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Water dilutes and alcohol concentrates urinary arsenic species when food is the dominant source of exposure

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46 **Abstract**

47 Exposure to certain arsenic (As) species has been associated with increased cancer risk and a wide range
48 of other health concerns, even at low levels. Here we used urine as a biomarker of As internal dose in a
49 well characterised cohort to relate diet, demographics and geography to exposure. As speciation in spot
50 urine samples was determined for 89 participants aged ≥ 50 years from the Northern Ireland Cohort for
51 the Longitudinal Study of Aging (NICOLA), stratified to cover the country. Principal component
52 analysis showed that all As species clustered together, suggesting that arsenobetaine, inorganic As and
53 the methylated species monomethylarsonic acid and dimethylarsinic acid forms have a common
54 source. Seafood and alcohol consumption were positively correlated with As species, while dairy
55 products (i.e. milk) and tap water were negatively correlated. Multiple regression analysis showed that
56 diet explained approximately 30% of the variability in urinary iAs concentrations. Geography was not
57 found to be a predictor of As exposure. Dairy consumption was negatively correlated and the best
58 predictor of iAs in urine, explaining 15.9% of the variability. The majority of the variation in As
59 biomarkers was not explained, suggesting the contribution of other sources and other non-predicted
60 variables on As metabolism and elimination.

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63 **Keywords:** arsenic, diet, metabolism, speciation, urine.

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70 1. Introduction

71 Human health is dependent on the quality of the food, air and water available. Long-term clinical health
72 conditions (such as cardiovascular diseases, diabetes, neurodegenerative diseases, respiratory diseases
73 and cancer) can be related to environmental and food exposure to toxic compounds (Wardrop and Le
74 Blond 2015). In the case of the metalloid arsenic (As), high concentrations of As in drinking water are
75 typically found in Bangladesh, West Bengal (India), Inner Mongolia (China), Taiwan, Argentina and
76 Chile (Chappell et al. 2003; Wu et al. 2018). In these areas, it is well known from epidemiological
77 studies that there is an association between inorganic As (iAs) exposure and urinary bladder, kidney
78 and lung cancer risk (Chappell et al. 2003; IARC 2012). Although those naturally high As regions are
79 of major concern, low-moderate chronic exposure to As have also been associated with a range of
80 detrimental health effects, including an increased risk of skin lesions, diabetes, cardiovascular effects,
81 neurotoxicity, lung disease, reproductive effects and cancer (WHO 2001).

82 Exposure to As can originate from different sources, such as inhalation (Xu et al. 2013; Subhani et al.
83 2015), dermal (Ouypornkochagorn and Feldmann 2010), drinking water (IARC 2004, Chappell et al.
84 2003; Navoni et al. 2014; Diaz et al. 2015) and food (Meharg and Raab 2010; Signes-Pastor et al.
85 2017a). The contribution of these sources depends on As levels in each of these matrices. Food can be
86 considered the main source of As exposure in the European Union and many other regions, where there
87 is high percentage of population with access to treated water supplies (Meharg and Raab 2010; Signes-
88 Pastor et al. 2017a). Rice consumption has been shown to be a concern for iAs exposure as it
89 accumulates iAs up to concentrations 10-fold higher than in other grains (Williams et al. 2006; Kordas
90 et al. 2016; Davis et al. 2017). Dietary studies, using urine spot samples as a biomarker of As exposure,
91 have shown that iAs in rice is both bioavailable and a dominant source in the diet (Signes-Pastor et al.
92 2017a; Wei et al. 2014). As species commonly found in urine include iAs, monomethylarsonic acid
93 (MMA) and dimethylarsinic acid (DMA), both of which are human metabolites of iAs, and the non-
94 toxic arsenobetaine (AsB), derived from seafoods (Navas-Acien et al. 2011; Popowich et al. 2016).
95 Other dietary sources of As, and how general dietary patterns (liquid, alcohol and vitamin intake etc.)
96 interplay with As metabolism, the methylation of iAs to MMA and DMA, are not well understood.

97 This study has a developed country focus, Northern Ireland (NI), where a high percentage of people
98 uses treated municipal water supplies with low levels of iAs (Signes-Pastor et al. 2017b) and where rice
99 is not the dominant staple (Harrington et al. 2001). Here we evaluate the association between As
100 exposure, nutrition and demographic variables in NI using a subset of participants aged ≥ 50 years from
101 the Northern Ireland Cohort for the Longitudinal Study of Ageing (NICOLA). The subset was
102 considered of settled geography since all participants were living in their own homes in Northern
103 Ireland. As has been shown to have strong environmental gradients in soils and waters over NI, as
104 revealed through detailed sampling (at 2 km² density) (GSNI 2007).

