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

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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. 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.

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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 Hoggood, 2007; Choi et al., 2010; EFSA, 2009).
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 Hogsrod, 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 (Rumpel 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 assumed 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 toxicity 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). Urinary DMA excretion was found following a blue mussel and salman 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 contraceptive. 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 1 to day 2) describe 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 seafood/potato ingredient. It was served as a breakfast between 8 and 9 pm on day 0.5 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 described 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 1300g 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 (days 1–2 and days 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.
Fig. 1. Study design. Thirty-seven subjects were randomized (the subjects were randomized, but not stratified by gender, and therefore by chance all subjects in the salmon group were females) to four intervention groups: cod (9), salmon (11), blue mussel (7 (originally 38 subjects were part of the study, but data is missing for one subject in the blue mussel group for period 2)) and control (10). During the seven days of run-in (day 7 to day 0) and throughout the intervention (through day 23) the volunteers kept restrictions regarding As-containing food. At day 0.0 and day 21.0 an identical controlled test meal were given and the following 72 h all urine were collected; the first 24 h in three batches (test meal-2 pm, 2–7 pm, 7 pm until morning urine next day), the two last days in 24 h-batches. During the 72 h urine-collection periods, all volunteers got an equal, strictly controlled diet, identical for both periods. The urinary As excretion after test meal 1 reflects the urinary As excretion after ingestion of a single meal of seafood, whereas the urinary As excretion after ingestion of test meal 2 reflects the As excretion after a 15 consecutive days with seafood (14 days with seafood/potato lunch followed by test meal 2). The arrows pointing upwards indicate the time the urine and plasma samples were collected. Regarding the urine samples, it is indicated whether the sample was a morning spot sample (spot) or part of a complete sampling (24 h).

2.6. Adjustment for creatinine

The morning spot urine samples on day 7 and 21 were adjusted for the urinary creatinine (CR) concentrations. The mean CR concentration on day 7 and day 21 was 1.51 (0.51) g/L and 1.65 (0.64) g/L, respectively (Table 1). For the 72 h after the last seafood meal (test meal 2), all urine was collected, thus the total amount (μg) As excreted was calculated and therefore not adjusted for the creatinine concentrations.

2.7. Analytical methods

The tAs in the food, plasma and urine was determined using inductively coupled plasma mass spectrometry (ICP-MS) as previously described (Jukkaaho et al., 2007; Molin et al., 2012a). The arsenicals DMA, MA and iAs were measured with HPLC-ICP-MS using an anion-exchange column, while AB was measured with HPLC-ICP-MS using a cation-exchange column as previously described (Molin et al., 2012a). In all cases external calibration was used for the quantification of tAs and individual arsenicals.

The accuracy of the determination of tAs in the food was evaluated by means of analysis of the certified reference materials (CRMs) DORM-2 Dogfish muscle (National Research Council of Canada) and BCR CRM 627 Tuna fish tissue (IRMM, Geel, Belgium); the latter reference material was also used in the evaluation of the certified reference analysis of the food. NIES No 18 Human urine (National Institute for Environmental Studies, Ibaraki, Japan) was used to evaluate tAs as well as iAs, MA, DMA and AB in the urine samples. All obtained results agreed well with the respective CRMs as previously described in Molin et al. (2012a).

For the urinary creatinine determination, the urine samples prior to analysis were defrosted at room temperature and centrifuged in an Eppendorf (5810R) centrifuge (15 min, 2000 × g) until analysis. An aliquot of 200 μL was transferred to the test tube and placed in the Maxmat carousel. All sample treatment was automatic; samples were diluted 1:20 with distilled water and reagents added. All samples were analyzed in one batch on the same day. Determination of CR was performed by a colorimetric enzymatic principle using MAXMAT PL II multidisciplinary diagnostic platform using the Creatinine PAP kit. (Maxmat S.A, Montpellier, France).

2.8. Statistical analyses

The IBM® SPSS® Statistics (version 19) (SPSS Inc., Chicago, IL, USA) and R 2.10.1 (http://cran.r-project.org/) were used for the statistical analyses. For maximal robustness, non-parametric tests were preferably used due to the small sample size: Mann Whitney U test were used to identify significant between-group differences, while Wilcoxon paired samples test were used to identify within-group changes. Coefficients of correlation were calculated by the Spearman’s rho test. In addition, ANOVA/ANCOVA models together with pairwise t-tests (with Holm’s correction for multiple comparisons) were used when appropriate, in particular when linear model estimates were needed. Non-quantified values (i.e. values below LOQ), were set at LOQ/2 as suggested by international guidelines (FAO/WHO, 1995; Kroes et al., 2002). The following values (LOQ/2) were used in the present study: iAs (0.15 μg/kg), DMA (0.25 μg/kg), and MA (0.15 μg/kg).

3. Results

3.1. As content of the diet

The average daily tAs intake was calculated by analysing two identical samples of each of the test meals and the duplicate portions of the strictly controlled diet. The daily tAs intake was 670, 180, 620 and 3.7 μg and the iAs intake was 2.8, 3.3, 13 and 4.4 μg in the semi-controlled diets of the cod, salmon, blue mussel and control group, respectively (Table 2, column 2).

