

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

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23 Running title: Differential response to arsenic in seafood.

Abbreviations: AB, Arsenobetaine ((CH₃)₃As+CH₂COO⁻); AC, Arsenocholine ((CH₃)₃As+CH₂CH₂OH); As (III), Inorganic
arsenite (As(O⁻)); As (V), Inorganic arsenate (O=As(O⁻)); As, Arsenic; CRM, Certified Reference Material; DMA, Dimethylarsinate
((CH₃)₂AsO(O⁻)); HPLC, High Performance Liquid Chromatography; iAs, Inorganic arsenic; ICPMS, Inductively Coupled Plasma Mass
Spectrometry; LOQ, Limit of Quantification; MA, Methylarsonate (CH₃AsO(O⁻)); tAs, Total arsenic; TETRA, Tetramethylarsonium ion
((CH₃)₄As⁺); TMAO, Trimethylarsine oxide ((CH₃)₃AsO); TMAP, Trimethylarsoniopropionate ((CH₃)₃As+CH₂CH₂COO⁻).

24 Abstract

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

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

48 This research was supported by the Norwegian Research Council (project nr.142468/140).
49 The study was approved by the National Committee for Research Ethics and was carried out
50 in accordance with The Code of Ethics of the World Medical Association. Written informed
51 consent was obtained from each participant.

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73 1. Introduction

74 Dietary arsenic (As), in particular from drinking water, has for some time been considered a
75 major food safety issue (JECFA 2010). Several studies have identified seafood as the
76 predominant food source of As, with rice, mushrooms and poultry as additional sources,
77 depending on growing and feeding conditions (Borak and Hosgood, 2007; Dabeka et al.,
78 1993; EFSA, 2009; Munoz et al., 2005; Schoof et al., 1999; Tao and Bolger, 1999). In areas
79 with low or no inorganic arsenic (iAs) exposure from drinking water, the variation in total As
80 (tAs) from food is mainly due to variations in seafood and rice consumption (e.g. 28 µg per
81 day for an adult American woman and 160–280 µg per day for an adult Japanese woman)
82 (Tao and Bolger, 1999; Tsuda et al., 1995; Uneyama et al., 2007).

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84 The toxicity of As is highly dependent on the chemical form and oxidation state, i.e. trivalent
85 or pentavalent. The most potent toxicological arsenic compounds are the trivalent
86 arsenic species, which easily generate reactive oxygen and reacts with sulfuric compounds
87 (Fowler et al., 2007; Hughes, 2011; Styblo et al., 2002). In mammals, arsenic
88 biotransformation occurs in alternating steps involving reduction and subsequent oxidative
89 methylation to less toxic methylated forms such as methylarsonate (MA) and dimethylarsinate
90 (DMA). In this process intermediate trivalent methylarsonous acid and dimethylarsinous acid
91 are formed, which are equally more toxic than the corresponding pentavalent forms (EFSA,
92 2009; Hughes, 2011; Styblo et al., 2002; Thomas et al., 2001; WHO, 2001). The dominating
93 organic arsenicals in most seafood species, arsenobetaine (AB) is, despite limited evidence,
94 considered harmless (Borak and Hosgood, 2007; Lai et al., 2004). Arsenosugars, the
95 predominant form in algae and seaweed, have showed cytotoxic effects. The trivalent form of
96 the arsenosugar is more toxic than the pentavalent counterpart, thus both forms are
97 significantly less toxic than arsenate, MA (III) and DMA (III) (Andrewes et al., 2004; Sakurai

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

101

102 In seafood both the total content of arsenic and presence of different chemical forms of
103 arsenicals vary greatly with marine species, trophic level, diet/environment and ability of the
104 species to metabolize arsenicals. In Atlantic cod, the total As (tAs) concentration range from
105 0.4-52.4 mg As/kg wet weight (Julshamn et al., 2004; National Institute of Nutrition and
106 Seafood Research, 2011). However, the amount of iAs in fish fillets of cod has been reported
107 to be less than 0.001 µg/kg wet weight (Sloth et al., 2005). In fillets of farmed Atlantic
108 salmon, the tAs concentration range from 0.6-4.8 mg As/kg wet weight (Julshamn et al.,
109 2004; National Institute of Nutrition and Seafood Research, 2011), and iAs is found only in
110 trace amounts (Sloth et al., 2005). AB is the predominant form of As both in cod and salmon
111 fillets (Dahl et al., 2010).