105

106 **2. Material and methods**

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108 *2.1 Study Population*

109 This study was conducted in a sub-sample (n = 89) of participants from the NICOLA study who were
110 also taking part in the NICOLA dietary validation study (NIDAS). Participants were randomly selected,
111 aged ≥ 50 years (born on or before September 30th 1962) and living in their own homes across NI.
112 NIDAS was primarily conducted to validate the dietary assessment methodology used in the NICOLA
113 study. Ethical approval for the both NICOLA and NIDAS studies were obtained from the School of
114 Medicine, Dentistry and Biomedical Sciences Research Ethics Committee, Queen's University Belfast
115 (Ethical Approval Numbers 15.18 and 12.23, respectively).

116 The inclusion criteria for this sub-sample were: a) participants in the NICOLA study; b) participants
117 willing to allow a researcher to visit them at their home. The exclusion criteria were: a) participants
118 unable to provide informed consent; b) participants with medical conditions that would limit their ability
119 to complete the protocol. Each participant signed a written informed consent prior to participating in
120 the study.

121 Spot urine samples were collected for As speciation analysis. All urine samples were stored at -80 °C
122 until analysis. All participants were asked to complete a 4-day food diary which involved them

123 recording their food and drink intake over a period of four days (two weekdays and two weekend days).
124 The dietary assessment and urine sampling were repeated after a period of 6 months to allow the capture
125 of any seasonal variation in diet. Demographic data (age, gender, location of residence, level of
126 education, smoking status and alcohol consumption) were obtained via a computer assisted personal
127 interview, while clinical data [weight, height, body mass index (BMI)] were obtained as part of the
128 NICOLA health assessment (Neville et al. 2019).

129

130 *2.2 As speciation analysis and sample preparation*

131 iAs, MMA, DMA and AsB were analysed in urine by ion chromatography interfaced with inductively
132 coupled plasma-mass spectrometry detection (IC-ICP-MS) using the method published by Signes-
133 Pastor et al. (2017c). Briefly, chromatographic separation was performed using a Dionex IonPacTM AS7
134 RFIC analytical column (2×250 mm, Thermo Scientific) protected by a Dionex IonPacTM AG7 guard
135 column (2×50 mm, Thermo Scientific) and a 12.5-minutes gradient mobile phase starting from 100%
136 20 mM ammonium carbonate (A) up to 100% 200 mM ammonium carbonate (B). Mobile phase flow
137 rate was 0.3 mL/min. Certified standards of AsB, DMA, tetramethylarsonium (TMAO), MMA and iAs
138 were used to determine their retention times. The concentration of As species were determined using a
139 DMA dilution series. SeronormTM Trace Elements Urine (Lot 0511545, Sero[®]) and Clinchek[®] Urine
140 Control, for As species, level 1, were used as certified reference material (CRM) for quality control.
141 Working standards, blank samples, urine samples and certified reference materials were included within
142 each analytical run.

143 The urine samples were prepared by appropriate dilution and centrifuged at $4500 \times g$ for 5 minutes.
144 Supernatants were transferred to 700 μ L polypropylene vials with 1% analytical grade hydrogen
145 peroxide (30%, Analar Normapur[®]) to convert all arsenite (AsIII) in arsenate (AsV). As species
146 concentrations in urine were statistically modelled with and without normalization by specific gravity
147 (SG).

149 2.3 Four-Day Food Diaries

150 Participants recorded all food and drink consumed over 2 weekdays and 2 weekend days (4 consecutive
151 days) by reporting the frequency, amount and method of food preparation. Amounts of foods were
152 reported in household measures (for example, 1 table spoon) or natural measures (for example, 1 slice
153 of bread). Researchers explained the dietary assessment protocols in detail to ensure close adherence.
154 Completed food diaries were reviewed by the researchers in order to check if any entries were unclear
155 and to clarify the information recorded. Mean food, energy and nutrient intakes from the food diaries
156 were calculated using a computerised food analysis database (Nutritics 2018).