3.2. Urinary and plasma As concentrations

Between the test meal 1 on day 0 and the start of the 15 days with seafood on day 7, the subjects ingested a diet practically free from As (Fig. 1). Thus, the morning urine As concentrations on day 7 reflects the concentration after a one week wash-out of As, while the morning urine As concentrations on day 21 (before the last meal of the 15 days with seafood) is the resulting balance obtained following 14 days of repeated intake and elimination (Table 1). The mean urinary tAs concentrations at day 7 was below 20 μg/g CR for all intervention groups, comparable to that observed in unexposed, healthy subjects from the UK, who refrained from seafood three days prior to urine sampling (17.5 μg/g CR) (Brima et al., 2006). The tAs concentrations in morning spot urine increased significantly (p < 0.05) for all seafood groups during the intervention, with the cod group showing the highest tAs concentration, followed by the blue mussel, salmon and control group, respectively (486 μg/g CR, 184 μg/g CR, 57 μg/g CR and 8.4 μg/g CR) (Table 1). The same pattern was seen for plasma, mean plasma tAs concentrations at day 7 were below 0.9 μg/L, but increased significantly (p < 0.05) after the intervention for all seafood groups, with the following plasma tAs concentrations on day 21: 12.6 μg/L, 4.6 μg/L, 2.9 μg/L and 0.7 μg/L for the cod, blue mussel, salmon and control group, respectively.

The AB spot urine concentrations were below 17 μg/g CR for all intervention groups on day 7 and increased significantly (p < 0.05) in all the seafood-consumming groups during the intervention. The
The urinary excretion of tAs and the arsenicals AB, iAs, DMA and MA during the 72 h follow-up period is shown in Table 1. The cod group excreted the largest amount of tAs (1390 μg, Table 2); 2-fold more than the blue mussel group, 4.5-fold more than the salmon group and 25-fold more than the control group (Table 2, column 6). The AB excretion was highest in the total excreted MA was excreted during the first 24 h. The iAs excretion in the blue mussel group (34 μg/L) was more than 20-fold higher than that found in the other intervention groups. 75–90% of the total excreted MA was excreted during the first 24 h.

3.3. Urinary excretion of tAs, AB, iAs, DMA and MA during the 72 h follow-up period

The urinary excretion of tAs and the arsenicals AB, iAs, DMA and MA during the 72 h follow-up period after test meal 2, is shown in Table 2. The data for day 21-23 is missing for one subject.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Total n = 38 (37)</th>
<th>Cod n = 9</th>
<th>Salmon n = 11</th>
<th>Blue mussel n = 8 (7)</th>
<th>Control (potato) n = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>tAs plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
</tr>
<tr>
<td>Before</td>
<td>0.70 (0.4)</td>
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<td>0.008*</td>
<td>0.9 (0.6)</td>
<td>0.08*</td>
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<tr>
<td>After</td>
<td>5.0 (5.8)</td>
<td>5.0 (5.0–5.0)</td>
<td>0.5 (0.5–2.1)</td>
<td>2.9 (1.6)</td>
<td>4.6 (1.8)</td>
</tr>
<tr>
<td>g/L</td>
<td>10.6 (7.8–30.5)</td>
<td>3.0 (0.5–4.8)</td>
<td>4.9 (2.5–7.9)</td>
<td>0.5 (0.5–2.1)</td>
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</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Total n = 38 (37)</th>
<th>Cod n = 9</th>
<th>Salmon n = 11</th>
<th>Blue mussel n = 8 (7)</th>
<th>Control (potato) n = 10</th>
</tr>
</thead>
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<tr>
<td>tAs plasma</td>
<td></td>
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</tr>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
</tr>
<tr>
<td>Before</td>
<td>1.51 (0.51)</td>
<td>1.43 (0.47)</td>
<td>NS</td>
<td>1.76 (0.48)</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>1.65 (0.64)</td>
<td>1.68 (0.75)</td>
<td>NS</td>
<td>1.58 (0.69)</td>
<td>NS</td>
</tr>
<tr>
<td>µg As/g CR</td>
<td>µg As/g CR</td>
<td>p-Value</td>
<td>µg As/g CR</td>
<td>p-Value</td>
<td>µg As/g CR</td>
</tr>
<tr>
<td>Before</td>
<td>13.7 (11.2)</td>
<td>11.6 (6.04)</td>
<td>0.008*</td>
<td>9.58 (6.06)</td>
<td>0.003*</td>
</tr>
<tr>
<td>After</td>
<td>10.4 (2.92–59.7)</td>
<td>11.7 (5.04–25.2)</td>
<td>7.83 (3.57–23.6)</td>
<td>11.8 (8.1–27.2)</td>
<td>12.8 (2.92–59.7)</td>
</tr>
<tr>
<td>AB urine</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
</tr>
<tr>
<td>Before</td>
<td>11.5 (10.8)</td>
<td>10.0 (4.59)</td>
<td>0.008*</td>
<td>7.17 (6.87)</td>
<td>0.004*</td>
</tr>
<tr>
<td>after</td>
<td>8.94 (12.1–49.2)</td>
<td>9.68 (4.61–18.3)</td>
<td>4.64 (1.46–22.4)</td>
<td>10.6 (6.82–23.0)</td>
<td>11.7 (12.4–49.2)</td>
</tr>
<tr>
<td>iAs urine</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
</tr>
<tr>
<td>Before</td>
<td>0.25 (0.17)</td>
<td>0.30 (0.29)</td>
<td>NS</td>
<td>0.20 (0.09)</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>0.20 (0.08–0.99)</td>
<td>0.18 (0.11–0.99)</td>
<td>0.19 (0.08–0.39)</td>
<td>0.18 (0.11–0.40)</td>
<td>0.24 (0.12–0.45)</td>
</tr>
<tr>
<td>DMA urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
</tr>
<tr>
<td>Before</td>
<td>2.25 (1.60)</td>
<td>1.85 (1.01)</td>
<td>0.008*</td>
<td>1.97 (0.58)</td>
<td>0.003*</td>
</tr>
<tr>
<td>After</td>
<td>1.84 (0.69–10.3)</td>
<td>1.55 (0.69–3.97)</td>
<td>1.84 (0.98–3.23)</td>
<td>1.97 (1.3–4.77)</td>
<td>1.9 (0.94–10.3)</td>
</tr>
<tr>
<td>MA urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
<td>p-Value</td>
<td>µg/L</td>
</tr>
<tr>
<td>Before</td>
<td>0.34 (0.20)</td>
<td>0.40 (0.29)</td>
<td>NS</td>
<td>0.33 (0.12)</td>
<td>NS</td>
</tr>
<tr>
<td>After</td>
<td>0.31 (0.14–1.06)</td>
<td>0.30 (0.15–1.06)</td>
<td>0.35 (0.14–0.50)</td>
<td>0.22 (0.15–0.68)</td>
<td>0.29 (0.14–0.77)</td>
</tr>
</tbody>
</table>