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

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

123 2009). Irrespective of food source, iAs is generally considered to be absorbed rapidly and
124 almost completely after ingestion, although solubility and matrix also may play a role (EFSA,
125 2009). In spite of the fact that the metabolic pathway of iAs has not yet being fully clarified,
126 there has been a general consensus that a large part will undergo methylation and end up
127 being excreted as DMA (Cui et al., 2008; Hayakawa et al., 2005; Tseng, 2009). MA and
128 DMA present in foods seem to be excreted in the urine largely in their unchanged forms and
129 only a minor proportion of MA is converted to DMA (Tseng, 2009). A possible further
130 metabolism of DMA has been observed as a urinary metabolite in Bangladeshi women in the
131 form of thio-DMA (Raml et al., 2007), and further metabolism of DMA to TMAO was
132 observed in one human subject who ingested a high dose of DMA (Marafante et al., 1987).

133

134 Many details about absorption and metabolism of As compounds in humans are still
135 unknown, in particular about organoarsenicals from seafood (Borak and Hosgood, 2007).

136 Several studies have noted that seafood intake increases the urinary excretion of DMA, the
137 main metabolite from iAs metabolism (Buchet et al., 1996; Heinrich-Ramm et al., 2002; Lai
138 et al., 2004; Mohri et al., 1990). Arsenosugars and arsenolipids are probable sources of the

139 increase in the urinary excretion of DMA, as earlier interventions in humans have identified
140 an increased urinary excretion of DMA after intake of these arsenicals (Francesconi et al.,
141 2002; Ma and Le, 1998; Schmeisser et al., 2006). The large amounts of AB found in seafood
142 have for years been considered to be readily absorbed and then rapidly excreted unchanged in
143 urine (Lai et al., 2004). This notion is largely based on one study using isotopically labeled
144 AB, showing rapid excretion and with less than 1 % of the radioactivity remaining in the body
145 24 days after ingestion (Brown et al., 1990). Notable, hardly any quantitative data exist on the
146 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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173 2.2. Study design

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

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187 2.3. Intervention diet

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

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

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204 2.4. Blood sampling

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

210

211 2.5. Urine samples

212 Following ingestion of the test meals, urine was collected in three periods during the first 24
213 hours: (0.1) between the test meal and 2 pm (approximately 0-5h after the test meal); (0.2) 2-7
214 pm (approximately 5-10h after the test meal); and (0.3) 7 pm until first urination on the
215 following day (approximately 10-24h after the test meal) (Figure 1). For the next 48 hours,
216 24-hour urine batches were collected. Morning spot samples of urine were collected at
217 baseline (day -7), and before ingestion of the test meal (0 h). All urine samples were kept at 4
218 °C until all urine from each day/period was pooled. The total volume was then measured, and
219 15 ml of the urine was distributed into aliquots and stored below -70 °C until analysis.

