Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood

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Running title: Differential response to arsenic in seafood.

Abbreviations: AB, Arsenobetaine ($(CH_3)_3As^+CH_2COO^-$); AC, Arsenocholine ($(CH_3)_3As^+CH_2CH_2OH$); As (III), Inorganic arsenite ($As(O^-)_3$); As (V), Inorganic arsenate ($O=As(O^-)_3$); As, Arsenic; CRM, Certified Reference Material; DMA, Dimethylarsinate ($(CH_3)_2AsO(O^-)$); HPLC, High Performance Liquid Chromatography; iAs, Inorganic arsenic; ICPMS, Inductively Coupled Plasma Mass Spectrometry; LOQ, Limit of Quantification; MA, Methylarsonate ($CH_3AsO(O^-)_2$); tAs, Total arsenic; TETRA, Tetramethylarsonium ion ($(CH_3)_4As^+$); TMAO, Trimethylarsine oxide ($(CH_3)_3AsO$); TMAP, Trimethylarsoniopropionate ($(CH_3)_3As^+CH_2CH_2COO^-$).

Abstract

Seafood is the predominant food source of several organoarsenic compounds. Some seafood species, like crustaceans and seaweed, also contain inorganic arsenic (iAs), a well-known toxicant. It is unclear whether human biotransformation of ingested organoarsenicals from seafood result in formation of arsenicals of health concern. The present controlled dietary study examined the urinary excretion of arsenic compounds (total arsenic (tAs), iAs, AB (arsenobetaine), dimethylarsinate (DMA) and methylarsonate (MA)) following ingestion of a single test meal of seafood (cod, 780 µg tAs , farmed salmon, 290 µg tAs or blue mussel, 690 µg tAs or potato (control, 110 µg tAs) in 38 volunteers. The amount of ingested tAs excreted via the urine within 0-72h varied significantly among the groups: Cod, 74 % (52-92 %), salmon 56 % (46-82 %), blue mussel 49 % (37-78 %), control 45 % (30-60 %). The estimated total urinary excretion of AB was higher than the amount of ingested AB in the blue mussel group (112 %) and also ingestion of cod seemed to result in more AB, indicating possible endogenous formation of AB from other organoarsenicals. Excretion of iAs was lower than ingested (13-22 % of the ingested iAs was excreted in the different groups). Although the ingested amount of iAs + DMA + MA was low for all seafood groups (1.2-4.5 % of tAs ingested), the urinary DMA excretion was high in the blue mussel and salmon groups, counting for 25 % and 11 % of the excreted tAs respectively. In conclusion our data indicate a possible formation of AB as a result of biotransformation of other organic arsenicals. The considerable amount of DMA excreted is probably not only due to methylation of ingested iAs, but due to biotransformation of organoarsenicals making it an inappropriate biomarker of iAs exposure in populations with a high seafood intake.

Key words: Arsenic, dimethylarsinate, seafood arsenic, arsenobetaine, dietary intervention.

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1. Introduction

Dietary arsenic (As), in particular from drinking water, has for some time been considered a major food safety issue (JECFA 2010). Several studies have identified seafood as the predominant food source of As, with rice, mushrooms and poultry as additional sources, depending on growing and feeding conditions (Borak and Hosgood, 2007; Dabeka et al., 1993; EFSA, 2009; Munoz et al., 2005; Schoof et al., 1999; Tao and Bolger, 1999). In areas with low or no inorganic arsenic (iAs) exposure from drinking water, the variation in total As (tAs) from food is mainly due to variations in seafood and rice consumption (e.g. 28 µg per day for an adult American woman and 160–280 µg per day for an adult Japanese woman) (Tao and Bolger, 1999; Tsuda et al., 1995; Uneyama et al., 2007).

The toxicity of As is highly dependent on the chemical form and oxidation state, i.e. trivalent or pentavalent. The most potent toxicological potent arsenic compounds are the trivalent arsenic species, which easily generate reactive oxygen and reacts with sulfuric compounds (Fowler et al., 2007; Hughes, 2011; Styblo et al., 2002). In mammals, arsenic biotransformation occurs in alternating steps involving reduction and subsequent oxidative methylation to less toxic methylated forms such as methylarsonate (MA) and dimethylarsinate (DMA). In this process intermediate trivalent methylarsonous acid and dimethylarsinous acid are formed, which are equally more toxic than the corresponding pentavalent forms (EFSA, 2009; Hughes, 2011; Styblo et al., 2002; Thomas et al., 2001; WHO, 2001). The dominating organic arsenicals in most seafood species, arsenobetaine (AB) is, despite limited evidence, considered harmless (Borak and Hosgood, 2007; Lai et al., 2004). Arsenosugars, the predominant form in algae and seaweed, have showed cytotoxic effects. The trivalent form of the arsenosugar is more toxic than the pentavalent counterpart, thus both forms are significantly less toxic than arsenate, MA (III) and DMA (III) (Andrewes et al., 2004; Sakurai

et al., 1997). No toxicological studies have addressed arsenolipids, lipid-soluble forms reported present in the fatty tissues of fish, and the human toxicology of these compounds remain unknown (Francesconi, 2010).

In seafood both the total content of arsenic and presence of different chemical forms of arsenicals vary greatly with marine species, trophic level, diet/environment and ability of the species to metabolize arsenicals. In Atlantic cod, the total As (tAs) concentration range from 0.4-52.4 mg As/kg wet weight (Julshamn et al., 2004; National Institute of Nutrition and Seafood Research, 2011). However, the amount of iAs in fish fillets of cod has been reported to be less than 0.001 µg/kg wet weight (Sloth et al., 2005). In fillets of farmed Atlantic salmon, the tAs concentration range from 0.6-4.8 mg As/kg wet weight (Julshamn et al., 2004; National Institute of Nutrition and Seafood Research, 2011), and iAs is found only in trace amounts (Sloth et al., 2005). AB is the predominant form of As both in cod and salmon fillets (Dahl et al., 2010).

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Arsenolipids have recently been identified in tuna fish and cod liver (Taleshi et al., 2010), and because of its high fat content, salmon fillet is likely to contain arsenolipids as well. In blue mussels from Norwegian fjords, the tAs concentration ranged from 1.2-13.8 mg As/kg wet weight (Sloth and Julshamn, 2008). In addition to relatively high levels of AB, DMA and arsenosugars, blue mussels may contain high concentrations of iAs, up to 5.8 mg As/kg wet weight (Sloth and Julshamn, 2008).

In general, bioavailability and kinetics of arsenic compounds will vary with their physiochemical properties (EFSA, 2009), environmental factors, their dose, as well as differences among humans in methylation capacity and handling of arsenosugars (Tseng,

2009). Irrespective of food source, iAs is generally considered to be absorbed rapidly and almost completely after ingestion, although solubility and matrix also may play a role (EFSA, 2009). In spite of the fact that the metabolic pathway of iAs has not yet being fully clarified, there has been a general consensus that a large part will undergo methylation and end up being excreted as DMA (Cui et al., 2008; Hayakawa et al., 2005; Tseng, 2009). MA and DMA present in foods seem to be excreted in the urine largely in their unchanged forms and only a minor proportion of MA is converted to DMA (Tseng, 2009). A possible further metabolism of DMA has been observed as a urinary metabolite in Bangladeshi women in the form of thio-DMA (Raml et al., 2007), and further metabolism of DMA to TMAO was observed in one human subject who ingested a high dose of DMA (Marafante et al., 1987).

Many details about absorption and metabolism of As compounds in humans are still unknown, in particular about organoarsenicals from seafood (Borak and Hosgood, 2007). Several studies have noted that seafood intake increases the urinary excretion of DMA, the main metabolite from iAs metabolism (Buchet et al., 1996; Heinrich-Ramm et al., 2002; Lai et al., 2004; Mohri et al., 1990). Arsenosugars and arsenolipids are probable sources of the increase in the urinary excretion of DMA, as earlier interventions in humans have identified an increased urinary excretion of DMA after intake of these arsenicals (Francesconi et al., 2002; Ma and Le, 1998; Schmeisser et al., 2006). The large amounts of AB found in seafood have for years been considered to be readily absorbed and then rapidly excreted unchanged in urine (Lai et al., 2004). This notion is largely based on one study using isotopically labeled AB, showing rapid excretion and with less than1 % of the radioactivity remaining in the body 24 days after ingestion (Brown et al., 1990). Notable, hardly any quantitative data exist on the absorption of AB in humans (EFSA, 2009).