### Notes
- The subjects followed restrictions regarding As-containing foods one week prior to day 7.
- The data for day 21-23 is missing for one subject.
- p-Value for significant within-group changes (Wilcoxon paired samples test).
- Significant between-group changes when compared with control group (Mann Whitney U test) are marked with an asterisk (*).
decreased, with the slope of the curve flattening throughout the rest of the 72 h period. Most subjects in the study groups had lower plasma tAs concentrations 48 h after test meal 2 than at 21.0 h (before ingestion of the 15th seafood meal/test meal 2). The mean percent of tAs excretion 2 h after test meal 2 was 47%, 179%, 105%, and 3.2-fold, respectively. The relative excretion was significantly (and as a consequence, tAs) in the cod group and DMA in the blue mussels group being the two notable exceptions.

The urinary tAs concentration patterns were qualitatively similar in the two periods (Fig. 3B). When plotting the tAs excretion for both periods cumulatively, the increased excretion rates at the end of period 2 became apparent: 4.7-fold; p < 0.0001, 1.5-fold; NS and 3.2-fold, p < 0.01 for day 23 vs day 2 in the cod, salmon and blue mussels groups, respectively (Fig. 3C). Fig. 3D shows the individual cumulative relative excretion curves in periods 1 and 2 in the cod and blue mussels group. Percentages are given relative to the single meal in period 1 or last of 15 meals in period 2. The mean period 2 relative amount of tAs excreted was 47%, 179%, 105%, and 113% in the control, cod, salmon and blue mussels groups, respectively (Fig. 2B–D).