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223 2.6. Analytical methods

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

228

229 The accuracy of the tAs determination for foodstuffs was evaluated by means of analysis of
230 two certified reference materials (CRM): DORM-2 Dogfish muscle (20.1 ± 1.0 mg/kg)
231 (National Research Council of Canada (NRCC)); and BCR CRM627 Tuna fish tissue ($5.1 \pm$
232 0.3 mg/kg) (IRMM, Geel, Belgium). The obtained results agreed well with the respective
233 certified values of 18.0 ± 1.1 mg/kg and 4.8 ± 0.3 mg/kg, respectively. The same BCR CRM
234 627 Tuna fish tissue was used for evaluation of the speciation analysis of the food samples.
235 The results were also compared with the results given in Sloth et al. (2003) for, TMAO
236 (trimethylarsine oxide), AC (arsenocholine), TETRA (tetramethylarsonium ion) and TMAP
237 (trimethylarsoniopropionate), as the CRM is only certified for AB and DMA (Maier EA,
238 1997). The obtained results (AB: 3.7 mg/kg, DMA: 0.15 mg/kg, TMAO: 0.016 mg/kg, AC:
239 0.016 mg/kg, TETRA: 0.034 mg/kg, and TMAP: 0.029 mg/kg), agreed well with the certified
240 values (AB: 3.9 ± 0.2 mg/kg; DMA: 0.15 ± 0.01 mg/kg) and the results given in Sloth et al.
241 (2003) (TMAO: 0.010 ± 0.002 mg/kg, AC: 0.012 ± 0.002 mg/kg, TETRA: 0.037 ± 0.002 mg/kg,
242 and TMAP: 0.033 ± 0.002 mg/kg). NIES No 18 Human urine (National Institute for
243 Environmental Studies, Ibaraki, Japan), is a CRM for tAs, AB and DMA, and was used to
244 evaluate the tAs of human urine samples. The obtained results (tAs 150 ± 6 µg/L and DMA
245 42 ± 6 µg/L), agreed well with the CRM (tAs 137 ± 11 µg/L and DMA 36 ± 9 µg/L).

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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 $\text{NH}_4\text{H}_2\text{PO}_4$ 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: $67.5 \pm 1.8 \mu\text{g As / L}$; certified: $69 \pm 12 \mu\text{g / L}$. DMA, measured: 42
258 $\pm 2 \mu\text{g As/L}$; certified: $36 \pm 9 \mu\text{g/L}$. Limit of quantification were taken as the concentration
259 of the lowest standard: $0.5 \mu\text{gL}^{-1}$.

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 $\mu\text{g/kg}$); DMA (0.25
263 $\mu\text{g/kg}$); and MA (0.15 $\mu\text{g/kg}$).

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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273 excreted measured as A_0 , A_1 and A_2 , the total excretion of the dose of arsenical can hence be
274 **(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
275 species, is considered to be mainly excreted in urine, the total excreted amount in urine can be
276 used as a crude estimate of the absorption of a single dose As ingested after an initial wash-
277 out period, as was the case here. The ratio k was estimated as the average day2/day1 excretion
278 ratio for the participants in the three seafood-consuming groups. The k-value was then applied
279 to individual excretion records, to produce individual estimates of total As **excretion**.

280

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

292

293 2.9. Statistical analyses

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

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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

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

326

327 The average day2/day1 excretion ratio for tAs in these groups was $k = 0.54 \pm 0.20$. This ratio
328 did not differ significantly among the groups. The observed slope of the logarithmic linear
329 relationship of day 2 vs day 1 urinary excretion was 0.86, and the corresponding slope for day
330 1 vs day 0 was 0.48. In a simple kinetic model the slope would be 1, thus our results indicate
331 both concentration and time dependency in the tAs excretion. Consequently, the tAs half-life
332 increased with decreasing body burden - in the cod group it was initially <16h. With a daily
333 tAs excretion of 50-100 μg , half-life seemed to be around 24h. The time course of urinary
334 excretion and plasma concentrations of tAs is illustrated by Figure 2 A-C. The plasma tAs and
335 urinary tAs concentrations peaked after 2h and 10h after ingestion of the test meal, however
336 the excretion pattern was similar for both urine and plasma (Figure 2 A-B).

337

338 Further analyses indicated that, rather than following a biphasic pattern typical of two-
339 compartment models, the As excretion changed more continuously, indicating a more
340 complex excretion and retention situation. The data do, however, not allow for a more
341 detailed modelling. We found that, in spite of the changing half-life for tAs excretion, treating
342 the tAs excretion after the test meal as a geometric series would still give a good
343 approximation of total excretion. Using the mean of the day2/day1 excretion ratios, 0.54, as
344 the constant k , total tAs excretion after day 2 could thus be crudely estimated as $0.54/(1-$
345 $0.54)=1.17$ times the day 2 excretion (Table 3).