157

158 2.4 Statistical analysis

159 All statistical analysis was performed using SPSS version 25.0 (SPSS Inc, Chicago, IL) and the
160 statistical significance was defined as $P < 0.05$. Descriptive statistics were obtained for all variables of
161 interest. The urinary concentrations of As species were not normally distributed with positive-skew
162 profile and were logarithmically transformed using base-10 for analysis. All analysis was carried out
163 using As species concentration in urine, both adjusted, or not, by SG. Analysis of variance (ANOVA)
164 was performed to evaluate the effect of geographical location on urinary concentrations of As species.
165 Principal component analysis (PCA) was performed with As species in urine and selected foods, drinks
166 and nutrients in order to analyse the relationships among these variables and to identify the main dietary
167 factors affecting As exposure. Due to the different units of measurement of variables, raw data was
168 standardized to receive equal weight in PCA. Variables were standardised by subtracting the mean
169 values from the raw data and dividing by the standard deviation. PCA was carried out using covariance
170 matrix, with extraction of two components and varimax rotation with Kaiser normalization. Multiple
171 linear regression using stepwise method was performed to predict exposure to As from demographic,
172 clinical and dietary variables.

174 3. Results and Discussion

175 A total of 89 participants [n = 42 males (47.2 %), n = 47 females (52.8 %)] were included in the analysis,
176 with ages ranging from 51.1 y to 89.5 y (average = 65.9 y; median = 66.6 y). Demographic and clinical
177 characteristics, including age, gender, weight, body mass index (BMI), smoking status and alcohol
178 consumption are presented in Table 1. BMI ranged from 20.9 to 43.9 kg/m² (average = 28.5 kg/m²;
179 median = 27.7 kg/m²) and 26.9 % of participants (n = 24) had BMI > 30 kg/m². Forty participants (44.9
180 %) were current or previous smokers (44.9 %). Alcohol was consumed by 61 participants (68.5 %),
181 with the mean consumption among consumers being 16.4 g/day (median: 4.81 g/day). All participants,
182 with the exception of two, completed the food diary. Mean food and nutrient intake are presented in
183 Table 2. The extent and spatial distribution of the participants in the study are shown in Figure 1.

184 The mean concentrations calculated for iAs, MMA, DMA and AsB in the CRMs were compared with
185 the mean certified values in order to calculate percentage recoveries (Supplementary Material - Table
186 S1). Recoveries of 108.3 %, 87.2 %, 94.7 % and 86.9 % resulted for iAs, MMA, DMA and AsB
187 respectively for ClinChek[®] Control (level I). For Seronorm[™] trace elements urine (lot 0511545), the
188 reference values were expressed in terms of total As only. The recovery of total As was 97.6 % and all
189 experimental concentrations were within the acceptable range. The limit of detection was (LOD) for As
190 speciation was 0.0067 µg/L, as estimated from the DMA calibration.

191 The median concentration (5-95th percentiles) of i-As, MMA, DMA and AsB were 0.33 (0.09-1.00)
192 µg/L, 0.32 (0.10-0.92) µg/L, 1.92 (0.58-7.53) µg/L and 2.28 (0.13-62.46) µg/L, respectively (Table 3).
193 TMAO was below the detection limit in ~70% of samples. No statistical differences were found between
194 the two urine samples collected 6 months apart, in terms of AsB, iAs, MMA, DMA (paired t-test, p >
195 0.05, data not shown). Therefore, the 2nd collected urine profile of each participant was considered for
196 further analysis. Although women have shown more efficient methylation capacity than men in previous
197 studies (Lindberg et al. 2008; Hudgens et al. 2016; Shen et al. 2016), gender was not a significant
198 predictor of As exposure in the current study.

199 As a naturally occurring element, the contribution of As exposure sources depends on the levels of As
200 within soil, water and food. Drinking water or food originating from regions with high concentrations
201 of As will result in a higher risk of adverse effects (Chappell et al. 2003; Wu et al. 2018). Results from
202 a detailed geochemistry study conducted by The British Geological Surveys (BGS) indicate that the
203 southern (South Armagh, Portaferry, Strangford and New Castle) and western (County Tyrone) regions
204 of NI have high soil As concentrations (> 10 mg/kg) (GSNI 2007). This geochemistry data has been
205 previously used to investigate potential associations with chronic diseases. The relationship of
206 nephrotoxicant elements (including As) and the prevalence of chronic kidney disease (CKD) was
207 investigated by Jackson et al. (2016). The main association with CKD was found with elevated
208 concentrations of the essential elements zinc and calcium in stream water (Jackson et al. 2016). A
209 potential association has also been observed between incidence of stomach cancer and high levels of As
210 in soils in the South Armagh area of NI (McKinley et al. 2013).