### Table 2

<table>
<thead>
<tr>
<th>Group/species</th>
<th>2 Daily amount ingested</th>
<th>3 Urine day 21</th>
<th>4 Urine day 22</th>
<th>5 Urine day 23</th>
<th>6 Urine day 21–23</th>
<th>7 % of tAs excreted day 21–23</th>
<th>8 % of tAs excreted day 0–3</th>
<th>9 p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cod group (n = 9)</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Total As</td>
<td>670</td>
<td>787 (202)</td>
<td>347 (118)</td>
<td>252 (119)</td>
<td>1390 (412)</td>
<td>100</td>
<td>100</td>
<td>NS*</td>
</tr>
<tr>
<td>AB</td>
<td>490</td>
<td>719 (150)</td>
<td>296 (114)</td>
<td>223 (120)</td>
<td>1240 (363)</td>
<td>89.3</td>
<td>88.5</td>
<td>NS*</td>
</tr>
<tr>
<td>Inorganic As</td>
<td>2.8</td>
<td>0.4 (0.1)</td>
<td>0.3 (0.1)</td>
<td>0.4 (0.1)</td>
<td>1.1 (0.4)</td>
<td>0.08</td>
<td>0.23</td>
<td>.008</td>
</tr>
<tr>
<td>DMA</td>
<td>1.3</td>
<td>11 (2.6)</td>
<td>5.0 (1.4)</td>
<td>3.5 (1.3)</td>
<td>19 (4.6)</td>
<td>1.37</td>
<td>1.48</td>
<td>NS</td>
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<tr>
<td>MA</td>
<td>&lt;0.15</td>
<td>0.7 (0.3)</td>
<td>0.5 (0.2)</td>
<td>0.5 (0.2)</td>
<td>1.6 (0.6)</td>
<td>0.12</td>
<td>0.68</td>
<td>.008</td>
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<td><strong>Salmon group (n = 11)</strong></td>
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<tr>
<td>Total As</td>
<td>180</td>
<td>167 (29)</td>
<td>77 (30)</td>
<td>59 (13)</td>
<td>303 (41)</td>
<td>100</td>
<td>100</td>
<td>NS*</td>
</tr>
<tr>
<td>AB</td>
<td>140</td>
<td>131 (25)</td>
<td>44 (21)</td>
<td>42 (11)</td>
<td>218 (39)</td>
<td>71.9</td>
<td>76.0</td>
<td>NS*</td>
</tr>
<tr>
<td>Inorganic As</td>
<td>3.3</td>
<td>0.3 (0.1)</td>
<td>0.3 (0.1)</td>
<td>0.4 (0.1)</td>
<td>1.0 (0.2)</td>
<td>0.33</td>
<td>0.56</td>
<td>.003</td>
</tr>
<tr>
<td>DMA</td>
<td>3.0</td>
<td>15 (1.8)</td>
<td>6.4 (3.1)</td>
<td>6.1 (1.6)</td>
<td>28 (3.7)</td>
<td>9.24</td>
<td>8.08</td>
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<tr>
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<td>0.9 (1.0)</td>
<td>0.3 (0.1)</td>
<td>0.4 (0.1)</td>
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<td>Total As</td>
<td>620</td>
<td>438 (107)</td>
<td>203 (44)</td>
<td>147 (60)</td>
<td>763 (114)</td>
<td>100</td>
<td>100</td>
<td>NS*</td>
</tr>
<tr>
<td>AB</td>
<td>160</td>
<td>167 (45)</td>
<td>64 (24)</td>
<td>76 (27)</td>
<td>299 (76)</td>
<td>39.2</td>
<td>66.5</td>
<td>.028*</td>
</tr>
<tr>
<td>Inorganic As</td>
<td>13.0</td>
<td>2.4 (0.9)</td>
<td>0.8 (0.1)</td>
<td>0.8 (0.2)</td>
<td>3.8 (0.9)</td>
<td>0.50</td>
<td>0.64</td>
<td>NS</td>
</tr>
<tr>
<td>DMA</td>
<td>15.0</td>
<td>109 (22)</td>
<td>41 (17)</td>
<td>36 (18)</td>
<td>180 (30)</td>
<td>23.6</td>
<td>21.6</td>
<td>NS</td>
</tr>
<tr>
<td>MA</td>
<td>&lt;0.15</td>
<td>27 (9.7)</td>
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<td>2.8 (1.5)</td>
<td>34 (11)</td>
<td>4.46</td>
<td>2.20</td>
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<tr>
<td>Total As</td>
<td>3.7</td>
<td>12 (9)</td>
<td>24 (9)</td>
<td>15 (4)</td>
<td>52 (17)</td>
<td>100</td>
<td>100</td>
<td>NS*</td>
</tr>
<tr>
<td>AB</td>
<td>0.8</td>
<td>5.0 (6.1)</td>
<td>17 (8.2)</td>
<td>9.6 (2.5)</td>
<td>31 (15)</td>
<td>59.6</td>
<td>71.5</td>
<td>.007</td>
</tr>
<tr>
<td>Inorganic As</td>
<td>4.4</td>
<td>0.4 (0.2)</td>
<td>0.4 (0.1)</td>
<td>0.4 (0.1)</td>
<td>1.1 (0.3)</td>
<td>2.12</td>
<td>2.0</td>
<td>NS</td>
</tr>
<tr>
<td>DMA</td>
<td>0.3</td>
<td>24 (0.9)</td>
<td>2.2 (0.6)</td>
<td>2.3 (0.5)</td>
<td>6.8 (1.9)</td>
<td>13.1</td>
<td>14.9</td>
<td>NS</td>
</tr>
<tr>
<td>MA</td>
<td>&lt;0.15</td>
<td>0.5 (0.2)</td>
<td>0.4 (0.1)</td>
<td>0.4 (0.1)</td>
<td>1.3 (0.4)</td>
<td>2.50</td>
<td>6.2</td>
<td>.013</td>
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<tr>
<td>Unknowns</td>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*p* Values for between-group differences in relative urinary excretion of arsenicals after test meal 1 (day 0–3) and test meal 2 (day 21–23). Significant changes with Mann Whitney U test for between group changes when compared with control group is marked with an asterisk (*).

7. **3.5. Comparison of the cumulative excretion of arsenicals in periods 1 and 2**

In all study groups, tAs excretion in period 2 was about twice that after one single dose of seafood (period 1). For all participants pooled (n = 37) there were strong correlations between the tAs, AB and DMA excretion in period 1 vs period 2 (r = 0.941; p < 0.001, r = 0.903; p < 0.001, r = 0.781; p < 0.001, respectively (Spearman)). Within each intervention group, the association was significant for the tAs and AB excretion rates only in the cod group (tAs<sub>per2</sub> = 443 + 1.6 × tAs<sub>per1</sub>, p<sub>per2</sub> < 0.0001, AB<sub>per2</sub> = 122 + 1.7 × AB<sub>per1</sub>, p<sub>per2</sub> < 0.0001, ANCOVA), and for DMA only in the blue mussels group (DMA<sub>per2</sub> = 32 + 1.9 × DMA<sub>per1</sub>, p<sub>per2</sub> < 0.0001, ANCOVA) (Figs. 3A, 4A and 5A). Thus, the majority of parameters were not significantly associated between periods 1 and 2 at the individual level within each group, AB (and as a consequence, tAs) in the cod group and DMA in the blue mussels group being the two notable exceptions.

The urinary tAs concentration patterns were qualitatively similar in the two periods (Fig. 3B). When plotting the tAs excretion for both periods cumulatively, the increased excretion rates at the end of period 2 became apparent: 4.7-fold; p < 0.0001, 1.5-fold; NS and 3.2-fold, p < 0.01 for day 23 vs day 2 in the cod, salmon and blue mussels groups, respectively (Fig. 3C). Fig. 3D shows the individual cumulative relative excretion curves in periods 1 and 2 in the cod and blue mussels group. Percentages are given relative to the single meal in period 1 or last of 15 meals in period 2. The mean period 2 relative amount of tAs excreted was 47%, 179%, 105%, and 113% in the control, cod, salmon and blue mussels groups, respectively (Fig. 2B–D).