346

347

348 3.4. Estimates of absorption based on total As excretion

349 The observed day 0-2 and estimated tAs species excretion, with corresponding percentages of
350 ingested amounts, are shown in Table 3, columns 6-9. Using **estimated** excreted tAs as an
351 estimate of absorption, the tAs absorption was estimated to be 81 % in the cod group, which
352 was significantly different ($p < 0.015$) from the salmon group, 64 % and the blue mussel group,
353 56 %. The difference in tAs absorption between the salmon and blue mussel groups was not
354 statistically significant. **Considerable inter-** individual differences were observed in all groups,
355 with ranges 58-99 %, 49-93 % and 41-86 % in the cod, salmon and blue mussel groups,
356 respectively (Table 3, column 9).

357

358 The data indicated larger inter-individual than inter-species absorption differences between
359 As species, consequently, each subject's tAs absorption estimate was used for all As species.

360 Such inter-individual differences are illustrated by the individual cumulative tAs excretion
361 curves (Figure 2D). Although absorption in the cod group was significantly higher than in the
362 salmon group, there was some overlap. In these groups, there were larger inter-individual
363 variations during the first hours than during the last.

364

365 3.5. Arsenobetaine (AB)

366 AB was the major As compound identified in the seafood investigated in this study,
367 comprising 73 %, 76 %, 33 % of the tAs ingested in the cod, salmon and blue mussel groups,
368 respectively (Table 3, column 2). The average urinary AB excretion rates during the first 72
369 hours showed a very pronounced peak of AB excretion around 10h in the cod group, but
370 smaller group-wise differences thereafter (Figure 3A). A considerable variation in the
371 individual relative cumulative excretion patterns in the cod and salmon groups appeared, with
372 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 % ($=0.64/0.76 \times 100$, 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 % ($((185-69)/219 \times 100$, 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

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

402

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

411

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

419

420

421

422 3.6. Dimethylarsinate (DMA)

423 In all groups, the excretion of DMA was higher than the amount ingested. The blue mussel
424 group excreted the highest amount of DMA. There was approximately a 2.5-fold increase in
425 excreted vs. ingested amount of iAs+DMA+MA, mostly excreted (90 %) as DMA (Table 3).
426 The amount of DMA excreted was about 4.5 times the ingested amount (71 vs. 16 µg). With
427 only 15 µg iAs ingested, the excreted DMA is likely to be mostly originating from
428 organoarsenicals. The DMA results and results on other urinary arsenicals in the blue mussel
429 group will be discussed further in a separate publication. In the cod group, excreted amount of
430 iAs+DMA+MA was about 1.5 times the ingested amount (13.2 vs 8.8 µg). The sum of these
431 metabolites only accounted for about 2 % of the tAs excreted in the cod group, as compared
432 to about 11 %, 25 % and 23 % in the salmon, blue mussel and control groups, respectively. In
433 the control group, DMA excreted (8 µg) was 5 times the amount ingested, and the sum of
434 iAs+DMA+MA excreted was about 1.3 times the amount ingested (i.e. 12.1 vs 9.2 µg) (Table
435 3, column 6).

436
437 The optimal ANCOVA model for excreted DMA vs estimated absorbed non-AB in Table 4
438 (see explanation above), expressed urinary excretion of DMA 0-72h as a linear function of
439 (estimated) absorbed non-AB As, with a common intercept and slopes specific for each group
440 ($R^2=0.93$). The slopes (coefficients) are approximations of group-wise excretion percentages
441 relative to non-AB As intake in the study. The standard deviations of the coefficients obtained
442 for DMA indicated considerable inter- individual variation within the cod, salmon and control
443 groups. Using the ANCOVA model, about 52 % and 43 % of non-AB As seems to have been
444 excreted as DMA in the salmon and blue mussel group respectively, as opposed to only about
445 10 % in the cod group (for individual comparisons of the cod and salmon groups, see Figure
446 4B). This indicates that there is a higher content of DMA precursor arsenic compounds,