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148 The aim of the present study was to examine the urinary As excretion following exposure to
149 various As compounds from different types of seafood given as a bolus dose. The study
150 design made it possible to investigate several basic assumptions often made about As
151 metabolism: 1. Absorption of AB is rapid and more or less complete. 2. Absorbed AB is
152 excreted rapidly, completely and unchanged, mainly in urine, and the process is basically
153 independent on the dietary source of AB. 3. There is no formation of AB in the body. 4. DMA
154 (and MA) is mainly produced by methylation of iAs, and urinary DMA (or MA) can therefore
155 be used as a marker of iAs load. Finally, the design of our study made it possible to explore
156 whether seafood consumption can increase the body load of iAs derived from
157 organoarsenicals.
158
159 2. Subjects and methods
160 2.1. Subjects
161 Study participants were students at Akershus University College, Norway, and the
162 intervention took place in March 2006. Of 48 potential subjects assessed for eligibility, 38
163 healthy volunteers (28 women and 10 men) aged 20–40 years were compliant with the
164 protocol throughout the study. Smokers, pregnant or lactating women, persons habitually
165 consuming seafood more often than three times a week (i.e. a higher seafood intake than
166 generally recommended in Norway), and persons using medical drugs other than
167 contraceptives were excluded. The study was approved by the National Committee for
168 Research Ethics and was carried out in accordance with The Code of Ethics of the World
169 Medical Association. Written informed consent was obtained from each participant.
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2.2. Study design

The study period lasted 10 days in total (Figure 1). During a one week washout period (day -7 until day 0) and throughout the study period, the subjects were asked to abstain from eating seafood, mushrooms, rice or rice products and dietary supplements. The consumption of cod liver oil, a food supplement commonly used in Norway, was discouraged, starting four weeks prior to day 0 and lasted throughout the study period. Four randomized treatment groups received a test meal at the University College on day 0. The meal consisted of 150 g of either cod (*Gadus morhua*) (n=9), farmed salmon (*Salmo salar*) (n=11), blue mussel (*Mytilus edulis*) (n=8) or potato (n=10) served for breakfast (8-10 am). Following intake of the four different test meals, all subjects consumed a strictly controlled diet prepared and served at the University College the following 72 hours (day 0-2). The supper was brought in bags and eaten in the subject's homes. The participants were requested to eat all of the food served and to maintain their normal physical activity routines.

2.3. Intervention diet

The blue mussels were purchased from Safjord shellfish (Varaneset, Norway), and examined for algal toxins by the Norwegian Food Safety Authority. The cod was bought from Ural Nor Fish AS (Moldtustranda, Norway), and the salmon from Coast Seafood AS (Måløy, Norway). The cod and the salmon were already cleaned and filleted when purchased. To ensure homogenous distribution of As in the test meals, homogenous mixtures of the filleted fish were made into puddings, which were stored at -20 ºC until meal preparation. The blue mussels were steamed for 10 minutes, removed from their shells, frozen separately and immediately stored at -20 ºC until meal preparation. The test meals for all four intervention groups consisted of pies, using an identical recipe except for the 150 g of seafood/potato. The strictly controlled diet was designed to be low in As from other foodstuffs and in accordance

with the Nordic recommended daily intake of energy (2100 kcal/8.8 MJ). Those who needed more energy were provided with “energy buns” without any restriction. Additionally, tap water (the tAs level in Norwegian groundwater is mostly below 0.2 µg As/L (Olsen and Morland, 2004)) was provided with no restriction. Samples of the test pies and other meals were homogenized and stored at -20 ºC for subsequent As determination.

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2.4. Blood sampling

Blood samples were collected from fasting subjects (minimum12 h) at the same time (8-10 am) on day 0. In addition blood samples were collected 2h, 4h, 24h and 48h after ingestion of the test meal. Plasma was obtained from EDTA tubes kept at room temperature (0-30 min) and centrifuged at 1300x g for 10 min. All plasma samples were kept frozen (-70 ºC) until analysis.

2.5. Urine samples

Following ingestion of the test meals, urine was collected in three periods during the first 24 hours: (0.1) between the test meal and 2 pm (approximately 0-5h after the test meal); (0.2) 2-7 pm (approximately 5-10h after the test meal); and (0.3) 7 pm until first urination on the following day (approximately 10-24h after the test meal) (Figure 1). For the next 48 hours, 24-hour urine batches were collected. Morning spot samples of urine were collected at baseline (day -7), and before ingestion of the test meal (0 h). All urine samples were kept at 4 ^{o}C until all urine from each day/period was pooled. The total volume was then measured, and 15 ml of the urine was distributed into aliquots and stored below –70 ^{o}C until analysis.

2.6. Analytical methods

The tAs in the food (test meals and 72-hour controlled diet), plasma and the urine were determined using Inductively Coupled Plasma Mass Spectrometry (ICPMS) as previously described (Julshamn et al., 2007; Sloth et al., 2005). The As speciation analysis of the food was performed using HPLC-ICPMS (Sloth et al., 2003; Sloth et al., 2005).

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The accuracy of the tAs determination for foodstuffs was evaluated by means of analysis of two certified reference materials (CRM): DORM-2 Dogfish muscle (20.1 ± 1.0 mg/kg) (National Research Council of Canada (NRCC)); and BCR CRM627 Tuna fish tissue (5.1 ± 0.3 mg/kg) (IRMM, Geel, Belgium). The obtained results agreed well with the respective certified values of 18.0 ± 1.1 mg/kg and 4.8 ± 0.3 mg/kg, respectively. The same BCR CRM 627 Tuna fish tissue was used for evaluation of the speciation analysis of the food samples. The results were also compared with the results given in Sloth et al. (2003) for, TMAO (trimethylarsine oxide), AC (arsenocholine), TETRA (tetramethylarsonium ion) and TMAP (trimethylarsoniopropionate), as the CRM is only certified for AB and DMA (Maier EA, 1997). The obtained results (AB: 3.7 mg/kg, DMA: 0.15 mg/kg, TMAO: 0.016 mg/kg, AC: 0.016 mg/kg, TETRA: 0.034 mg/kg, and TMAP: 0.029 mg/kg), agreed well with the certified values (AB: 3.9 ± 0.2 mg/kg; DMA: 0.15 ± 0.01 mg/kg) and the results given in Sloth et al. (2003) (TMAO: 0.010±0.002 mg/kg, AC: 0.012±0.002 mg/kg, TETRA: 0.037±0.002 mg/kg, and TMAP: 0.033±0.002 mg/kg). NIES No 18 Human urine (National Institute for Environmental Studies, Ibaraki, Japan), is a CRM for tAs, AB and DMA, and was used to evaluate the tAs of human urine samples. The obtained results (tAs 150 ± 6 µg/L and DMA 42 ± 6 µg/L), agreed well with the CRM (tAs 137 ± 11 µg/L and DMA 36 ± 9 µg/L).

248 2.7. Determination of arsenobetaine, DMA, MA and iAs in urine

249 Prior to the measurements the samples were filtrated (0.2 µm) and subsequently injected
250 undiluted into the HPLC system. AB was measured with cation- exchange chromatography
251 on a Zorbax 300 SCX (4.6 x 250 mm) column. A 10 mM aqueous pyridine solution at pH 2.3
252 and a flow rate of 1.0 $mLmin^{-1}$ was used as mobile phase. The injection volume was 5 µL and
253 the column temperature was 30°C. DMA, MA and iAs was measured with anion-exchange
254 chromatography on a PRP-X100 (4.6 x 150 mm) with 20 mM $NH_4H_2PO_4$ solution at pH 6.0
255 used as a mobile phase. The injection volume was 10 µL and the column temperature was
256 40°C. For quality assurance the NIES CRM No 18 human urine was used as reference
257 material. AB, measured: 67.5 ± 1.8 µg As / L; certified: 69 ± 12 µg / L. DMA, measured: 42
258 ± 2 µg As/L; certified: 36 ± 9 µg/L. Limit of quantification were taken as the concentration
259 of the lowest standard: 0.5 μgL^{-1}.
260
261 Non-quantified values (i.e. values below LOQ), were set at LOQ/2 (Kroes et al., 2002). The
262 following values (LOQ/2) were used in the present study: iAs (0.15 µg/kg); DMA (0.25
263 µg/kg); and MA (0.15 µg/kg).
264
265 2.8. Estimation of total As absorption
266 In a one-compartment model (Beckett WS, 2007) the daily amount of total As excreted in
267 urine after a single dose of seafood arsenical can be approximated by a geometric progression
268 with a constant ratio k between successive terms, $A_{n+1} = kA_n$. Therefore we estimated the total
269 remaining excretion of As, i.e. the amount remaining in the body after the last urine collection
270 on day 2, by the tail sum of a geometric series, which is k/(1-k) times the excretion the last
271 day measured. For example, with k = 0.5, the sum of the remaining days will be equal to the
272 excretion the last days measured. With a low baseline excretion and the three days' amount

