the weekend.

2.4. Blood samples

Blood samples were collected from fasting subjects (minimum 12 h) at the same time (8–10 am) on day 0 and day 21. In addition blood samples were collected 2 h, 4 h, 24 h, and 48 h after ingestion of test meal 1 and 2 (Fig. 1). Plasma was obtained from EDTA tubes kept at room temperature (0–30 min) and centrifuged at 1300*g* for 10 min. All plasma samples were kept frozen (−70 °C) until analysis.

2.5. Urine samples

Following ingestion of the test meals on day 0.0 and 21.0, urine was collected in three periods during the first 24 h: (0.1 and 21.1) between the test meal and 2 pm, (0.2 and 21.2) 2–7 pm, (0.3 and 21.3) 7 pm until first urination on the following day (Fig. 1). For the next 48 h (day 1–2 and day 22–23), 24 h-urine batches were collected. Furthermore, morning spot samples of urine were collected at baseline (day −7), day 0.0, day 7 and day 21.0 (Fig. 1). All urine samples were kept at 4 °C