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

449

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

463

464 3.7. Inorganic As (iAs) and MA

465 The intake of iAs was generally low for all groups, but in absolute amounts, twice as high for
466 the blue mussels group when compared with the other groups. The iAs ingested contributed
467 0.8 %, 2.3 % and 2.2 % of total As ingested in the cod, salmon and blue mussel group
468 respectively. The iAs content in the cod and salmon test meal was somewhat higher than
469 earlier studies have reported, while the iAs content in the blue mussel meal was in accordance
470 with earlier studies (Borak and Hosgood, 2007; Sloth and Julshamn, 2008; Sloth et al., 2005;
471 Uneyama et al., 2007). The urinary excretion of iAs was low in all seafood groups, between

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472 14-22 % of the ingested amount of iAs (Table 3). The MA content in the diet was below
473 LOQ. Some previous human intervention studies have reported the presence of small amounts
474 of MA in seafood (Buchet et al., 1994; Hsueh et al., 2002; Mohri et al., 1990), while others
475 have not detected any MA (Heinrich-Ramm et al., 2002). All subjects in the seafood groups
476 excreted small amounts of MA, accounting for 0.7 %, 2.3 %, and 2.2 % of the tAs compounds
477 excreted in the cod, salmon and blue mussel group respectively (Table 3).

478

479 4. Discussion

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

486

487 The excretory pattern of tAs is consistent with findings from several other studies of As
488 following seafood intake, where 50 %-86 % of the ingested tAs was excreted in urine within
489 two days (Arbouine and Wilson, 1992; Buchet et al., 1996; Buchet et al., 1994; Le et al.,
490 1994; Tam et al., 1982). Also, in agreement with our findings, biological half-life of As
491 ingested in seafood appears, initially, to be less than 20 hours (Fowler et al., 2007). Also, the
492 plasma data (Figure 2C) indicate rapid absorption and subsequent somewhat slower excretion,
493 consistent with some degree of biotransformation. Together, the plasma and urine data
494 indicate a strong association between absorption and excretion, retention playing a minor role
495 quantitatively.

496

497 4.1. Origin and metabolic fate of arsenobetaine (AB)

498 AB was the dominating dietary As species analyzed, accounting for 73 %, 76 %, 33 % and 70
499 % of tAs in the cod, salmon, blue mussels and control groups, respectively. Constant AB
500 absorption, regardless of source, would necessarily imply similar relative absorptions in the
501 cod, salmon and control groups, possibly with a less efficient absorption at the highest AB
502 doses (Arbouine and Wilson, 1992). Our results show the opposite: Not only did estimated
503 overall individual absorption of tAs vary between 39 % and 99 % , but, in the groups with
504 similar AB percentage of intake, average group-wise absorption increased with tAs ingested
505 (mean: cod 81 %, salmon 64 %, control 58 %). In the subjects with highest absorption,
506 urinary excretion must necessarily be the dominating excretion pathway, and when AB is the
507 dominating species, tAs and AB absorption cannot possibly be too far apart from each other.
508 If the lowest absorbing subjects in the cod and salmon groups had zero absorption of non-AB
509 As, their estimated AB absorption could still not exceed 78 % (447/570) and 64 % (141/220).
510 With 30 % of non-AB absorption, which seems more likely based on As species in urine, AB
511 absorption would be 68 % and 55 % for these subjects. It should be noted that with low non-
512 AB absorption, in some cases the observed AB excretion cannot in many cases fully account
513 for all AB absorbed. Therefore, based on our data, some biotransformation of AB to other
514 arsenic species cannot be entirely ruled out. On the other hand, for the highest absorbers in the
515 cod and salmon groups, estimated total As excretion was 769 and 267 µg, respectively. If
516 these subjects had 100 % absorption of non-AB As, that would account for 210 and 69 µg,
517 respectively. That would leave 559 (98 % of ingested AB) and 198 µg (91 % of ingested AB),
518 respectively as lower bounds for absorbed AB in these subjects. Hence, our data indicate large
519 variations in AB absorption, both individually and with food source; from almost 100 % and
520 down to 60 % or lower. Furthermore, while not providing any conclusive evidence, they do

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

523

524 In this study, initial AB excretion, particularly in the cod group, was rapid and seemed
525 somewhat faster than initial excretion of other As species. Our results are consistent with
526 findings in earlier studies which indicate that As from seafood containing mostly AB, like
527 cod, is eliminated faster than As from seafood containing a higher proportion of arsenosugars
528 and arsenolipids, like blue mussels and salmon (Arbouine and Wilson, 1992; Le et al., 1994).