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excreted measured as A_0, A_1 and A_2, the total excretion of the dose of arsenical can hence be (crudely) estimated as $A_0+A_1+A_2 + A_2k/(1-k) = A_0+A_1+A_2/(1-k)$. Since As, irrespective of species, is considered to be mainly excreted in urine, the total excreted amount in urine can be used as a crude estimate of the absorption of a single dose As ingested after an initial wash-out period, as was the case here. The ratio k was estimated as the average day2/day1 excretion ratio for the participants in the three seafood-consuming groups. The k-value was then applied to individual excretion records, to produce individual estimates of total As excretion.

Assuming that 1) only a small amount of As is excreted by other routes (EFSA, 2009) and 2) there is no accumulation of As after the bolus dose, the excretion estimate may be used as a (conservative) estimate of As absorption. Linear regression of logarithms of daily urinary tAs excretion day 2 vs day 1 and day 1 vs day 0 was performed to check how much tAs excretion deviated from this basic, simple model where the excreted daily amount is a fixed fraction of total circulating As. If the observed slope of the logarithmic linear relationship does not approach 1, which the exponential model implies, there is systematic deviation from the model. Then there would be a concentration-dependent elimination rate with the simplest form of the basic relation being, $A_{n+1} = k\ A_n^C$. Furthermore, if the observed regression slopes of the log-transformed excretion data are different for different pairs of consecutive days, there may in addition be a time-dependent elimination rate.

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2.9. Statistical analyses

The SPSS 14.0 software package (SPSS Inc., Chicago, IL; USA) and R 2.10.1 (http://cran.r-project.org/) were used for the statistical analyses. The non-parametric test Mann Whitney, ANCOVA and t-tests with Holm's correction for multiple comparisons were used to identify any significant differences in the urinary As excretion between the seafood intervention

298 groups and the control group. In particular, ANCOVA models were applied to assess possible
299 group wise differences in AB and DMA excretion relative to AB and non-AB As absorption,
300 respectively (Table 4). In all tests, p-values < 0.05 were considered significant.
301
302 3. Results
303 3.1. Baseline characteristics and urinary tAs concentrations
304 The baseline characteristics and urinary tAs concentrations of the study subjects are shown in
305 Table 1. The mean urinary concentration of tAs a week before the test meal (day -7) was 83
306 µg/L. After the 7 day wash-out period, at the start of intervention period (day 0), the mean
307 urinary concentrations were below 30 µg/L (average 20 µg/L) in all four groups. This is
308 similar to the concentrations of urinary tAs reported in non-exposed individuals (Fowler et al.,
309 2007).
310
311 3.2. As content of the diet
312 The tAs contents of the test meals of cod and blue mussel were about three times higher than
313 the tAs content of the salmon test-meal (Table 2). The tAs content of the control test-meal
314 without seafood was low (Table 2). In the cod and salmon groups, unidentified arsenicals
315 accounted for approximately 16–25 % of tAs while the blue mussel diet consisted of 60 %
316 unidentified As compounds. The tAs intake during the 72 h controlled diet (no seafood)
317 consumed by all groups following the test meal was 110 µg (Table 2).
318
319 3.3. Measured tAs excretion and crude estimates of total tAs excretion
320 The initial 24 h total As excretion varied markedly between the groups (Table 3). In the cod
321 group, 77 % of the urinary tAs excreted day 0-2 was excreted during the first 24 h after intake
322 of the test meal (446 of 576 µg tAs, mostly AB). This was five times higher than the excretion

during the next 24h. The excretion rate was much slower initially in the two other seafood groups (Figure 2A), and it also declined less rapidly. In the three seafood-consuming groups, tAs excretion was about twice as high on day 1 compared to day 2.

The average day2/day1 excretion ratio for tAs in these groups was k= 0.54 ± 0.20. This ratio did not differ significantly among the groups. The observed slope of the logarithmic linear relationship of day 2 vs day 1 urinary excretion was 0.86, and the corresponding slope for day 1 vs day 0 was 0.48. In a simple kinetic model the slope would be 1, thus our results indicate both concentration and time dependency in the tAs excretion. Consequently, the tAs half-life increased with decreasing body burden - in the cod group it was initially <16h. With a daily tAs excretion of 50-100 µg, half-life seemed to be around 24h. The time course of urinary excretion and plasma concentrations of tAs is illustrated by Figure 2 A-C. The plasma tAs and urinary tAs concentrations peaked after 2h and 10h after ingestion of the test meal, however the excretion pattern was similar for both urine and plasma (Figure 2 A-B).

Further analyses indicated that, rather than following a biphasic pattern typical of two-compartment models, the As excretion changed more continuously, indicating a more complex excretion and retention situation. The data do, however, not allow for a more detailed modelling. We found that, in spite of the changing half-life for tAs excretion, treating the tAs excretion after the test meal as a geometric series would still give a good approximation of total excretion. Using the mean of the day2/day1 excretion ratios, 0.54, as the constant k, total tAs excretion after day 2 could thus be crudely estimated as 0.54/(1-0.54)=1.17 times the day 2 excretion (Table 3).

3.4. Estimates of absorption based on total As excretion

The observed day 0-2 and estimated tAs species excretion, with corresponding percentages of ingested amounts, are shown in Table 3, columns 6-9. Using estimated excreted tAs as an estimate of absorption, the tAs absorption was estimated to be 81 % in the cod group, which was significantly different ($p<0.015$) from the salmon group, 64 % and the blue mussel group, 56 %. The difference in tAs absorption between the salmon and blue mussel groups was not statistically significant. Considerable inter- individual differences were observed in all groups, with ranges 58-99 %, 49-93 % and 41-86 % in the cod, salmon and blue mussel groups, respectively (Table 3, column 9).

The data indicated larger inter-individual than inter-species absorption differences between As species, consequently, each subject's tAs absorption estimate was used for all As species. Such inter-individual differences are illustrated by the individual cumulative tAs excretion curves (Figure 2D). Although absorption in the cod group was significantly higher than in the salmon group, there was some overlap. In these groups, there were larger inter-individual variations during the first hours than during the last.

3.5. Arsenobetaine (AB)

AB was the major As compound identified in the seafood investigated in this study, comprising 73 %, 76 %, 33 % of the tAs ingested in the cod, salmon and blue mussel groups, respectively (Table 3, column 2). The average urinary AB excretion rates during the first 72 hours showed a very pronounced peak of AB excretion around 10h in the cod group, but smaller group-wise differences thereafter (Figure 3A). A considerable variation in the individual relative cumulative excretion patterns in the cod and salmon groups appeared, with a slower initial AB excretion in all subjects in the salmon group, but also a lower rate of

373 excretion relative to ingested amount of AB in that group (Figure 3B). More AB than the
374 amount ingested was estimated excreted by most subjects in the blue mussel group (average
375 112 %, Table 3, column 9). The average observed day 0-2 excretion of AB in the cod and
376 blue mussels groups were 90 % and 99 % of the ingested amount respectively, while, the
377 corresponding average apparent absorption percentages in these groups were 81 % and 56 %.
378 When observed AB excretion day 0-2 was plotted as a percentage of estimated AB
379 absorption, most subjects in the cod and blue mussels were therefore placed above 100 %
380 (Figure 3C). The same relationship could be inferred from the result of the ANCOVA
381 analysis (Table 4).
382
383 Average estimated total AB excreted in the cod group was 556 µg (range 409-640, Table 3,
384 column 8). In comparison, only about 139 µg (range 11-198) of ingested AB was excreted
385 during the three day intervention period in the salmon group. The estimated apparent total As
386 absorption was 64 % in the salmon group, and the average AB absorption therefore has an
387 upper bound of 84 % (=0.64/0.76 x 100, assuming zero absorption of non-AB As) in this
388 group. This would imply that much less AB is excreted than absorbed: On the average 75 %,
389 139 vs 185 µg. A lower bound estimate is obtained assuming 100 % absorption of non-AB As
390 (69 µg), then average AB absorption would be 53 % ((185-69)/219 x 100, Table 3, columns 1
391 and 8). In this case, average AB excretion would be 120 % of absorption.
392
393 Thus, most subjects in the cod and blue mussel groups, and possibly also some in the salmon
394 group, seemingly excreted as much or even more AB than they absorbed, and several
395 individuals even more than they ingested.
396

The test meal of the control group was void of fish or seafood components (Table 2). But since the controlled diet on day 1 contained some As, probably originating from the lunch meal consisting of roasted chicken, the control group ingested 110 µg tAs throughout the 3 day intervention period. AB constituted 77 µg (70 %) of the tAs (Table 3), and 45 % of ingested tAs was excreted day 0-2.