211 This current investigation of As exposure in Northern Ireland was performed in a population that was
212 widely distributed throughout the country (Figure 1). The effect of geographical location was
213 investigated by classifying participants according to their area of residence: Belfast ($n = 21$), West of
214 Bann ($n = 14$), South Down & Armagh ($n = 11$), East Derry & North Antrim ($n = 12$), South Antrim (n
215 $= 18$), North Down ($n = 13$). No differences were observed in terms of $\log_{10}$ -transformed urinary
216 concentrations of iAs, DMA or AsB across the various locations. Thus, the NI areas with high
217 concentrations of As in soils were not reflected in terms of iAs exposure in its residents. MMA
218 concentrations were lower in N. Down ($\log_{10}$: -0.63) and in S. Antrim ($\log_{10}$: -0.64) compared to Belfast
219 ($\log_{10}$: -0.37) and the West of the Bann ($\log_{10}$: -0.34, $p < 0.05$). A marginal difference ($p = 0.053$) was
220 observed for the $\sum \text{iAs} + \text{MMA} + \text{DMA}$, with similar profile to the one observed for MMA (Figure 2). The
221 education level had no effect on As exposure in the investigated population.

222 Post-industrialized cities, such as Belfast and Derry, may be expected to have higher environmental
223 pollutant burdens, including As (Shi et al 2012; Strosnider et al. 2017). However, previous reports show
224 higher As levels in rural residents when compared to residents from urban communities (Zailina et al.

2014; Pang et al. 2016; Zhang et al. 2018). In the current study we found no difference in As exposure between metropolitan versus non-metropolitan areas (Greater Belfast versus other areas, or Greater Belfast + Greater Derry versus other areas) (Supplementary Material - Figure S1). Indeed, the results showed that residing in a city/urban area does not add additional sources of arsenic exposure as assayed through urinary As.

Considering the substantial number of food and nutrient variables, PCA was performed to evaluate the relationships between the dietary variables and to identify the main covariates of As exposure. Due to the different units of measurement (concentration, food/drink/nutrient intake), all variables were standardized to receive the same weight within the PCA. Two factors were generated from the PCA which accounted for 35.5% of the variability (Figure 3, Supplementary material - Table S2). Results from the PCA analysis showed that all As species clustered together, suggesting that the variability in urinary concentrations AsB and the inorganic species have a common source. The principal component 2 separates As species, with seafood consumption being more strongly related with As species, followed by alcohol consumption. Dairy products and tap water consumption were negatively related to As species, being diametrically opposite to the As species cluster on plots of PCA1 versus PCA2. Most of the nutrients (protein, energy, vitamins B1, B2, B3, B5, B6, B7, B9, selenium, carbohydrates) were not related to As species, being orthogonal in the PCA plot. Given that food intake was observed to be a better predictor of As exposure, as opposed to nutrient intake, subsequent multiple linear regression analysis were performed using foods as potential covariates (Figure 3). Nutrients were excluded from the regression analysis to avoid multicollinearity.

Multiple regression analysis showed that variation in urinary concentrations of iAs was explained mainly by dairy products (15.9 %), alcohol (8.9 %) and fortified cereals consumption (4.0 %). The total contribution of these covariates explained around 30% of iAs variability. Dairy product consumption showed a negative association with As exposure, suggesting that the higher the intake the lower the iAs concentration in urine. Since the urinary concentrations of inorganic As species are positively correlated with the urinary flow rate (Middleton et al., 2016), urinary concentrations adjusted for SG were used

for PCA or multiple linear regression analysis. All analysis were carried out using both SG-normalized or non-normalized concentrations without changing significantly the results.

DMA is the main metabolite of iAs found in urine and accounts for approximately 60% - 90% of $\sum \text{iAs} + \text{MMA} + \text{DMA}$. Therefore, the variables which contributed significantly to the association in the multiple regression analysis were relatively similar for DMA and the $\sum \text{iAs} + \text{MMA} + \text{DMA}$. Dairy product consumption was the main predictor of DMA and $\sum \text{iAs} + \text{MMA} + \text{DMA}$, explaining approximately 15% of variation for each. Beside the apparent dilution effect of dairy products, seafood was a significant source of exposure accounting for 7-8%, followed by a dilution effect of tap water which accounted for 6-7% of the total variability (Table 4).

Seafood consumption, including both shellfish, oily fish and white fish, is known to be the main source of AsB (Signes-Pastor et al. 2017a; Jones et al. 2016). AsB in urine comes only from seafood consumption (Popowich et al. 2016) in contrast to DMA which is derived from As metabolism, seafood (Jones et al. 2016) and rice consumption (Punshon et al. 2018). In the present work, seafood was the main predictor of AsB, explaining 16% of the total variation in AsB urinary levels (Table 4). Milk consumption was considered a significant predictor in the current analysis, although it only explained 4.1% of the total variation in AsB concentration. Overall, while milk and seafood consumption together explained 20% of the variation in AsB, the majority of the variation remains unexplained. Although minor, when compared to AsB, seafood consumption was also a significant predictor of DMA, and the $\sum \text{iAs} + \text{MMA} + \text{DMA}$, explaining approximately 7.5 - 8% of the total variation. Linear modelling showed that a daily increase of 100 g of seafood was associated with 6-fold increase in urinary DMA concentration (Table 4). Other variables such as age, weight, BMI, smoking status or education level were not found to be predictors of iAs, DMA, MMA or AsB.