AB was the main arsenical excreted in all intervention groups, constituting 39–89% of tAs excreted after 15 consecutive days with seafood, and 67–89% of tAs excreted after a single dose of seafood (Table 2, column 7–8). The urinary AB excretion relative to tAs was highest in the cod group, constituting 89% after both the 15 consecutive days with cod and the single dose (Table 2, column 7–8); followed by the salmon and blue mussels groups, respectively. The relative AB excretion was significantly higher than the relative excretion in the control group for all study groups, and it was significantly higher in the cod in comparison with the salmon and blue mussels groups.

 periods 1 and 2 at the individual level within each group, AB (and as a consequence, tAs) in the cod group and DMA in the blue mussels group being the two notable exceptions.
The urinary AB concentrations patterns were qualitatively quite similar in the two periods (Fig. 4B). Since AB was the dominant arsenical excreted, the relationship between the AB excretion rates at the end of periods 2 and 1 was similar to that for tAs excretion, with significantly higher rates for the cod and salmon groups (Fig. 4B and C). Individual curves for AB excretion in period 2 relative to AB ingested in the cod and blue mussels groups are shown in Fig. 4D. Note the wide excretion range, from less than the test meal content (about 50% of total AB intake) to about four times the content of the last meal.

For DMA, no significant differences in relative excretion were found in any of the intervention groups after 15 consecutive days with seafood compared with that after a single dose of seafood (Table 2, column 7–8). The relative iAs excretion was below 1% for all seafood groups, for the single dose as well as for the 15 consecutive days with seafood (Table 2, column 7–8).

The DMA excretion pattern was similar for period 1 and 2 for the salmon and cod groups (Fig. 5B). However, for the blue mussels group it was significantly higher throughout period 2. The difference is significant on day 23 (DMA_{day23} = 3.7 + 3.8 \times DMA_{day2}, p (slope \leq 1) < 0.001, ANCOVA) (Fig. 5A–C). Individual curves for period 2 excretion of DMA + iAs + MA relative to potential precursors (tAs minus dietary AB, which is considered to be relatively metabolically inert) ingested in the cod and blue mussels groups are shown in Fig. 5D. Note the wide excretion range, from approximately 10% excreted as iAs + MA + DMA in individuals in the cod group to about 20–60% excreted as iAs + MA + DMA of the non-AB ingested in some blue mussel eaters. Also, the individual variation was larger in the blue mussel group when compared to the cod group.

The blue mussel group excreted more of iAs + DMA + MA relative to intake for both periods, in comparison with the other seafood groups (Fig. 5D). The group means were 27%, 11%, 44%, and 48% in the control, cod, salmon and blue mussels groups, respectively. Except for the blue mussel vs salmon groups (0.1 < p < 0.2), all other group means were significantly (p < 0.0001) different.

Compared to the other groups, the MA excretion was remarkably high immediately following the 15 consecutive days with blue mussels (period 2). However, the MA concentration rapidly decreased after intake of the last blue mussel meal, especially during the first 48 h (Fig. 6B). Of the non-AB ingested with test meal 2, 3–10% was apparently excreted as MA in the blue mussel group, in comparison with less than 1% in the cod group (Fig. 6C). The MA excretion on day 23 was not significantly different from that on day 2 in any group.
3.6. Excretion patterns

The data do not allow any detailed examination of As kinetics from seafood, but provide some information on individual and group-wise differences in the pattern of arsenical excretion and accumulation. If the ratio of day 0/day 2 tAs excretion (after the single dose of seafood) is used as an indicator of individual excretion rate, strict first order kinetics with a half-life between 16–24 h would give a ratio between 4 and 8. The actual ratios for the various seafood groups are shown (x-dimension) in Fig. 3E where they range from 6–15, 3–13 and 5–9 in the cod, salmon and blue mussel groups, respectively (pooled median 6.3).

In the cod group, those with slow elimination of the first single meal tended to end up with higher plasma As levels at the end of the study following 15 days of seafood. Hence, there was a significant negative association between rapid initial tAs excretion, and both plasma tAs on day 23 (Fig. 3E) and tAs excretion during period 2 (Fig. 3F). This would indicate As accumulation in parts of period 2. In the other groups, there were no such significant associations, and by ANCOVA, for the plasma model ($r^2 = 0.54$) differences between the cod and salmon/blue mussel groups were significant ($p < 0.05$). In Fig. 3E the plasma association is shown, with regression lines for the cod group and combined blue mussel and salmon groups.

Fig. 3. Urinary total As (tAs) in periods 1 and 2. (A) Total tAs excretion during period 2 vs total tAs excretion during period 1. In all study groups, total As excretion in period 2 was about twice that of period 1. Within each study group, period 1 and 2 excretion was not strongly correlated. (B) Average urine tAs concentration during periods 1 and 2. Morning spot values for the first day in each period are not shown, as morning spot and full collection values are not comparable. Final urine concentrations were 2–4 times higher in period 2 compared to period 1. (C) Cumulative tAs excretion for each group during periods 1 (dotted lines) and 2 (bold dotted lines) test meals excreted in the cod group (black) and blue mussels group (blue). Note the wide excretion range in period 2, from less than the test meal content (about 5% of total As intake days 8–21) to three times the meal content, corresponding to about 20% of total As intake. (E) Plasma tAs day 23 (period 2) vs ratio of day 0/day 2 tAs excretion (period 1). Regression lines for cod group (black) and the other groups pooled (green) shown. In the cod group, there was a significant ($P < 0.01$) association between rapid As excretion after the first test meal, as measured by the day 0/day 2 excretion ratio, and lower plasma tAs at the end of the study. A similar relationship was not observed for the control, salmon and blue mussels groups, where the slope differed significantly from the cod group ($P < 0.001$) and was positive ($P < 0.01$). (F) Excretion on day 21/intake of tAs in the last meal (day 21) vs excretion during the whole period 1/intake of single meal. The regression line (not shown) is $y = 0.35 + 0.88x$, which indicates an increased base level of excretion on day 21, but otherwise a fairly close association with tAs excretion during period 1 ($r^2 = 0.22, p < 0.007$). The $y = x$ line is shown for comparison between the periods: Period 1 excretion relative to intake on day 0 may be taken as an indicator of absorption, and if a subject excretes a higher fraction on day 21 of the amount ingested in the last meal, i.e. plots above the $y = x$ line, this implies that the subject either has increased absorption, or that the subject is in negative balance (or both). The $y = 1$ line is shown for comparison excretion/intake on day 21. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
3.7. Associations between uptake and elimination in the two test periods