The differences between the groups in the handling of AB were quantified and analyzed by ANCOVA modelling of excreted AB vs estimated absorbed AB (Table 4). Modelling AB excretion with separate coefficients for AB for each group was significantly better than modelling with common coefficients (ANOVA test, data not shown). In the model, the intercept (43 µg per 72 h) can be interpreted as a common baseline AB excretion, i.e. about 14 µg/day. The coefficients are estimates of group-wise excretion fractions (minus baseline excretion) relative to AB absorption in the study. Coefficients significantly above 1 thus indicate de novo AB formation in the body.

The ANCOVA model for AB excretion covered most of the variation (R^2=0.99). In the blue mussel group and cod group, about 1.6 µg and 1.1 µg AB was excreted for each µg AB absorbed, respectively. This would indicate additional formation of AB in the body following consumption of blue mussels and cod. Because of the relatively high baseline excretion, in spite of the low coefficient (0.68), the model does not indicate AB degradation in the salmon group. In the control group, the individual variability was higher, and there was no statistically significant association between average AB excretion and absorbed AB.

3.6. Dimethylarsinate (DMA)

In all groups, the excretion of DMA was higher than the amount ingested. The blue mussel group excreted the highest amount of DMA. There was approximately a 2.5-fold increase in excreted vs. ingested amount of iAs+DMA+MA, mostly excreted (90 %) as DMA (Table 3). The amount of DMA excreted was about 4.5 times the ingested amount (71 vs. 16 µg). With only 15 µg iAs ingested, the excreted DMA is likely to be mostly originating from organoarsenicals. The DMA results and results on other urinary arsenicals in the blue mussel group will be discussed further in a separate publication. In the cod group, excreted amount of iAs+DMA+MA was about 1.5 times the ingested amount (13.2 vs 8.8 µg). The sum of these metabolites only accounted for about 2 % of the tAs excreted in the cod group, as compared to about 11 %, 25 % and 23 % in the salmon, blue mussel and control groups, respectively. In the control group, DMA excreted (8 µg) was 5 times the amount ingested, and the sum of iAs+DMA+MA excreted was about 1.3 times the amount ingested (i.e. 12.1 vs 9.2 µg) (Table 3, column 6).

The optimal ANCOVA model for excreted DMA vs estimated absorbed non-AB in Table 4 (see explanation above), expressed urinary excretion of DMA 0-72h as a linear function of (estimated) absorbed non-AB As, with a common intercept and slopes specific for each group (R^2=0.93). The slopes (coefficients) are approximations of group-wise excretion percentages relative to non-AB As intake in the study. The standard deviations of the coefficients obtained for DMA indicated considerable inter- individual variation within the cod, salmon and control groups. Using the ANCOVA model, about 52 % and 43 % of non-AB As seems to have been excreted as DMA in the salmon and blue mussel group respectively, as opposed to only about 10 % in the cod group (for individual comparisons of the cod and salmon groups, see Figure 4B). This indicates that there is a higher content of DMA precursor arsenic compounds,

possibly arsenosugars and/or arsenolipids in salmons and blue mussels, which can be converted into DMA in humans.

The average total DMA urinary excretion rate in each group during the first 72 hours peaked between 12 and 18 hours (Figure 4A). The individual cumulative average DMA excretion curves relative to the non-AB fraction ingested (Figure 4B) for the salmon and cod groups clearly show both inter-individual variations, particularly in the salmon group, as well as group differences with a much lower relative excretion in the cod group. The cod group ingested about 200 µg unknown As species during day 0-2 (Table 2), but these species appear mostly not to be converted into DMA in the body. A differential pattern emerged for the four groups when the estimated total non-AB As absorbed was plotted against DMA excretion as a fraction of estimated amount non-AB absorbed (Figure 4C). In the salmon group, a large fraction was excreted as DMA, but with large individual variation. In the cod group, only a small fraction of absorbed non-AB As was excreted as DMA in all subjects. Because of large individual variation, there was no significant association between non-AB absorption and DMA excretion in the control group.

3.7. Inorganic As (iAs) and MA

The intake of iAs was generally low for all groups, but in absolute amounts, twice as high for the blue mussels group when compared with the other groups. The iAs ingested contributed 0.8 %, 2.3 % and 2.2 % of total As ingested in the cod, salmon and blue mussel group respectively. The iAs content in the cod and salmon test meal was somewhat higher than earlier studies have reported, while the iAs content in the blue mussel meal was in accordance with earlier studies (Borak and Hosgood, 2007; Sloth and Julshamn, 2008; Sloth et al., 2005; Uneyama et al., 2007). The urinary excretion of iAs was low in all seafood groups, between

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14-22 % of the ingested amount of iAs (Table 3). The MA content in the diet was below LOQ. Some previous human intervention studies have reported the presence of small amounts of MA in seafood (Buchet et al., 1994; Hsueh et al., 2002; Mohri et al., 1990), while others have not detected any MA (Heinrich-Ramm et al., 2002). All subjects in the seafood groups excreted small amounts of MA, accounting for 0.7 %, 2.3 %, and 2.2 % of the tAs compounds excreted in the cod, salmon and blue mussel group respectively (Table 3).

4. Discussion

The main finding of this study was the large apparent differences in the pattern of As species excreted following consumption of a single meal of different seafood. Another, and somewhat unexpected, finding was the high excretion of AB relative to intake, strongly indicating that some of the AB excreted is a result of biotransformation from other organic As species. More expected, but still striking, was the considerable amount of DMA formed, of which only a minor part can be explained by methylation of ingested iAs.

The excretory pattern of tAs is consistent with findings from several other studies of As following seafood intake, where 50 %-86 % of the ingested tAs was excreted in urine within two days (Arbouine and Wilson, 1992; Buchet et al., 1996; Buchet et al., 1994; Le et al., 1994; Tam et al., 1982). Also, in agreement with our findings, biological half-life of As ingested in seafood appears, initially, to be less than 20 hours (Fowler et al., 2007). Also, the plasma data (Figure 2C) indicate rapid absorption and subsequent somewhat slower excretion, consistent with some degree of biotransformation. Together, the plasma and urine data indicate a strong association between absorption and excretion, retention playing a minor role quantitatively.

4.1. Origin and metabolic fate of arsenobetaine (AB)

AB was the dominating dietary As species analyzed, accounting for 73 %, 76 %, 33 % and 70 % of tAs in the cod, salmon, blue mussels and control groups, respectively. Constant AB absorption, regardless of source, would necessarily imply similar relative absorptions in the cod, salmon and control groups, possibly with a less efficient absorption at the highest AB doses (Arbouine and Wilson, 1992). Our results show the opposite: Not only did estimated overall individual absorption of tAs vary between 39 % and 99 % , but, in the groups with similar AB percentage of intake, average group-wise absorption increased with tAs ingested (mean: cod 81 %, salmon 64 %, control 58 %). In the subjects with highest absorption, urinary excretion must necessarily be the dominating excretion pathway, and when AB is the dominating species, tAs and AB absorption cannot possibly be too far apart from each other. If the lowest absorbing subjects in the cod and salmon groups had zero absorption of non-AB As, their estimated AB absorption could still not exceed 78 % (447/570) and 64 % (141/220). With 30 % of non-AB absorption, which seems more likely based on As species in urine, AB absorption would be 68 % and 55 % for these subjects. It should be noted that with low non-AB absorption, in some cases the observed AB excretion cannot in many cases fully account for all AB absorbed. Therefore, based on our data, some biotransformation of AB to other arsenic species cannot be entirely ruled out. On the other hand, for the highest absorbers in the cod and salmon groups, estimated total As excretion was 769 and 267 µg, respectively. If these subjects had 100 % absorption of non-AB As, that would account for 210 and 69 µg, respectively. That would leave 559 (98 % of ingested AB) and 198 µg (91 % of ingested AB), respectively as lower bounds for absorbed AB in these subjects. Hence, our data indicate large variations in AB absorption, both individually and with food source; from almost 100 % and down to 60 % or lower. Furthermore, while not providing any conclusive evidence, they do

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not rule out the possibility that the low AB apparent absorption in some individuals is in part due to AB being biotransformed.