The effect of alcohol on As exposure is not clear in the literature. Some authors have shown that alcohol consumption does not effect As speciation or As methylation capacities (Hopenhayn-Rich et al. 1996; Tseng et al. 2005; Huang et al. 2008), while others found that the consumption of alcoholic beverages is related to increased levels of iAs in urine (Saoudi et al. 2012, Mori et al. 2016). Wine has been

277 previously reported as source of exposure by the regular use of arsenic as pesticide in vineyards in the
278 past (Saoudi et al., 2012). We found here a positive association between alcohol consumption and
279 urinary iAs (Figure 3, Table 4). This association suggests inhibition of As primary methylation, since a
280 negative correlation was observed between alcohol consumption and MMA/iAs ratio (Spearman
281 coefficient: -0.280, p-value: 0.009). The linear modellings showed that an increase of 10 g/day in
282 alcohol was associated with a 9.6% increase in urinary iAs concentration. These findings are consistent
283 with those reported in a systematic review and meta-analysis by Shen et al. (2016) whereby primary
284 methylation of iAs was 0.13-fold lower in alcohol drinkers compared to non-drinkers (Shen et al. 2016).
285 Based on the present findings and on previous data (Hopenhayn-Rich et al. 1996; Tseng et al. 2005;
286 Huang et al. 2008; Saoudi et al. 2012, Mori et al. 2016), we hypothesize that the alcohol effect on As
287 exposure can only be observed when As exposure is relatively low and/or food is a dominant source.
288 Beer consumption was positively associated with increased total As in urine in Japanese adults (Mori
289 et al. 2016). Increased urinary concentrations of iAs+MMA+DMA were associated with higher wine
290 consumption in a French cohort (Saoudi et al. 2012). Considering that similar effects are observed
291 despite the alcoholic beverage (Saoudi et al. 2012, Mori et al. 2016), we suggest that the alcohol
292 regulates As methylation. However, this effect is not observed at higher levels of As exposure
293 (Hopenhayn-Rich et al. 1996; Tseng et al. 2005; Huang et al. 2008).

294 Drinking water is a relevant source of iAs in areas with high natural levels in public or private water
295 supplies (Chappell et al. 2003; Kordas et al. 2016; Wei et al. 2014; Navoni et al. 2014; Diaz et al. 2015).
296 In the city of Belfast, the concentration of iAs, DMA and TMAO in tap water ranged from 0.039-0.047
297 µg/L, 0.027-0.031 µg/L and 0.050-0.057 µg/L, respectively (Signes-Pastor et al. 2017b). These levels
298 are very low compared to the parametric value of 10 µg/L for human consumption in the EU (CEU,
299 1998). Tap water consumption showed a moderate negative correlation with urine SG (Pearson
300 correlation coefficient: -0.462, p<0.001). Moreover, tap water intake showed a dilution effect on urinary
301 As excretion, either adjusting or not the urinary concentrations by SG (Supplementary material - Figure
302 S2).

303 A similar dilution effect on As species excretion was observed for dairy products or milk consumption
304 (Supplementary material - Figure S2). The average daily intake of dairy products reaches approximately
305 250 g (Table 2). Dairy products are generally low in iAs (Jackson et al. 2012; Sugár et al. 2013). As a
306 result, low levels of As species can be expected in NI. In the linear models, an increase in dairy product
307 consumption by 100 g per day was associated with a 26% decrease in iAs urinary concentration (Table
308 4). However, the dilution effect was not observed for the variable “water from drinks” which included
309 water intake calculated from all drinks consumed including juices, infusions, dairy products and soft
310 drinks. Similarly to tap water, a negative correlation was observed between “water from drinks” with
311 the log-transformed iAs in urine normalized by SG. These findings corroborate the dilution effect of tap
312 water on iAs levels in urine.