There was a significant association ($r^2 = 0.22$, $p < 0.01$) between the amount of As excreted (during days 0–2) relative to that ingested of the single dose (range 0.41–0.99, median 0.63), and the corresponding ratio for day 21 (range 0.32–1.86, median 0.9) (Fig. 3F). Most of the subjects in the cod group and half the subjects in the salmon group excreted as much as or more than they ingested in the last of 15 meals. With regard to plasma concentrations for tAs these were equal at zero and 20 h except for the cod group showing a lower value at 20 h (Fig. 2A). All but two excreted more As relative to the amount ingested on day 21 than they did during the whole period 1 after the single dose with seafood. There was an estimated 4 h overlap between the 24 h excretion periods of days 20 and 21, leading to somewhat higher expected excretion on day 21, but this could not account for the high values observed.

4. Discussion

In this study, accumulation of As was found in urine and plasma after 15 days of repeated intake of seafood. In addition, considerable differences in the pattern, metabolism and excretion of As from different sources of seafood As was found. The potentially most important finding in relation to seafood safety was the strong increase in MA excretion, particularly within the first 24 h after ingestion of blue mussels for 15 consecutive days. Ingestion of blue mussels for 15 consecutive days also resulted in a 4-fold increase in the absolute excretion of iAs; however the amount of iAs relative to tAs excreted after a single meal and following 15 consecutive days with seafood were similar. This was also the case for DMA in all seafood consuming groups.

4.1. Urinary and plasma tAs concentrations

A single dose or fifteen consecutive days with seafood were both reflected in increased urinary and plasma tAs concentrations, and tAs concentrations in plasma were to some extent dose-dependent.

Non-exposed subjects seemed to excrete some As, despite very low ingestion of As for one week (below 20 µg/g CR) (Table 1). This is in line with the results in non-exposed populations with low seafood intake (Brima et al., 2006; Caldwell et al., 2009; Fowler et al., 2007; Navas-Acien et al., 2009). The day 21 plasma and urinary tAs concentrations for the control group were also low (0.7 and 8.4 µg/L, respectively), which would indicate good compliance of the subjects regarding the food restrictions during the study period.

After 15 consecutive days with seafood, mean urinary tAs concentration was 173 µg/g CR (Table 1). This is comparable with the levels reported in a healthy population with frequent seafood consumption (Sirot et al., 2009), where mean urinary tAs concentrations of 94.8 and 59.7 µg/g CR was found in females and males, respectively, which would indicate good compliance of the subjects regarding the food restrictions during the study period.

Following wash-out, on day 7 the urinary AB concentration (11.5 µg/g CR) was about sevenfold higher than the urinary AB concentrations reported in two populations with low seafood intake; 1.54 µg/g CR (Caldwell et al., 2009) and 0.5 µg/g CR (Navas-Acien et al., 2009). The DMA urinary concentration
(2.25 µg/g CR) was, however, somewhat lower than the DMA urinary concentrations reported by the same studies, 3.69 µg/g CR (Caldwell et al., 2009) and 5.0 µg/g CR (Navas-Acien et al., 2009).

Contrary to a Taiwanese study, which found little change in concentrations of urinary iAs, DMA and MA excreted before and after refraining from seafood for three days (Hsueh et al., 2002), we found that the urinary DMA concentrations increased significantly for all seafood consuming groups. However, the iAs and MA concentrations in urine increased strongly only in the blue mussel group after 15 consecutive days with seafood (Table 1). The reason for this is unknown.

4.2. Absolute excretion of tAs, AB, iAs, DMA and MA

Similar to period 1 and like in other seafood studies, AB was the main arsenical excreted with DMA as the second most abundant arsenical (Buchet et al., 1996; Heinrich-Ramm et al., 2002; Lai et al., 2004; Le et al., 1994). In agreement with previous studies, which have suggested that urinary DMA excretion may in part be formed from arsenosugars and/or arsenolipids (Francesconi et al., 2002; Ramil et al., 2005, 2009; Schmeisser et al., 2006), the highest DMA excretion was found in the blue mussel group (180 µg); the intervention group that probably consumed the highest amount of arsenosugars. Recently, also a large cross-sectional study (NHANES) identified DMA as one of the main arsenicals excreted after seafood intake (Navas-Acien et al., 2011). Urinary DMA may result from both the methylation process of iAs (EFSA, 2009), ingested DMA and from bioconversion of organoarsenicals present in seafood like arsenosugars and/or arsenolipids.

While the excretory pattern of most arsenicals was quite similar in periods 1 and 2, the absolute excretion of MA after 15 consecutive days with blue mussel was 4.5-fold higher when compared with the MA excretion after one single dose of blue mussel.