In this study, initial AB excretion, particularly in the cod group, was rapid and seemed somewhat faster than initial excretion of other As species. Our results are consistent with findings in earlier studies which indicate that As from seafood containing mostly AB, like cod, is eliminated faster than As from seafood containing a higher proportion of arsenosugars and arsenolipids, like blue mussels and salmon (Arbouine and Wilson, 1992; Le et al., 1994). This knowledge and our observation that particularly the blue mussel group excreted more AB than absorbed, strongly indicate formation of AB from other organic As species of seafood origin in humans.

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With apparent individual tAs absorption ranging from 58 % to 99 % (Table 3, column 9) in the cod group, the data indicate an absorption of about 125-200 µg non-AB arsenic in this group. Since only a fraction of non-AB arsenic could have been excreted as iAs+DMA+MA, and a significant amount of As is to be found in the unidentified fraction only on day 0. AB seems to be one likely candidate metabolite for excretion of the remaining non-AB arsenic ingested. With a mean estimated tAs absorption of 81 %, and similar AB absorption (see above), an average of about 450- 480 µg AB (could have been absorbed. This would account for approximately 80-85 % of the estimated total excretion of 556 µg AB (Table 3), hence, leaving about 15-20 % of the AB excreted by the cod group to be of non-AB origin. Similarly, a possibly larger fraction of urinary AB could be of non-AB arsenic in the blue mussel group.

The assumption that some of the excreted AB originates from the non-AB fraction of the cod is further supported by the ANCOVA model results (Table 4). In the cod group, a significantly lower proportion (10 %) of absorbed non-AB As was excreted as DMA in comparison with the fraction in the other groups.

In the blue mussel group, the regression coefficient (Table 4) describing the relation between excreted and estimated absorbed AB was significantly higher than those of the other groups. There was little individual variation in the coefficient within the blue mussels group. Excreted amount of As day 0-2, expressed as a fraction of ingested varied twofold in this group (37-78 %, Table 3), and taken together, these observations could indicate that the absorption of non-AB arsenic and AB was similar in the blue mussel group.

In a recent study it was found that 3 out of 5 volunteers consuming an AB-free diet, excreted AB in their urine. The authors speculated this could be due to either a long-term excretion of accumulated AB from the pre-trial diet or that AB is a human metabolite of DMA or iAs in the trial-food (Newcombe et al., 2010). Since our participants did not ingest any seafood seven days prior to the seafood test meal, and excreted very little As at the start of the study, it is not likely that accumulated lipid-soluble arsenicals contributed significantly to urinary excretion of AB. Most of the AB additional to that ingested and absorbed is probably a result of biotransformation of other arsenicals present in the test meals into AB.

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The suggested biotransfomation would explain most of the observations on the kinetics and metabolism of AB. At first sight, AB does indeed appear as a readily absorbed, metabolically inert and rapidly excreted As compound. At closer scrutiny, we get a somewhat more complex picture, with a possibility for biotransformation, accounting for a significant part of

As absorbed or excreted. Thus, AB excretion does not always reflect AB intake very well. Moreover, our data indicate that biotransformation of a small part of ingested AB cannot be entirely ruled out.

4.2. DMA formation and excretion

Consistent with previous human seafood consumption studies (Arbouine and Wilson, 1992; Buchet et al., 1996; Buchet et al., 1994; Heinrich-Ramm et al., 2002; Lai et al., 2004; Le et al., 1994; Mohri et al., 1990), DMA accounted for 8 % of the total tAs excreted in urine in the salmon group and 22 % in the blue mussels group, but only about 1 % cod group (Table 3). In two studies by Buchet and co-workers (Buchet et al., 1996; Buchet et al., 1994), the proportions of DMA relative to tAs excreted in urine after cod consumption were higher than in our study, 7 % and 12 %. In the same two studies, the proportions of DMA excreted after intake of mussels were 33 % and 42 %, which are comparable to our results.

Mohri et al. (1990) observed high amounts of trimethylarsenic compounds (probably mainly AB), in urine on the day after the participants ate dinners consisting mainly of fish, whereas high amounts of DMA in urine were linked to the ingestion of seaweed (Mohri et al., 1990). Previous studies have shown a range of metabolites in the urine of humans after ingestion of seaweed, mollusks or synthetic arsenosugar, with DMA being the major metabolite (Francesconi et al., 2002; Le et al., 1994; Ma and Le, 1998; Raml et al., 2009). The mechanism by which DMA is formed, and whether toxic intermediates, i.e. iAs or trivalent methylated species, are formed, is at present unknown. Francesconi et al. (2002) found that the proportion of DMA excreted by one volunteer after ingesting a synthetic arsenosugar was 67 %. Eleven other As compounds, nine of them unidentified, were also excreted (Francesconi et al., 2002). Francesconi et al. also noted a delayed excretion of DMA, with a

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595 peak at about 24 hours after intake of a synthetic arsenosugar. This is similar to our results
596 with regard to the excretion pattern for DMA following intake of blue mussel (which contains
597 arsenosugars). In a study in which two volunteers ingested cod liver oil (which contain
598 arsenolipids) (Schmeisser et al., 2006), the main metabolite excreted in urine was also DMA.
599
600 Consumption of seafood high in arsenolipids and/or arsenosugars, which are metabolized into
601 DMA - the same main metabolite, which is formed from iAs - could, provided it is formed in
602 a similar way, be an indication of health concern which should be further investigated. Most
603 epidemiologic studies exploring seafood intake and impact on human health do not
604 distinguish between different seafood and fish species and have not taken As in seafood into
605 consideration.
606
607 In the salmon and the blue mussel groups, approximately 40-50 % of non-AB As absorbed
608 seemed to be excreted as DMA, while in the cod group, this fraction was only about 10 %
609 (Table 4). In the cod group, much of the remaining non-AB As absorbed might have been
610 metabolized into AB. Although the very good fit (R^2=0.93) of the ANCOVA model it could
611 be a modeling artifact. This does not seem very likely: Absorption was estimated from total
612 As (tAs) excretion and AB intake was determined independently of urine analyses.
613 Furthermore, DMA excretion was analyzed independently, and did not correlate strongly with
614 tAs excretion.
615
616 The sum of excreted arsenite, arsenate, MA and DMA have commonly been used as a
617 biomarker for recent iAs exposure (Mandal and Suzuki, 2002; Steinmaus et al., 2009). This
618 assumption does not take into account that preceding seafood intake may influence the DMA
619 excretion, particularly intake of seafood that contains arsenosugars and/or arsenolipids. Our

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results strongly support the notion that DMA excretion may poorly reflect iAs ingestion in subjects exposed to seafood arsenic.

4.3. Possible iAs formation and MA excretion

All groups excreted far less iAs than ingested, 22 %, 14 %, 14 % during day 0-2 in the cod, salmon and blue mussel groups, respectively. Nor did any individuals excrete more iAs than ingested, so no indications for formation of iAs in the body after seafood ingestion were obtained in the present study.