313 Results from the National Health and Nutrition Examination Survey (NHANES) and the US Department
314 of Agriculture’s Food Intakes Converted to Retail Commodities Database (FICRCD) showed that diet
315 accounted for 11.5% of the variation in the $\sum$ iAs+MMA+DMA (Davis et al. 2014). In the current study,
316 diet explained 30% of the variation for the same urine As species. Unlike previous studies where rice-
317 based products were reported to be a major cause of As exposure (Kordas et al. 2016; Wei et al. 2014)
318 this was not evident in NI. Rice is not a food staple in NI (18), and its consumption in the studied
319 population was very low (Table 2). The mean consumption of rice-based products was 20.2 g/day,
320 which is equivalent to 6.0 g of dried grains/day (Table 2). Rice consumption did not show any significant
321 association with urinary As species in the current population.

322 Despite the lack of a significant association between As exposure and nutrient intake in the current
323 study, As hepatic metabolism has previously been shown to depend on the folate cycle and methionine
324 cycle. The levels of SAM and glutathione, which are required for the serial As methylation and reduction
325 reactions, respectively, are regulated by methionine and folate pathways while vitamin B6 and B12 are
326 cofactors (Kurzius-Spencer et al. 2017). A previously randomized placebo-controlled trial showed that
327 folate supplementation resulted in increased percentage of DMA and reduced percentages of MMA and
328 iAs (Gamble et al. 2006). In NHANES 2003-2004, dietary folate, vitamin B6 and protein intake were

329 correlated to lower percentage iAs and higher percentage DMA, in urine (Kurzius-Spencer et al. 2017).
330 The high consumption of meat was also associated with higher percentage DMA in urine, probably due
331 to the higher sources of protein and methionine, which are SAM precursors (Kordas et al. 2016).

332 The current study has some limitations. The results are based on small sample size of older adults which
333 therefore limits the generalisability of the results. Due to the limited number of participants in this study,
334 other clinical variables relating to liver and renal function could not be included in the models. The
335 intra-individual variability on As kinetic disposition is likely to be higher in older adults than in young
336 adults, due to the variable age-related reduction in renal and hepatic function, comorbidities and
337 potential drug-arsenic interaction. Ageing is related to physiological reduced hepatic and renal
338 clearance, but also age-related diseases (Hilmer 2008). An older population may be more representative
339 for the assessment of residential area effect compared to younger adults who are more likely to travel
340 or move (Wilding et al. 2018). The present study shows that H₂O, alcohol and seafood are important
341 predictors of As levels in urine. It is, however, possible that other variables not evaluated in this work
342 such as genetic polymorphisms related to As toxicokinetics may be important contributors and may
343 increase the predictability of the regression models (Gribble et al. 2015).

344

345 **Conflict of interest**

346 All authors declare that there is no conflict of interest

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517 **Table 1.** Demographic and clinical characteristics of study participants (n = 89).

Characteristics	
Gender	Male: 42 (47.2%)
	Female: 47 (52.8%)
Age (years)	65.9 (51.1-89.5)
	66.6 (59-72.7)
Weight (kg)	77.3 (53.8-130)
	75.6 (66.2-86.4)

BMI (kg/m²)	28.5 (20.9-43.9)
	27.7 (25.5-30.4)
BMI status	<25 kg/m ² : 18 (20.2%)
	≥25 and < 30 kg/m ² : 47 (52.8%)
	≥30 kg/m ² : 24 (26.9%)
Smoking status	Current or previous: 40 (44.9%)
	Never: 49 (55.1%)
Alcohol consumption	Current: 61 (68.5%)
	Ex: 12 (13.5%)
	Never: 16 (17.9%)
	Average (min-max) consumption among users: 16.4 g/day (0-109.02 g/day)
Education level	No education/primary education: 11 (12.3%)
	Secondary education: 37 (41.6%)
	Third/higher education: 41 (46.0%)

518 Data presented as n (%) for categorical variables or as average (min-max); median (25th-75th
519 percentiles) for continuous variables

520

521 **Table 2.** Intake of selected food and nutrients as reported in 4-day food diaries (n = 87).