529 This knowledge and our observation that particularly the blue mussel group excreted more
530 AB than absorbed, strongly indicate formation of AB from other organic As species of
531 seafood origin in humans.

532

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

544

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

549

550 In the blue mussel group, the regression coefficient (Table 4) describing the relation between
551 excreted and estimated absorbed AB was significantly higher than those of the other groups.
552 There was little individual variation in the coefficient within the blue mussels group. Excreted
553 amount of As day 0-2, expressed as a fraction of ingested varied twofold in this group (37-78
554 %, Table 3), and taken together, these observations could indicate that the absorption of non-
555 AB arsenic and AB was similar in the blue mussel group.

556

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

565

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

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

573

574 4.2. DMA formation and excretion

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

583

584 Mohri et al. (1990) observed high amounts of trimethylarsenic compounds (probably mainly
585 AB), in urine on the day after the participants ate dinners consisting mainly of fish, whereas
586 high amounts of DMA in urine were linked to the ingestion of seaweed (Mohri et al., 1990).

587 Previous studies have shown a range of metabolites in the urine of humans after ingestion of
588 seaweed, mollusks or synthetic arsenosugar, with DMA being the major metabolite
589 (Francesconi et al., 2002; Le et al., 1994; Ma and Le, 1998; Raml et al., 2009). The

590 mechanism by which DMA is formed, and whether toxic intermediates, i.e. iAs or trivalent
591 methylated species, are formed, is at present unknown. Francesconi et al. (2002) found that
592 the proportion of DMA excreted by one volunteer after ingesting a synthetic arsenosugar was
593 67 %. Eleven other As compounds, nine of them unidentified, were also excreted
594 (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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620 results strongly support the notion that DMA excretion may poorly reflect iAs ingestion in
621 subjects exposed to seafood arsenic.

622

623 4.3. Possible iAs formation and MA excretion

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

628

629 Another finding, consistent with previous studies on seafood As exposure, was that all the

630 groups excreted a small amount of MA, although the amount of dietary MA, which is largely

631 excreted unchanged or as DMA, was below LOQ (Arbouine and Wilson, 1992; Buchet et al.,

632 1996; Buchet et al., 1994; Mohri et al., 1990). A lower degree of methylation, i.e. a higher

633 amount of urinary MA, has in epidemiological studies been associated with increased health

634 risk, as e.g. cancer in the lung. It is documented that the methylation capacity is less efficient

635 in men than women (Vahter et al., 2007), but our data sample was too small to determine

636 gender difference. As regards evaluation by reference to the relative amounts of As

637 metabolites in urine, low MA in urine is thought to indicate a higher rate of iAs elimination

638 (Buchet et al., 1994; Vahter, 2002). In the present study, the relative percentages of iAs, MA

639 and DMA excreted by the seafood groups were 0.2 %–0.6 % (iAs), 0.7 %–2.3 % (MA) and 2

640 %–31 % (DMA) (Table 3). These results are similar to the results of a comparable study in

641 which volunteers ate different kinds of seafood, and in which the relative urinary As

642 percentages were 0.04 %–0.94 % (iAs), 0 %–0.48 % (MA) and 1 %–22 % (DMA) (Buchet et

643 al., 1994).