Another finding, consistent with previous studies on seafood As exposure, was that all the groups excreted a small amount of MA, although the amount of dietary MA, which is largely excreted unchanged or as DMA, was below LOQ (Arbouine and Wilson, 1992; Buchet et al., 1996; Buchet et al., 1994; Mohri et al., 1990). A lower degree of methylation, i.e. a higher amount of urinary MA, has in epidemiological studies been associated with increased health risk, as e.g. cancer in the lung. It is documented that the methylation capacity is less efficient in men than women (Vahter et al., 2007), but our data sample was too small to determine gender difference. As regards evaluation by reference to the relative amounts of As metabolites in urine, low MA in urine is thought to indicate a higher rate of iAs elimination (Buchet et al., 1994; Vahter, 2002). In the present study, the relative percentages of iAs, MA and DMA excreted by the seafood groups were 0.2 %–0.6 % (iAs), 0.7 %–2.3 % (MA) and 2 %–31 % (DMA) (Table 3). These results are similar to the results of a comparable study in which volunteers ate different kinds of seafood, and in which the relative urinary As percentages were 0.04 %–0.94 % (iAs), 0 %–0.48 % (MA) and 1 %–22 % (DMA) (Buchet et al., 1994).

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4.4. Strengths and weaknesses of the study

The strength of this study is the study design with a controlled diet period with speciation of both dietary and urinary As, including full collection of three-day urine excretion after the bolus dose. The study design made it possible to shed light on several basic assumptions often made about As metabolism. Although some previous studies have provided data on both ingested and excreted As compounds (Buchet et al., 1996; Buchet et al., 1994; Mohri et al., 1990), these studies had fewer subjects (between five and nine), and generated fewer speciation data related to the As compounds ingested. In addition, the present study controlled the As intake by including not only restrictions on seafood consumption, but also restrictions on other foodstuffs with a potential As content.

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One weakness of the study was that it included an unknown source of As on day 1 in the strictly controlled diet period, which was supposed to be As-free. The main As compound in this extra loading was AB, probably originating from the roasted chicken meal (Table 2). As it is prohibited to use As-containing feed additives in Europe, the As might originate from the use of fish meal in chicken fodder. Several diet studies have found poultry to be a major contributor to As intake (Dabeka et al., 1993; Lasky et al., 2004; Tao and Bolger, 1999).

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5. Main findings and Conclusion

The first assumption, that absorption of AB is rapid and more or less complete, is refuted by our results. Our data show that there are large individual variations in the estimated absorption of AB, from around 50 to close to 100 %.

The second assumption, that absorbed AB is excreted rapidly and unchanged mainly in urine, and that the process is basically independent of the dietary source of AB, is also questionable

in light of our results. AB in salmon was less well absorbed and apparently more slowly excreted than AB in cod.

The third assumption, that there is no formation of AB in the body, is also cast into doubt by our results. The majority of the subjects in the blue mussel group apparently excreted far more AB than they had ingested, and overall AB excretion in both the blue mussel and the cod group was higher than expected from the estimated absorption.

The fourth assumption, that DMA (and MA) mainly is produced by methylation of inorganic As, and that urinary DMA (or MA) therefore can be used as a marker of inorganic As load, is strongly contradicted by our results in all three seafood groups, but to least degree in the cod group. Our results show that a small portion of DMA excreted in seafood consumers comes from dietary iAs. Hence, the assumption that DMA can serve as a biomarker of iAs intake is only valid when iAs, e.g. from drinking water, is the dominating species ingested and other dietary sources are removed or taken into account.

The last question we posed was if seafood consumption can increase the body load of iAs through metabolism of organoarsenicals. Provided that the formation of DMA from organoarsenicals does not take place via released iAs, our data do not indicate that organoarsenicals from seafood increases the load of iAs.

It should, however, be noted that the high loads of As associated with a high fish/seafood intake, in particular from seaweed and mussels where iAs can be released, might be of some concern and should be further studied. Future research should also address the possible health impact of ingesting arsenosugar/arsenolipid containing seafood, due to the high excretion of

695 the main metabolite of inorganic As, DMA. Finally, the mechanism of how DMA is formed
696 from these arsenicals and how AB can be formed by ingested organoarsenicals should be
697 further explored.
698
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Legends to figures:

Figure 1

Study design. The arrows pointing upwards indicate the time the urine and blood samples were collected. Regarding the urine samples, it is indicated whether the sample was a spot sample (spot) or part of a complete sampling (24h).

Figure 2

Time course of urinary tAs excretion and plasma tAs 0-72 hours after test meal.

A: Group means of urine tAs excretion rate (µg /h).

B: Group means of plasma tAs concentrations (µg/L) (Last measurement at 48h). Bars indicate standard deviation.

C: Group means of cumulative urinary tAs excretion as fraction of total amount of tAs ingested. Excreted fraction of tAs ingested were significantly higher in the cod group than in the salmon ($P<0.005$) and blue mussels ($P<0.0005$) groups. The latter two groups did not differ significantly.

D: Cumulative urinary tAs excretion as fraction of tAs ingested. Individual curves for the cod and salmon groups.

Figure 3

A: Group means of urine AB excretion rate (µg /h) after test meal 0-72h.

B: Cumulative urinary AB excretion as percentage of AB ingested. Individual curves for the cod and salmon groups (actual data shown, no corrections or estimations). AB excretion as a fraction of AB ingested were significantly higher in the cod and blue mussels groups (see Table 4) ($P<0.0001$) than in the salmon and control groups.

C: Total excreted AB 0-72h (observed) as a percentage of estimated absorbed AB, plotted against estimated absorbed AB for each of the study groups. For each individual, AB absorption is assumed to be equal to total As (tAs) absorption, which is estimated as estimated total tAs excretion divided by tAs ingested.

Figure 4

A: Group means of urine DMA excretion rate (µg /h) after test meal.

B: Cumulative urinary DMA excretion 0-72h as percentage of non-AB As (non-AB) ingested after test meal, individual curves for the cod and salmon groups (actual data shown, no corrections or estimations).

C: Total excreted DMA 0-72h (observed) as a percentage of estimated absorbed non-AB As, plotted against estimated absorbed non-AB As for each of the study groups. The percentage of DMA excreted was significantly smaller ($P<0.0001$) for the cod group than for the salmon and blue mussels groups. The latter two groups did not differ significantly. For each individual, non-AB As absorption is assumed to be equal to total As (tAs) absorption, which is estimated as estimated total tAs excretion divided by tAs ingested.

Table 1: Baseline characteristics and urinary total arsenic (tAs) concentrations of the study population (mean (± SD) and *median (min-max)*).

	Total	**Cod**	**Salmon**	**Blue mussel**	**Control**
Baseline characteristics					
Gender (male/female)	10/28	3/6	0/11	3/5	4/6
Age (years)	25.4 (5.0)	23.2 (3.4)	25.0 (3.1)	27.9 (6.3)	25.9 (6.3)
	24 (20-40)	*22 (20-31)*	*25 (21-32)*	*25.5 (22-37)*	*24.5 (20-40)*
BMI (kg/m^2)	23.9 (3.3)	23.4 (2.4)	22.4 (1.9)	24.6 (4.3)	25.5 (3.8)
	23.4 (19.5-33.3)	*23.3 (20.2-29.2)*	*22.5 (19.5-25.3)*	*23 (21.1-32.5)*	*24.3 (20.8-33.3)*
Urinary tAs concentrations					
Urinary tAs (µg/L)	83 (150)	72 (56)	149 (270)	75 (82)	27 (28)
(day -7, baseline)	*27 (5-910)*	*67 (7-188)*	*23 (7-910)*	*28 (19-209)*	*15.5 (5-90)*
Urinary tAs (µg/L)	20 (15)	15 (7.8)	20 (12)	29 (24)	18 (13)
(day 0, study start)	*16 (4-77)*	*14 (4-31)*	*16 (5-39)*	*19.5 (6-77)*	*17 (5-39)*

Table 2: The content of arsenic compounds in the test meals and in the duplicate portions of the strictly controlled diet (mean[1]).

	Seafood meal				Duplicate portions (72h)[2]
	Cod	**Salmon**	**Blue mussels**	**Control**	**All intervention groups**
tAs (µg)	670	180	620	3.7	110
iAs (µg)	2.8	3.3	13.0	4.4	3.2
DMA (µg)	1.3	3.0	15.0	0.1	1.5
MA	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15
AB (µg)	490	140	160	0.8	76
TMAO (µg)	3.0	6.0	4.4	< 0.5	< 0.5
DMAE (µg)	< 0.5	< 0.5	6.6	< 0.5	< 0.5
TMAP (µg)	2.9	< 0.5	42	< 0.5	< 0.5
TETRA (µg)	1.2	< 0.5	5.4	< 0.5	< 0.5
AC (µg)	3.8	< 0.5	5.4	< 0.5	< 0.5
Unknowns[3] (µg)	170	28	368	0	29

[1] The mean value was calculated for two identical samples of each of the test meals and the duplicate portions of the strictly controlled diet.