Characteristics	Range	Mean (95% CI)	Median (5-95 th Percentile)
Food and drinks			
Tap water ^a (L/day)	0-2.85	1.11 (1.00-1.21)	1.08 (0.46-1.93)
Alcohol (g/day)	0-109	16.4 (11.1-21.7)	4.8 (0 – 75.5)
Meat ^b (g/day)	0-406.7	127.2 (110.5-143.9)	128.5 (6.3-268.5)
Fish and seafood ^c (g/day)	0-191	28.6 (20.2-36.9)	21 (0-97)
Poultry ^d (g/day)	0-281	44.0 (33.0-54.9)	75.5 (52.5-124.6)
Rice ^e			
<i>Rice-based products (g/day)</i>	0-158	20.2 (12.9-27.6)	0 (0-93)
<i>Rice (g of dried grains/day)</i>	0-52.1	6.0 (3.72-8.28)	0 (0-29.1)
Potatoes (g/day)	0-557.5	129.7 (106.8-152.6)	103.7 (0-319.9)
Eggs (g/day)	0-145.0	27.6 (21.1-34.1)	18.8 (0-81.9)
Dairy ^f (g/day)			
<i>Total dairy products</i>	0-658.7	249.3 (218.7-280.0)	233.9 (30.3-497.8)
<i>Milk</i>	0-641.2	180.5 (151.9-209.0)	164.5 (0-394.5)
<i>Cheese and yogurts</i>	0-240.0	44.7 (34.1-55.3)	26.3 (0-143.1)

<i>Cream products</i>	0-217.8	24.1 (17.0-31.3)	14.6 (0-75.1)
<i>Cheese, yogurt and cream</i>	0-261.3	68.9 (56.2-81.5)	52.6 (2.1-176.8)
Eggs and dairy (g/day)	0-686.2	276.9 (246.1-307.7)	271.8 (61.7-521.0)
Whole grains ^g (g/day)	0-510.0	82.8 (66.4-99.1)	68.0 (0-213.0)
Legumes ^h (g/day)	0-165.0	22.8 (15.9-29.6)	13.3 (0-97.9)
Seeds and nuts ⁱ (g/day)	0-98.0	6.2 (3.0-9.3)	0 (0-28.2)
Dark leafy vegetables ^j (g/day)	0-105.0	19.2 (13.8-24.6)	11.0 (0-74.0)
Fortified cereals ^k (g/day)	0-285.0	15.9 (7.8-24.0)	0 (0-58.5)
Nutrients			
Energy (kcal/day)	980-3450	1948 (1837-2058)	1883 (1248-2931)
Protein (g/day)	32.3-159.7	79.9 (74.8-84.9)	75.5 (52.5-124.6)
Carbohydrate (g/day)	65.0-389.6	210.2 (197.6-222.7)	200.1 (133.5-319.9)
Fat (g/day)	36.2-160.1	76.2 (70.9-81.5)	75.7 (38.5-125.8)
Thiamine (B1, mg/day)	0.5-3.4	1.7 (1.5-1.8)	1.6 (0.9-2.8)
Riboflavin (B2, mg/day)	0.6-3.9	1.8 (1.7-2.0)	1.8 (0.9-3.1)
Niacin (B3, mg/day)	11.4-108.1	37.7 (34.7-40.7)	35.1 (21.2-57.6)
Pantothenate (B5, mg/day)	1.5-18.2	6.2 (5.7-6.7)	5.8 (3.8-9.5)
Pyridoxine (B6, mg/day)	0.4-8.4	2.1 (1.9-2.4)	1.8 (1.1-3.9)
Biotine (B7, mg/day)	12.3-85.9	43.8 (40.6-47.1)	43.0 (22.6-70.1)
Folates (B9) (µg/day)			
<i>Total folates</i>	105.0-797.8	267.8 (243-1-292.4)	242.5 (145.1-508.7)
<i>Dietary Folate Equivalent (DFE)</i>	105.0-857.8	279.8 (251.7-307.9)	260.0 (148.5-499.7)
<i>Natural folates</i>	71.7-573.7	239.0 (218.5-259.5)	216.5 (139.2-430.1)
<i>Synthetic folates</i>	0-348.0	27.2 (15.8-38.6)	0 (0-110.8)
Cobalamine (B12, µg/day)	0.3-39.6	5.5 (4.5-6.6)	4.9 (1.5-9.7)
Methionine (mg/day)	0-2490.6	120.2 (46.6-193.8)	16.8 (0-428.3)
Cysteine (mg/day)	0-939.6	70.4 (41.1-99.7)	23.9 (0-275.5)
Selenium (µg/day)	14.5 -153.3	45.1 (40.6-49.5)	41.3 (18.9-78.5)

^aWater from the tap for drinking, used to prepare powdered drinks or infusions, boiled or not.