4.3. Excretion pattern

The urinary excretion patterns were basically similar after 15 consecutive days with seafood and after a single dose, except for the delayed excretion of AB in the cod group and DMA in the blue mussel group (Figs. 4D and 5D).

Like Choi et al. (2010), we found a rapid decrease in the urinary arsenicals the first 24 h following the last seafood meal, but we observed a slower decrease during the second day. This might be a result of 15 consecutive days of seafood consumption for our subjects resulting in a higher body burden of arsenicals than the 6 consumption days of subjects of Choi et al. They returned to baseline levels after five days, while our subjects had still elevated urinary tAs at the end of day 23 (Choi et al., 2010).

4.4. Associations between uptake and elimination in the two test periods

The large increase in plasma tAs concentration and amount of tAs excreted in urine from periods 1 to 2 indicate increased As
body burden. However, the high excretion particularly in the cod group on day 21, following the last As-rich meal, cannot be explained by accumulation alone, as several subjects on that day excreted considerably more than they ingested in the last As-rich meal. Unless As absorption increased a lot during the 15 days on seafood, most subjects may have excreted more than they absorbed on day 21 (Fig. 3F). The shorter interval of about 20 h between meal 14 and 15 than the previous of 24 h could explain sorbed on day 21 (Fig. 3F). The shorter interval of about 20 h be-
seafood, most subjects may have excreted more than they ab-
creted considerably more than they ingested in the last As-rich
plained by accumulation alone, as several subjects on that day ex-
group on day 21, following the last As-rich meal, cannot be ex-
reason for this is not known. This could be related both to
changes in absorption and elimination kinetics. In view of the high
apparent absorption observed in period 1, increased excretion capacity with prolonged As intake seems to be a more likely explana-
tion for this phenomenon than increased absorption. The gut
microflora may be of importance both for absorption and metabo-
lism and might change less rapidly upon repeated exposure to
arsenicals from seafood than would As metabolising enzymes in
the liver. Relative to absorption expected from the results of period
1, there are no obvious group patterns notable in Fig. 3F.

A negative association between initial As excretion rate (defined as the ratio of day 0 and day 2 tAs excretion) and subsequent As levels was observed only in the cod group. Here, it might seem that the combination of high absorption and repeated As intake resulted in significant increase in As body burden. The excretion rate may have increased during the seafood period in all study groups, and 7 out of 9 subjects in the cod group on day 21 excreted as much or more As than they ingested in the last seafood meal (Fig. 3F).

The overlap of the cumulative excretion curves for the blue mussel group in the two test periods (Fig. 3D) indicate that increased absorption is not a likely explanation for the high excre-
tion, supporting increased excretion rate as explanation for the
relatively high As excretion on day 21.

If As accumulation is slow in absolute terms, higher initial excretion rate may be indicative of higher absorption, resulting in no or a positive rather than a negative association between ini-
tial excretion speed and subsequent increase in body burden. This may apply to the salmon and blue mussel groups. In these groups, the average total As excretion was approximately 2-fold after 15 consecutive days with seafood (period 2) in comparison with that after a single dose (period 1) (Fig. 3C), but the total 72 h As excre-
tion was only a little higher than the As content in the last test meal (Table 2).

4.5. Chronic As exposure from seafood

Only one previous human study has assessed the urinary As excretion after a repeated intake (6 days) of a controlled seafood diet (Choi et al., 2010). Their diet consisted of approximately 2/3 seaweed and 1/3 fish and shellfish, which is comparable to the arsenical composition of the blue mussel meal in the present study (Choi et al., 2010). Since little data exists on the urinary As excre-
tion after a repeated seafood intake and since the blue mussel group ingested relatively high amounts of iAs, our results in the following are compared also with results in populations chroni-
cally exposed to iAs via drinking water or occupation.

The urinary MA concentration in the blue mussel group after 15 consecutive days of consumption was 24.6 µg/g CR. In comparison, the MA concentrations in a population not exposed to iAs in drinking water and with a low seafood intake was below 1.5 µg/g CR in all three separate measurements during a ten-year period (Navas-Acien et al., 2009). The 75th percentile of MA concentration in a large population in the NHANES study was 1.33 µg/g CR (Cald-
well et al., 2009). Also the MA concentration within a Korean Community with frequent rice, seaweed and shellfish intake was 1.5 µg/g CR, substantially lower than the subjects in the blue mussel group on day 21 (Cleland et al., 2009). The MA concentrations in urine after 15 consecutive days with cod or salmon consumption (0.48 and 0.73 µg/g CR, respectively) were lower, but still comparable with the results from these studies (Caldwell et al., 2009; Cleland et al., 2009; Navas-Acien et al., 2009).

Interestingly, even a population exposed to high levels of iAs from As-contaminated coal combustion and workers at a copper melting plant, had MA concentrations that were about fivefold lower (4.69 µg/g CR) and approximately twofold lower (14.4 µg/g CR), respectively (Gao et al., 2011; Xi et al., 2011) than the MA concentration found following repeated blue mussels intake. Similarly,
the relative urinary MA excretion (4.46%, Table 2, column 7) after the 15 consecutive days with blue mussels was comparable with that in subjects occupationally exposed to iAs in a steel foundry (4.9%) (Soleo et al., 2008).