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645 4.4. Strengths and weaknesses of the study

646 The strength of this study is the study design with a controlled diet period with speciation of
647 both dietary and urinary As, including full collection of three-day urine excretion after the
648 bolus dose. The study design made it possible to shed light on several basic assumptions often
649 made about As metabolism. Although some previous studies have provided data on both
650 ingested and excreted As compounds (Buchet et al., 1996; Buchet et al., 1994; Mohri et al.,
651 1990), these studies had fewer subjects (between five and nine), and generated fewer
652 speciation data related to the As compounds ingested. In addition, the present study controlled
653 the As intake by including not only restrictions on seafood consumption, but also restrictions
654 on other foodstuffs with a potential As content.

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

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663 5. Main findings and Conclusion

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

667

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

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

672

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

677

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

685

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

690

691 It should, however, be noted that the high loads of As associated with a high fish/seafood
692 intake, in particular from seaweed and mussels where iAs can be released, might be of some
693 concern and should be further studied. Future research should also address the possible health
694 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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836

837 Legends to figures:

838

839 Figure 1

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

843

844 Figure 2

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

846 A: Group means of urine tAs excretion rate ($\mu\text{g/h}$).

847 B: Group means of plasma tAs concentrations ($\mu\text{g/L}$) (Last measurement at 48h). Bars
848 indicate standard deviation.

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

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

855

856 Figure 3

857 A: Group means of urine AB excretion rate ($\mu\text{g/h}$) after test meal 0-72h.

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

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

866

867 Figure 4

868 A: Group means of urine DMA excretion rate ($\mu\text{g/h}$) after test meal.

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

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

878

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

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

Table 2: The content of arsenic compounds in the test meals and in the duplicate portions of the strictly controlled diet (mean¹).

	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 Ingestion¹ 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 (\pm 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 models¹ 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, (R²=0.99,SD=22.3)	Dimethylarsinate, (R²=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}

¹As_{excr} = b₀ +b_{1i} As_{absorbed}, i=1,..,4 (i representing the different seafood/potato groups).