^bRed meat, poultry, fish and seafood

^cFatty fish (salmon, sardines, trout, mackerel, tuna, anchovies), white fish (cod, haddock, hake, monkfish, seabass, whiting, sole), shellfish (scallops, prawns, crayfish, mussels, squid, lobster)

^dChicken and turkey

^eWhite and brown rice, boiled, steamed or in rice-based products (noodles, rice cakes, breakfast cereal flakes, pudding);

^fMilk (whole, semi skimmed or skimmed) from cow, goat or sheep, cheeses, yogurts, creams (crème fraîche, sour cream, double cream, whipping cream and ice cream)

^gBrown rice, wholewheat, barley, millet, quinoa, oats, popcorn, whole rye, wheat berry

^hLegumes: beans, chickpeas, green peas and lentils

ⁱSunflower seeds, cashew nuts, pistachios, peanuts, almonds, pine nuts, coconut, walnuts, pecan nuts, Brazil nuts, quinoa, flax seed almonds

^jFortified cereals (breakfast cereals, breads, cakes fortified with B vitamins)

^kDark, leafy vegetables: kale, Brussel's sprouts, spinach, rocket, broccoli, mustard

Table 3. Urinary concentration of arsenic (As) species in the study population (n = 89), with and without specific gravity normalization

	iAs	MMA	DMA	AsB	Total As	ΣiAs+MMA +DMA
<i>Urinary concentrations (µg/L)</i>						
Mean	0.42	0.40	2.78	12.01	15.66	3.60
(95% CI)	(0.35-0.48)	(0.34-0.46)	(2.24-3.32)	(7.16-16.9)	(10.5-20.8)	(2.98-4.21)

Median (5-95th percentiles)	0.33 (0.09-1.00)	0.32 (0.10-0.92)	1.92 (0.58-7.53)	2.28 (0.13-62.46)	5.44 (1.33-69.53)	2.57 (0.80-9.46)
Range	0.07-1.68	0.06-1.36	0.26-14.64	0.05-127.31	0.64-135.23	0.45-14.85
<i>Urinary concentrations, specific gravity normalized (µg/L)</i>						
Mean (95% CI)	0.41 (0.35-0.48)	0.39 (0.34-0.45)	2.73 (2.21-3.26)	11.78 (7.04-16.52)	15.37 (10.37-20.37)	3.54 (2.93-4.14)
Median (5-95th percentiles)	0.32 (0.08-0.98)	0.31 (0.10-0.91)	1.90 (0.58-7.35)	2.24 (0.13-61.19)	5.33 (1.32-67.91)	2.51 (0.79-9.23)
Range	0.07-1.64	0.06-1.33	0.26-14.49	0.05-124.56	0.64-132.32	0.45-14.71

iAs: inorganic As; MMA: monomethylarsonic acid; DMA: dimethylarsinic acid; AsB: arsenobetaine, Total As: sum of all species in urine, Σ iAs+MMA+DMA: sum of iAs, MMA and DMA concentrations in urine; 95% CI: 95 % confidence interval

Table 4. Multiple regression analysis showing the contributors to urinary arsenic (As) species in terms of demographic, clinical and dietary variables (nutrients excluded).

Variable	iAs	MMA	DMA	iAs+MMA +DMA	AsB
Dairy products	16.0%***	-	14.8%**	15.7%**	
Alcohol	9.0%***	-	-	-	-

Fortified cereals	4.0%*	-	-	-	-
Tap water	-	15.3%***	5.8%*	6.9%**	-
Milk	-	4.5%*			4.1%*
Seafood	-	-	7.5%**	7.8%**	16.0%***
Poultry	-	-	3.5%*	-	-
Total	29.0%	19.8%	31.6%	30.4%	20.1%
contribution					
P-value ^a	<0.001	<0.001	<0.001	<0.001	<0.001

551 Urinary As species variables were log-transformed and normalized by specific gravity.

552 $\text{Log [iAs]} = -0.366 - 0.001 \times \text{Dairy products (g/d)} + 0.004 \times \text{Alcohol (g/d)} + 0.002 \times \text{Fortified cereals}$
553 (g/d)

554 $\text{Log [MMA]} = -0.161 - 0.222 \times \text{Tap water(L/d)} - 0.000461 \times \text{Milk (g/d)}$

555 $\text{Log [DMA]} = 0.651 - 0.001 \times \text{Dairy products (g/d)} + 0.003 \times \text{Seafood(g/d)} - 0.168 \times \text{Tap water(L/d)} -$
556 $0.001 \times \text{Poultry (g/d)}$

557 $\text{Log [iAs+MMA+DMA]} = 0.736 - 0.001 \times \text{Dairy products(g/d)} + 0.002 \times \text{Seafood (g/d)} - 0.172 \times \text{Tap}$
558 water (L/d)

559 $\text{Log [AsB]} = 0.432 + 0.008 \times \text{Seafood (g/d)} - 0.001 \times \text{Milk (g/d)}$

560 *P< .05, ** P<0.01, ***P<0.001

561 ^aP-value for the F test