[2] Menu of the diet: day 0; seafood/control meal for breakfast, sandwich with ham and cheese for lunch and pasta with minced meat for dinner, day 1; Greek salad for lunch and roasted chicken with potato salad, day 2; ham and pasta salad for lunch and Greek meatballs with couscous for dinner. All other meals consisted of bread with cheese/meat/jam and orange/apple juice.

[3] Unidentified peaks in the chromatogram and unextracted arsenicals.

Table 3 Ingestion[1] of tAs, iAs, DMA, MA and AB and urinary excretion 72h following intake of the test meal (mean (± SD)).

Group/ Species	1[i] Amount ingested	2 Percentage of total As ingested	3 Urine day 0	4 Urine day 1	5 Urine day 2	6[ii] Urine day 0-2	7[iii] Urine day 0-2: % of the As species ingested excreted	8[iv] Total excreted: estimated	9[v] Total excreted: estimated % of As species ingested
	µg	%	µg (SD)	µg (SD)	µg (SD)	µg (SD)	% (Range)	µg (Range)	% (Range)
Cod group									
Total As	780	100	446 (90)	83 (21)	46 (13)	576 (94)	74 (52 - 92)	630 (447 - 769)	81 (58-99)
Inorganic As	6	0.8	0.4 (0.3)	0.5 (0.2)	0.5 (0.3)	1.3 (0.6)	22 (8 – 44)		
DMA	2.8	0.4	5.4 (1.1)	1.7 (0.5)	1 (0.3)	8 (1)	301 (204- 373)		
MA	<0.15	0	1.9 (3.2)	0.5(0.2)	1.5 (1.1)	3.9 (3.1)	0 (0-0)		
AB	570	73	388 (71)	78 (19)	42 (12)	507 (73)	90 (64 - 105)	556 (409 - 640)	99 (72-113)
Non-AB As	210	27	58 (41)	5(8)	5 (8)	68 (41)	32 (5-64)	74 (34-135)	35 (16-64)
Salmon group									
Total As	290	100	101 (24)	40 (11)	20 (7)	161 (30)	56 (46 - 82)	185 (141 - 267)	64 (49-93)
Inorganic As	6.5	2.3	0.3 (0.1)	0.3(0.1)	0.3 (0.1)	0.9(0.3)	14 (8- 23)		
DMA	4.5	1.6	8.6 (1.7)	2.7 (0.3)	1 (0.4)	13 (2)	283 (232 - 355)		
MA	<0.15	0	1.2 (0.9)	2.1 (3.7)	0.4 (0.2)	3.7 (3.6)	0 (0-0)		
AB	220	76	70 (7)	36 (10)	15 (6)	121 (19)	56 (48 - 77)	139 (111 - 198)	64 (51-90)
Non-AB As	70	24	31 (19)	4(2)	5(4)	40(21)	57 (34-141)	46 (25-107)	66 (36-155)
Blue mussels group									
Total As	690	100	219 (38)	74 (11)	36 (8)	328 (47)	49 (37 - 78)	371 (297 - 449)	56 (41-86)
Inorganic As	15	2.2	1.0 (0.9)	0.4 (0.1)	0.7 (1.1)	2.1 (1.6)	14 (5 - 30)		
DMA	16	2.3	46 (17.7)	17.2 (8.3)	7 (3.4)	71 (21)	451 (355 – 611)		
MA	<0.15	0	2.5 (0.9)	2.4 (1.8)	2.3 (2.0)	7.2 (2.7)	0 (0-0)		
AB	230	33	143 (27)	50 (6)	25 (9)	217 (30)	99 (82 - 151)	247 (200 - 313)	112 (87-173)
Non-AB As	460	67	76 (17)	24 (10)	11 (10)	111 (29)	25 (14-35)	124 (71-184)	28 (14-37)
Control group									
Total As	110	100	14 (6)	23 (7)	13 (3)	50 (12)	45 (30 – 60)	65 (44 - 84)	58 (39-75)
Inorganic As	7.6	6.8	0.3 (0.1)	0.4(0.3)	0.3 (0.1)	1.0 (0.4)	13 (6-20)		
DMA	1.6	1.4	4.3(3.4)	2(1.9)	1 (0.6)	8 (5)	485 (266- 1185)		
MA	<0.15	0	1.0 (0.5)	1.4 (2.5)	0.7 (0.4)	3.1 (2.5)	0 (0-0)		
AB	77	70	6 (4)	20 (6)	9 (3)	36 (10)	46 (31 – 65)	46 (30 - 63)	60 (39-82)
Non-AB As	33	30	8 (4)	3(3)	4 (1)	14 (7)	43 (24-100)	19 (13-40)	56 (39-119)

[1] Ingested both from test meal on 0h and from the diet (measured from double-portions) 72h after the meal. Two participants in the blue mussel group did not eat all seafood in the meal; 100/150g and 125/150g. This is taken into account when presenting the data.

[i] Column 1-5: Ingestion and urinary excretion 0-72h of tAs, iAs, DMA, MA and AB following intake of the test meal (mean (± SD)).

[ii] Column 6: Total of As species excreted in urine day 0-2 (measured), mean and SD.

[iii] Column 7: Column 6 as percentage of column 1, mean and range.

[iv] Column 8: Estimated total of As species excreted after a bolus dose, column 6 plus estimated residual, mean and range.

[v] Column 9: Estimated total of As species excreted after a bolus dose, column 8, as percentage of species ingested, column 1, mean and range. For tAs, estimated amount excreted is also used as an estimate for amount absorbed, making the ratio of estimated amount tAs excreted to amount ingested an estimate of fraction of As absorbed.

Table 4

Coefficients (SD in parentheses) of optimal ANCOVA regression models[1] using common intercepts and different slopes, for (1) 0-72h urinary excretion of arsenobetaine (AB) against estimated absorbed AB, and (2) urinary 0-72h excretion of dimethylarsinate (DMA) against estimated absorbed non-AB As. Coefficients with different superscripts are significantly different ($p<0.05$). In the model for AB, a coefficient above 1 indicates that AB may be formed in the body, as more AB is excreted than apparently absorbed. In the model for DMA, the coefficient indicates the tendency for non-AB As to be excreted as DMA (assuming no appreciable amount of dietary AB is excreted as DMA.) Thus, the three study groups differed significantly in 0-72h AB excretion relative to intake, the salmon group excreting less than absorbed, the cod group somewhat more, and the blue mussels group 1.62 times more than absorbed. For DMA, only 10% of absorbed non-AB As seemed to be excreted as DMA, significantly different from 43% in the blue mussels group. The coefficient was even higher (52%) in the salmon group, but the large SD rendered the differences to the other groups non-significant.

	1	**2**
	Arsenobetaine, (R^2=0.99,SD=22.3)	**Dimethylarsinate, (R^2=0.93,SD=10.1)**
Intercept	43.13 (20.9)	- 6.06 (9.63)
Cod	1.10 (0.05)[a]	0.10 (0.06)[a]
Salmon	0.68 (0.15)[b]	0.52 (0.22)[ab]
Blue mussels	1.62 (0.18)[c]	0.43 (0.04)[b]
Control	0.10 (0.47)[ab]	0.92 (0.49)[ab]

[1] $As_{excr} = b_0 + b_{1i}\ As_{absorbed}$, i=1,..,4 (i representing the different seafood/potato groups).