Figure 1

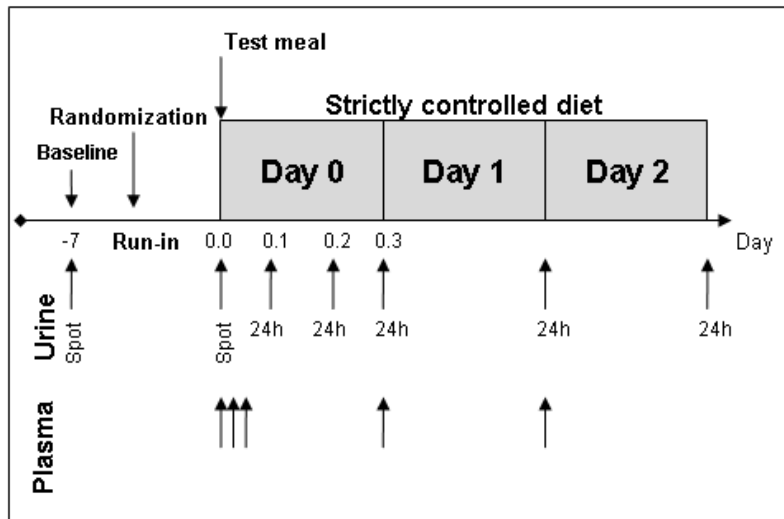


Figure 2

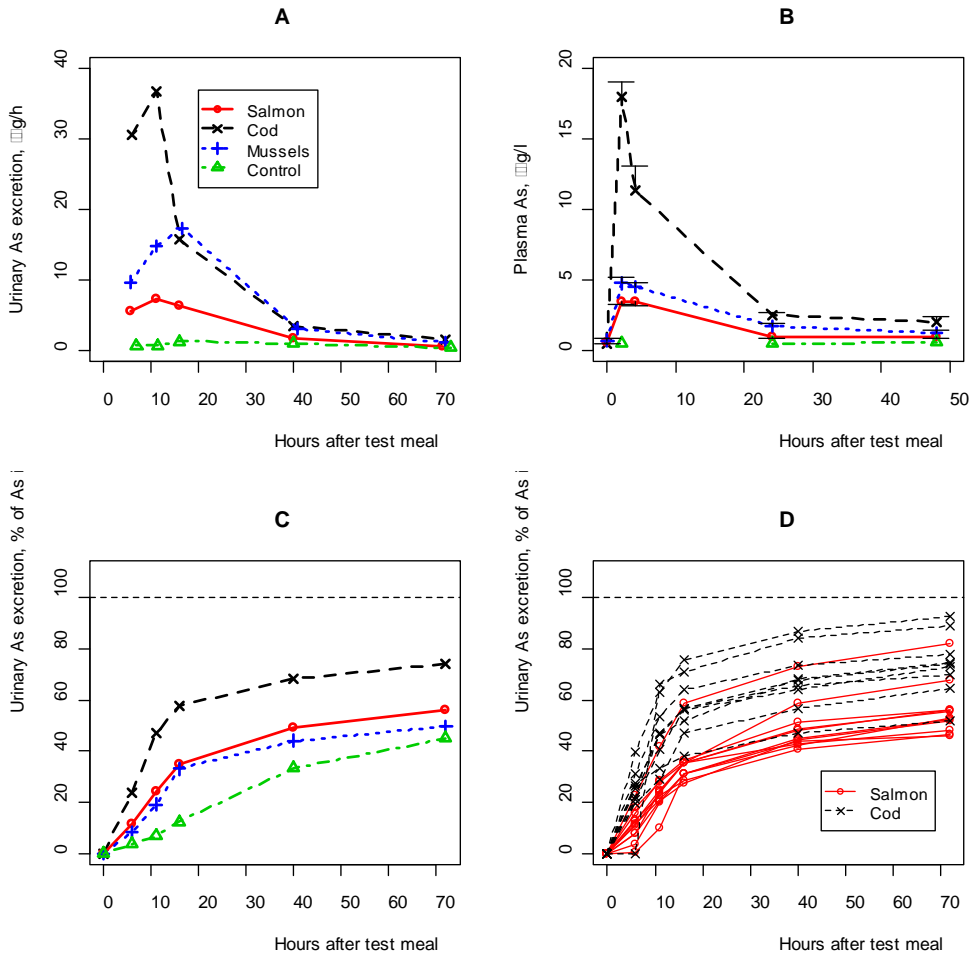


Figure 3

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

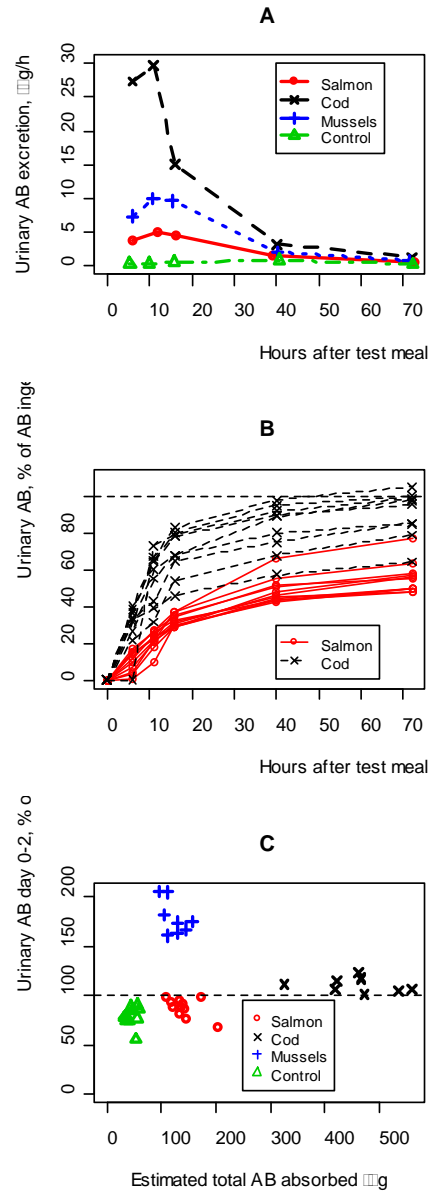


Figure 4