Only the blue mussel group excreted significantly more MA after 15 consecutive days with seafood than after the single dose. Earlier studies of populations chronically exposed to iAs from drinking water, indicate that the relative proportion of urinary MA might increase with increasing dose of iAs exposure (Del Razo et al., 1997; Hopenhayn-Rich et al., 1996; Kurtto et al., 1998; Li et al., 2008; Styblo et al., 1999). The observed decrease in methylation capacity associated with the increased exposure to As-contaminated drinking water, has been proposed to be a result of the inhibition of the second methylation step (limited capacity of the As3MT enzyme) when exposed to excessive iAs (Tseng, 2009). We do not know whether such a mechanism is relevant in the case of MA excretion following repeated intake of blue mussels since the pathway of formation and whether iAs might occurs as an intermediate is unknown. Neither is it known whether As bound as arsenosugars is converted directly mainly to DMA or whether iAs and MA are involved as intermediary metabolites.

4.6. Relative urinary excretion of iAs, DMA, MA

The relative distribution of iAs, MA and DMA in urine in various populations is considered to be fairly constant and in the range of 10–30%, 10–20% and 60–80%, respectively (Hopenhayn-Rich et al., 1996; Vahter, 1999). In line with this and similar to the relative distribution after three days with seafood restrictions (8%, 6% and 86%) found by Choi et al. (2010), the mean relative distribution of iAs, MA and DMA after one week’s seafood restrictions (day 7) was 9%, 12% and 79% in our study (Table 1). After 15 consecutive days with seafood in our study group, excluding AB and other minor or organic arsenicals the percentage was lower for iAs (1.6%) but comparable for MA (20.1%) and DMA (78%) (Table 1).

4.7. Inter-individual variations

We found large individual variations in the percentage ingested non-AB in the last seafood meal that was excreted as iAs + MA + D-MA in the blue mussel group (Fig. 5D). This is in line with previous studies of humans ingesting a single dose of arsenosugars in the form of algae (Le et al., 1994; Ma and Le, 1998) or synthetic arsensugar (Raml et al., 2009) and also after 6 consecutive days with algae/fish (Choi et al., 2010). In addition, large inter-individual variations in AB and MA excretion were found in the blue mussel group and for AB in the cod group.

Both race, gender, age, nutritional status as well as genetic polymorphisms in the regulation of the enzymes (i.e. arsenic methyl transferase (As3MT)) involved in As metabolism, seem to have an impact on the urinary excretion of methylated arsenicals (EFSA, 2009; Hughes, 2011). The observed inter-individual differences found in the metabolism of arsenicals in the present study, in particular in the blue mussel group, might in addition to these factors be explained by variation in intestinal flora.

4.8. Safety of arsenicals in seafood

Although no signs of As toxicity have been reported in humans after consumption of large quantities of seafood (Edmonds and Francesconi, 1993) or seaweed (Andrewes et al., 2004), the toxic action of iAs is believed to be related to its metabolism, i.e. the formation of methylated trivalent As species (Borak and Hosgood, 2007), hence high urinary excretion of DMA and MA could be associated with formation of trivalent species and therefore be of human health concern. MA (III) and DMA (III) are rapidly oxidized to their pentavalent counterparts (Styblo et al., 2002; Thomas et al., 2001), and these intermediates can initiate toxic effects, like DMA-damage (Mass et al., 2001; Nesnow et al., 2002), or be indirectly genotoxic (Hiorno et al., 2004; Vahter, 2002). However, due to their reactivity and low stability, these could not be determined in the present study.

A repeated dose of MA has been shown to have effects on the GI-tract, kidney, thyroid and the reproductive system (ATSDR, 2007), and a higher proportion of urinary MA has been associated with increased risks of As-related diseases (Chen et al., 2005; Hsueh et al., 1997; Maki-Paakkanen et al., 1998; Steinmaus et al., 2006; Tseng et al., 2005; Wu et al., 2006; Yu et al., 2000). DMA (V) has in animal studies been reported to promote carcinoogenic effects in the urinary bladder, kidney, liver and the thyroid gland (Wanibuchi et al., 2004; Yamamoto et al., 1995) and has in addition effects on the foetal development (ATSDR, 2007). Recently, both MA and DMA were classified as “possibly carcinoogenic” to humans by the International Agency for Research on Cancer (IARC) (IARC, 2009). Taken together with the possibility that toxic trivalent intermediates might occur in the formation of DMA and MA, the high DMA and MA excretion might be of possible concern and the implication of this finding should be further investigated.

4.9. Strengths and weaknesses of the study

The main strength of this study is the study design, which enabled a direct comparison if the urinary arsenical excretion pattern after a single dose and after 15 consecutive days with the identical seafood species. Using a control group, other possible sources of As were minimized, all groups following restrictions of food items high in As starting from seven days before study start (day 0) and throughout the study period. With three different seafood species providing large, but realistic doses of As, we obtained different excretion patterns with individual differences therein. Also, using each subject as his or her own control in the statistical analysis could reduce variance.

One weakness of the study is that our laboratories could not provide analysis of the arsenosugars and arsenolipids content on the food matrix so these species could not be quantified in the ingested foods.

5. Conclusion

We found clear indications of As accumulation after repeated seafood intake, and after 15 days on a seafood diet. Only with high AB intake (the cod diet), a relation between slow initial As excretion speed and higher plasma and urinary As concentrations two days after last seafood meal, was found.

While iAs excretion was relatively low in all study groups, the very high MA excretion after repeated intake of blue mussels should be further investigated. Also, since chronic low-level iAs exposure and high urinary excretion of MA has been related to higher risk for adverse As-related health effects, a possible risk associated with excessive blue mussel intake cannot be ruled out.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found, in the online version.
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