Figure 1

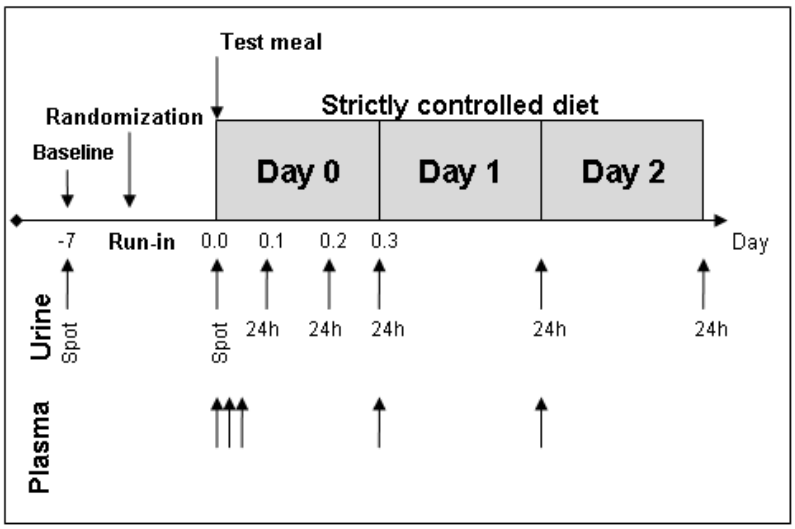

Test meal
Randomization
Baseline
Strictly controlled diet
Day 0
Day 1
Day 2
-7
Run-in
0.0
0.1
0.2
0.3
Day
Urine
Spot
Spot
24h
24h
24h
24h
24h
Plasma

Figure 2

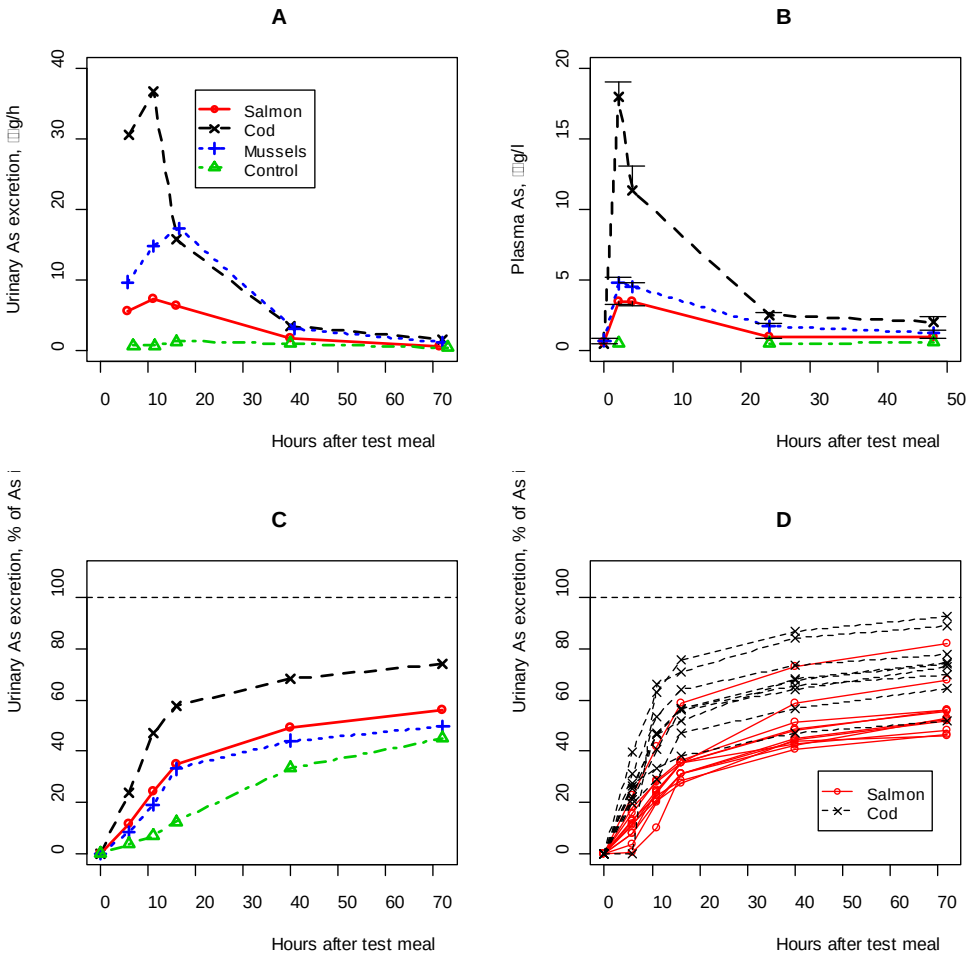

A
B
C
D
Urinary As excretion, □g/h
Plasma As, □g/l
Urinary As excretion, % of As i
Hours after test meal
Salmon
Cod
Mussels
Control

Figure 3

Merknad [m1]: Trond, vi må endre på teksten i figur 3B. Holder det å endre teksten til ”urinary arsenic excretion, % of AB ingested”?

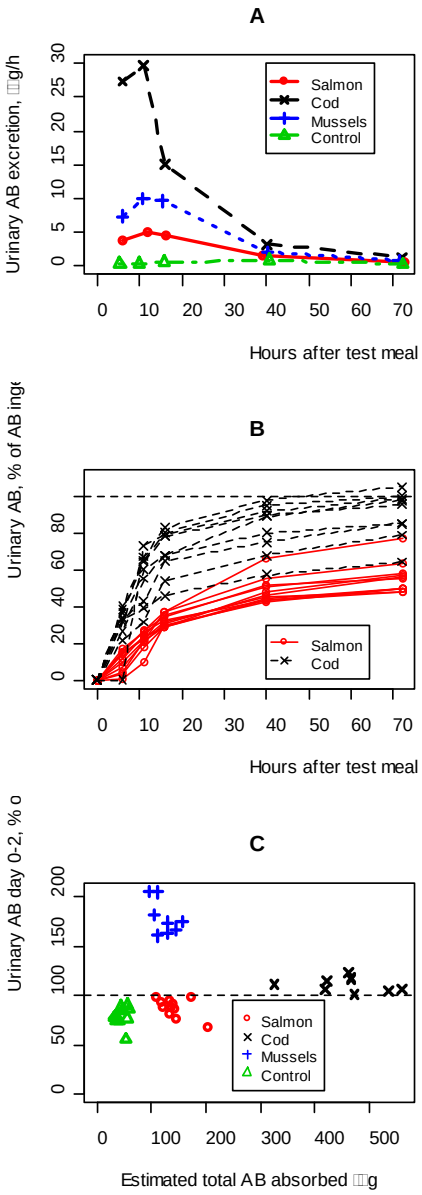
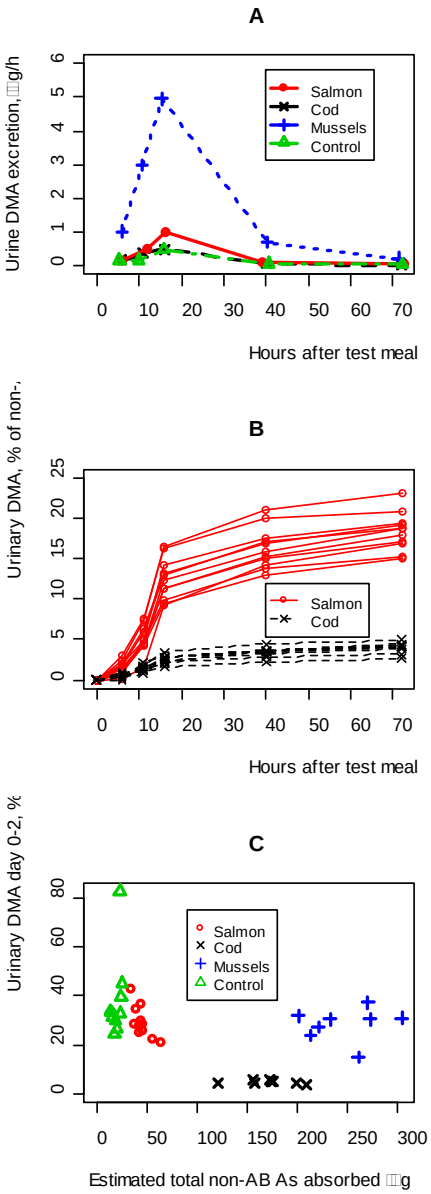


Figure 4


A
Urine DMA excretion, µg/h
0 1 2 3 4 5 6
Salmon
Cod
Mussels
Control
0 10 20 30 40 50 60 70
Hours after test meal
B
Urinary DMA, % of non-
0 5 10 15 20 25
Salmon
Cod
0 10 20 30 40 50 60 70
Hours after test meal
C
Urinary DMA day 0-2, %
0 20 40 60 80
Salmon
Cod
Mussels
Control
0 50 100 150 200 250 300
Estimated total non-AB As absorbed µg